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**A COMPARISON OF METHODS** <sup>93</sup>  
<sub>6466</sub>  
**FOR THE DIAGNOSIS OF BOVINE SUBCLINICAL MASTITIS**  
**WITHIN NEW ZEALAND DAIRY HERDS**

A thesis presented in partial fulfilment of the requirements  
for the degree of Doctor of Philosophy in Veterinary Clinical Science  
at Massey University.

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## ABSTRACT

During the 1986-1987 and 1987-1988 dairy seasons, milk samples were taken from cows within three New Zealand dairy herds. Individual quarter foremilk samples were taken using aseptic precautions, and whole udder, composite samples were obtained using a milk meter, at monthly intervals.

The three herds differed with respect to herd size, milking shed design, use of teat spraying, and the incidence of mastitis, both past and present.

The bacteriological status of each quarter was determined, and the ability of the following parameters to distinguish between infected and uninfected quarters, and between infected and uninfected cows was ascertained.

- (1) Somatic cell count
- (2) Sodium concentration
- (3) Potassium concentration
- (4) Electrical conductivity
- (5) pH
- (6) Lactose concentration
- (7) N-acetyl- $\beta$ -d-glucosaminidase activity
- (8)  $\alpha$ 1-antitrypsin concentration

With the exception of the antitrypsin concentration, the concentration of each parameter in the milk changed during the course of milk removal. The effect of this variation on the composition of the composite milk sample is discussed.

The stage of lactation at which the sample was taken exerted a significant effect on the level of each parameter in quarter foremilk samples. Similar effects were observed with composite samples, although statistical significance was not reached in every instance. Both infected and uninfected udder quarters were affected by the stage of lactation.

The age of the cow exerted a significant effect on the levels of a number of the parameters within quarter foremilk samples. The effect of age of the cow on the level of each parameter within composite milk samples was generally not significant.

The bacteriological status of the udder quarter exerted a significant effect on the level of each parameter in at least one of the three herds. The effect of the bacteriological status on the pH and on the potassium concentration of the milk was smaller in degree than was the effect on the remaining parameters.

The herds differed with respect to bacteriological findings. Herd A showed a lower incidence of infection than did herds B or C. The incidence of infection with minor pathogens was highest within herd B, while the incidence of infection with major pathogens was highest within herd C. The incidence of infection tended to increase with the age of the cow.

While each of the eight parameters showed high specificity, the sensitivity was generally lower. An exception to this finding was the somatic cell count which showed both high sensitivity and high specificity.

The somatic cell count was able to correctly classify more than 75 % of quarter foremilk samples in each of the three herds, being more consistent in this respect than were any of the other parameters, the diagnostic abilities of which varied between herds.

The addition of second parameter to a model containing the somatic cell count generally did not increase the diagnostic accuracy of the system.

The threshold value was found to vary both between herds, and between stages of the lactation, this variation being greatest for the somatic cell count. Providing that the threshold is adjusted for the stage of lactation, the somatic cell count is able to accurately predict the infection status throughout the dairy season. The selected threshold should take into account the level of bacterial infection within the particular herd to which it will be applied.

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## CHAPTER ONE

### THE SIGNIFICANCE OF BOVINE MASTITIS

#### (1) A DEFINITION OF MASTITIS

The term " mastitis " may be simply defined as " inflammation of the mammary gland ", the term being derived from the Greek word " mastos " meaning " breast " and the suffix " itis " meaning " inflammation of " ( Blackiston, 1972 ).

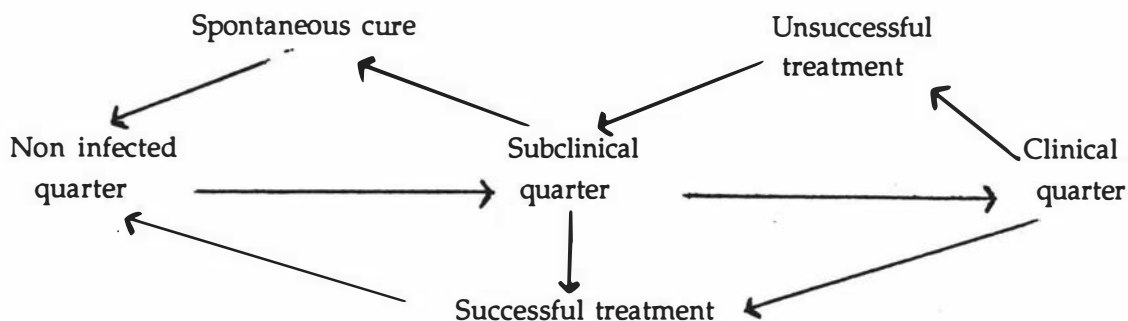
Mastitis is not, however, a simple disease, but rather a disease complex resulting from the interplay between microbial infections and managerial practices ( Schalm and Woods, 1953 ). This interplay results in variations in causative agents, duration, severity, pathogenesis and prognosis both between herds and between cows within herds. Central to the disease complex however, is inflammation of the mammary gland.

Inflammation of a tissue is characterized by the cardinal signs - heat, swelling, redness, pain and often a partial loss of function. The loss of function may be seen as obvious differences in the appearance of the milk which include flakes, clots and blood. Any combination of these signs may be present in bovine mastitis, in which case the condition is termed " clinical ". Clinical mastitis may be divided into a number of categories, depending on the severity of the condition. " Peracute mastitis " describes the case in which the abovementioned cardinal signs are accompanied by systemic disturbances which may include loss of appetite, shivering, fever and depression ( Schalm et al, 1971 ).

In the acute form of the disease, systemic signs are present, but less severe than is the case with peracute mastitis, while subacute mastitis refers to the presence of obvious signs of inflammation, but an absence of systemic effects.

Clinical mastitis is a severe and on occasions life threatening disease of dairy cows. Bovine mastitis may also occur in a more insidious form in which there are no systemic signs and no obvious indications of inflammation. This form of the disease complex is termed " subclinical mastitis ".

While at any given time, the quarter of an infected cow may be classified as being either clinical or subclinical, it must be remembered that the classification may change with time, or with the application of antibiotic therapy. Thus mastitis is a dynamic disease as is shown in the following diagram ( adapted from Dodd and Neave, 1970 ).



In practice, the clinically infected quarter is that which is apparent to the stockman, although this will depend on both his vigilance and experience.

While clinical mastitis is undeniably a problem in its own right, it is readily recognised, allowing appropriate treatment to be given. The detection of subclinical mastitis is more difficult, and in the absence of specific testing, the condition can remain hidden until one of the following outcomes occurs:

- (1) Spontaneous recovery,
- (2) The condition becomes clinically apparent,
- (3) Therapy is given either during the lactation or at the commencement of the dry period.

Subclinical mastitis may have implications at each level in the dairy industry from producer to consumer.

## (2) EFFECTS OF SUBCLINICAL MASTITIS ON THE PRODUCER

The dairy farmer may be affected by subclinical bovine mastitis in the following ways:

- (1) There may be a decrease in the volume of milk produced by affected cows.
- (2) The composition of the milk may change.
- (3) The condition may become clinical, resulting in treatment costs and inconvenience.
- (4) Infected cows may act as a source of pathogenic bacteria for other uninfected members of the herd.
- (5) The bacteria excreted from infected quarters will add to the total number of bacteria in bulk milk, which may lead to financial penalties being applied.

The effects of subclinical mastitis on milk production have been reviewed by Hoare ( 1982 ).

Historically, three types of studies have been carried out in an effort to determine the effect of mastitis on milk yield:

- (1) Between quarter comparison, ie within a cow, infected vs uninfected quarters
- (2) Between cow comparison, ie infected vs uninfected cows within a herd
- (3) Comparisons between herds with varying levels of infection.

Milk yield comparisons between dairy herds are subject to error through uncontrolled variables such as breeds of cow, age, stage of lactation and management factors. Within such a study, Natzke *et al* ( 1972b ) found that herds on a mastitis control program produced on average, an extra 477 kg of milk per cow per year for each of three years, than did the average New York dairy herd. The increased milk yield was associated with a decrease in the number of infected quarters in a herd from 28.1 % to 7.1 %. Similarly, Benson and Dettman ( 1977 ), found an increase of 343 kg of milk per cow per year with the introduction of a mastitis control program, over a three year period. In this study herds were closely matched for size of herd, calving pattern, age distribution and level of production prior to commencement of the trial. For an increase in bulk milk somatic cell count of 100,000 cells per ml, there was a loss of between 0.24 and 0.30 litres per cow per day ( Holmes and Gill, 1978 ).

Within herd comparisons eliminate some of the variability, but unless the cows are closely matched for age/parity, stage of lactation and breed, then the scope for error is still present. Within such a trial, a decrease of 0.065 kg of milk per cow per day was associated with a 1 mm increase in the Wisconsin Mastitis Test score ( Daniel and Fielden, 1971 ). The decrease in milk yield over a season, when one quarter was infected averaged 9.15 % ( 395 kg of milk per cow ), ( Hopkirk, 1972 ), while the daily milk yield decreased by 0.14 litres per cow with an increase in somatic cell count of 100,000 cells per ml on a between cow basis ( Holmes and Gill, 1978 ).

A decrease in the Production Index is associated with increased cell counts, particularly in older cows ( Duirs and MacMillan, 1979; MacMillan et al, 1980 ). Production index allows cows to be ranked within a herd on the basis of their age - corrected milk production, with the herd average being 100 .

While the two types of studies cited above are subject to large errors, between quarter, within udder comparisons provide good experimental control in that two laterally adjacent quarters produce very similar volumes of milk in the absence of infection. In such a trial, California Mastitis Test scores of 1, 2 and 3 were associated with losses of 0.19, 0.30 and 0.67 kg of milk per quarter per milking respectively ( Natzke et al, 1965 ). In a similar study, the respective losses for California Mastitis Test scores of trace, 1, 2 and 3, were 0.42 kg ( 9.0 % ), 0.95 kg ( 19.5 % ), 1.72 kg ( 31.6 % ) and 2.33 kg ( 43.4 % ) per quarter per day ( Forster et al, 1967 ).

Infections lasting over three months depressed the relative yield of affected quarters by about 35 %, while transient infections depressed yield later in lactation by 13 % ( Morris, 1973 ).

While this type of analysis may control between cow and between herd variability, it suffers from the possibility that the loss of yield in an infected quarter may be compensated for by other quarters within the udder. This would exaggerate the apparent effect of mastitis on milk yield.

In an early study, evidence of yield compensation was claimed, however the results were based on one cow only ( Swett et al, 1938, cited by Morris, 1973 ). McLeod ( 1958 ) reported that in five pairs of identical twin cows, one of each pair having a naturally infected quarter, the infected cows produced 96 % to 110 % of the yield of their uninfected twin, with an average figure of 99.6 %. In two further pairs of identical twin cows, where the infection was of a more chronic nature, there was a reduction in yield in comparison with their uninfected mate.

More recently, Woolford et al ( 1983 ) carried out a within udder comparison using identical twins. In mature cows there was a nonsignificant loss of 1.7 %

yield, while in heifers there was a significant loss (  $p < 0.01$  ) of 7.8 %. For mature cows, infected quarters produced 20.4 % less milk than did their paired quarter in the control group. However uninfected quarters in the infected group produced 13.3 % more milk than did their matching quarter in the control group. These figures are both highly significant and provide strong evidence for the existence of yield compensation by uninfected udder quarters in mature cows. There was no evidence of compensation in heifers.

The within udder comparisons cited above may therefore overestimate effects of subclinical mastitis on a cow's milk yield.

While the milk yield losses attributable to subclinical mastitis may have been overestimated by some studies, Woolford *et al* ( 1983 ) did not minimise the importance of this form of the disease and noted that yield compensation does not occur in younger animals. Clearly further work is required on the importance of yield compensation before a clear picture of the true effects of mastitis on total milk yield can emerge.

The New Zealand seasonal supply dairy farmer is paid on the basis of the amount of milkfat or of milkfat and protein produced. Subclinical mastitis has been shown to cause small changes in the fat composition of milk. During a lactation in which a cow was infected, the milkfat concentration averaged 12.4 percentage points lower than during previous lactations in which the cow was not infected ( O'Donovan *et al*, 1960 ). For milk with California Mastitis Test scores of trace, 1, 2 and 3, the milkfat concentration was 0.09 %, 0.11 %, 0.22 % and 0.45 % lower than that of milk which was negative to the California Mastitis Test ( Ashworth *et al*, 1967 ).

However, in some cases of mastitis, the milkfat concentration may actually rise, and the variable results may be attributed to the relative falls in fat production and in total milk yield. If the reduction in milk yield of the affected quarter is greater than that of fat production, then the concentration of fat will increase. Over a period of time, the total output of fat from a quarter is likely to be reduced, because of the lower volume of milk ( Schultz, 1977a ).

The total protein concentration in milk which scored trace, 1, 2 or 3 to the California Mastitis Test differed by -0.01 %, -0.01 %, +0.05 % and +0.21 % from that of milk which was negative to the California Mastitis Test. Only the results for the milk with scores of 2 or 3 were significantly different from those of the negative samples ( Ashworth *et al*, 1967 ).

However, other workers have found that the total protein content of skim milk does not change significantly as the leukocyte count increases ( Haenlein *et al*, 1973 ).

Milk with high leukocyte counts contains less casein, a lower ratio of casein to total protein and a higher concentration of total whey proteins than does milk with a lower leukocyte count ( Haenlein et al, 1973 ). The increase in total whey proteins appears to compensate for the drop in the casein fraction, resulting in little change in total protein until the subclinical mastitis becomes relatively severe, when the leakage of plasma proteins over-compensates for the losses of locally produced proteins, with the result that the total protein content of the milk increases.

If, because of compensation, the milk yield losses resulting from subclinical mastitis are not as great as have been suggested by many workers, and the disease causes only small changes in the content of total protein and milkfat, then under the present payment system, there will be little financial incentive for the dairy farmer to control this disease. Perhaps the manufacturing industry should encourage the production of low cell count milk, by basing the payment on the amount of casein produced, rather than on the amount of total protein.

That subclinical mastitis can lead to the clinical form of the disease is well recognized. Woolford et al ( 1983 ) noted that approximately 50 % of clinical episodes of mastitis were associated with a pre-existing subclinical mastitis.

Costs associated with clinical mastitis may arise as follows ( Dobbins 1977 ):

- (1) Milk production loss
- (2) Veterinary fees
- (3) Antibiotic costs
- (4) Loss due to discarded milk during the withholding period
- (5) Replacement costs of culled animals
- (6) Loss of genetic potential

In addition to the financial considerations, which in this form of the disease are both considerable and more apparent, the treatment of cows with clinical mastitis is both time consuming and disruptive to the normal milking routine.

Animals with subclinical mastitis may shed large numbers of virulent organisms in their milk, thereby forming a reservoir of infection for the other quarters in the infected cow, and other cows within the herd.

Neave ( 1975 ) reported the following results in subclinically infected quarters:

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| Organism                          | % of quarters exceeding<br>10,000 colony forming units<br>per ml of milk. |
|-----------------------------------|---------------------------------------------------------------------------|
| <u>Staphylococcus aureus</u>      | 79.6                                                                      |
| <u>Streptococcus dysgalactiae</u> | 91.1                                                                      |
| <u>Streptococcus uberis</u>       | 78.8                                                                      |
| Coliforms                         | 40.0                                                                      |

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These organisms will contaminate teat cups and milk lines, and they may impact against the teats of cows milked after those that are infected, particularly during the reverse flow of milk in the teat cup. The excretion of large numbers of mastitis organisms in milk adds to the total number of bacteria in bulk milk, regardless of the degree of care taken with plant hygiene. In England, Jeffrey and Wilson ( 1987 ) tested 9,066 bulk milk samples, 754 of which had total bacterial counts > 45,000 colony forming units per ml. Of these 754 samples, 330 ( 43.8 % ) had greater numbers of mastitis related bacteria than of spoilage organisms usually associated with inadequately sterilized milking equipment. It was further shown that 44 % of producers who were penalized for total bacterial counts, were penalized as a result of mastitis related bacteria. Of the producers who failed because of these mastitis organisms, 78 % had bulk somatic cell counts < 500,000 per ml and 59 % were < 400,000 cells per ml, thus a lower bulk cell count does not guarantee that subclinical mastitis will not cause problems with the bacterial content of milk. There was a seasonal variation, the penalties being more frequent when the cows were kept indoors and were not grazed on pasture.

Comparable figures for New Zealand are not currently available. This aspect of the effect of subclinical mastitis requires more research under differing conditions to establish what role the disease has in countries such as New Zealand, where cows are grazed on pasture throughout the year.

### (3) EFFECT OF SUBCLINICAL MASTITIS ON THE MANUFACTURER

#### OF DAIRY PRODUCTS

The dairy manufacturer is affected by subclinical mastitis. Changes occur not only in the concentrations of fat, protein and other major milk constituents, but also in the chemical form of these components, leading to undesirable characteristics in milk and in milk based products.

The flavour quality of pasteurized milk decreases with increasing somatic cell count. Janzen ( 1972 ) showed that the 0 to 14 day shelf life of the pasteurized products was influenced adversely by the cell count of the raw milk. With an increase in the somatic cell count, there was an increase in the chloride concentration, but a decrease in the total solids in milk.

Janzen and Northern ( 1972 ) reported a decrease in total solids of 0.85 % and an increase in chlorides of 0.046 % when the somatic cell count increased from < 500,000 to > 2,000,000 cells per ml. The combination of lower total solids and higher chlorides which are associated with the increase in the somatic cell count, can affect the desirable flavour characteristics of milk, and may be detrimental to milk consumption ( Janzen and Northern, 1972 ).

The addition of milk from quarters affected by subclinical mastitis to that of healthy quarters invariably alters the heat stability of skim milk and of skim milk powder ( Feagan et al, 1966a ).

The solubility index of spray-dried milk powder made from high cell count milk is extremely high, and the reconstituted milk develops a slightly burnt flavour when freshly mixed, changing to a tallowy flavour on storage ( Brus and Jaartsveld, 1971a ).

Little change is evident in the total fat concentration of milk from mastitic cows, but in milk with a raised Wisconsin Mastitis Test score there is an increase in the lipase activity and a decrease in the phospholipid concentration ( Randolph and Erwin, 1974 ). The two factors are thought to be responsible for the higher levels of free fatty acids and total short chain fatty acids found in this milk ( Randolph and Erwin, 1974 ).

The level of free fatty acids is positively correlated with the somatic cell count. Gudding ( 1982 ) showed that the milk from cows with cell counts of > 400,000 cells per ml contains a higher concentration of free fatty acids than does milk with a content of < 250,000 cells per ml. Off flavours due to lipolysis were detected at a somatic cell count level of 500,000 cells per ml.

The above changes may be responsible for a decrease in quality of some products made from the lipid fraction of the milk. Butter made from milk with a high cell count showed signs of oxidation after two months of storage (Brus *et al*, 1966). More recently, Needs *et al* (1988) added high cell count milk to bulk milk to give a range of milks containing 10 %, 20 %, 30 %, and 100 % high cell count milk. Whipping time and stiffness increased as the proportion of high cell count milk increased, although up to the 30 % level, the effects were not statistically significant.

Infection of the udder has been shown to affect some of the proteins in the milk. The infusion of *Streptococcus agalactiae*, or of bacterial endotoxin into individual quarters of cows has little effect on levels of  $\alpha$ -lactalbumin or of  $\beta$ -lactoglobulin, but levels of serum albumin and of immunoglobulins increase markedly, while levels of  $\alpha$ s1-casein and  $\beta$ -casein decrease (Anderson and Andrews, 1977). An increase in the level of para-k-casein occurs and this can be attributed to high levels of proteolytic enzymes in the milk. Andrews (1983) confirmed that high levels of several proteinases are present in milk with a high cell count and that one of these enzymes is plasmin. It was suggested that much of the decrease in concentration of various caseins is due to proteolytic breakdown rather than to a decrease in production.

The effects of the altered protein content are evident in the manufacture of cheese. Gouda cheese which was made from high cell count milk received a lower grade than that made from low cell count milk, (Brus and Jaartsveld, 1971b), although it was concluded that further trials are needed to fully assess the effects of mastitis on the quality of Gouda cheese. The use of high cell count milk during the preparation of Cheddar cheese is associated with a reduction in the coagulum strength and an increased moisture content in the final product. Both the cheese texture and the flavour are adversely affected and even a small increase in the somatic cell count of the milk is detrimental to cheese composition and quality (Grandison and Ford, 1986).

Politis and Ng-Kwai-Hang (1988a) collected monthly milk samples which were used to make Cheddar cheeses under standardised conditions. A direct link was shown between cell count, milk protein composition, cheese composition and the loss of milk components in the whey. The high cell count milk was characterized by a relative inability to incorporate protein into the curd. Improvements in cheese composition and quality can be expected by maintaining somatic cells in individual cows below 500,000 and possibly below 300,000 cells per ml.

The yield of cheese may also be affected by the somatic cell count. The decrease in yield is progressive, rather than being detected only at very high cell count levels and improvements in cheese yield are possible by using milk with a cell count of

under 500,000, and possibly under 300,000 cells per ml ( Politis and Ng-Kwai-Hang, 1988b ).

De Rham and Andrews ( 1982 ) suggested that some of the problems encountered during cheesemaking such as the yield losses, poor curd strength and off flavour development may be due to the increased proteolysis found in mastitic milk.

The milk lactose concentration generally decreases as the somatic cell count increases. Ashworth *et al* ( 1967 ) found lactose concentrations of 4.88 %, 4.83 %, 4.72 %, 4.50 %, and 3.95 % for milk from quarters which scored negative, trace, 1, 2, or 3 respectively in the California Mastitis Test. While the decrease in the concentration of lactose may not be as important commercially as are the changes with respect to some of the other parameters, the decrease in the lactose concentration has been tested as a means of diagnosing subclinical mastitis in several studies ( Renner, 1975; Mijnen *et al*, 1982 ).

While the above changes in milk composition due to mastitis have been demonstrated experimentally, their importance with respect to commercial production is largely unknown. Any reduction in the quality of dairy products in a highly competitive market must have harmful effects for the industry as a whole.

#### (4) EFFECTS OF SUBCLINICAL MASTITIS ON THE CONSUMER

The consumer of milk and milk products may be affected by subclinical mastitis. Dairy products made from milk with high somatic cell counts have been shown by a number of workers to develop undesirable flavours and to have a short shelf life ( see preceeding section ). With the advent of ultra-heat treated milk products, these undesirable effects may become increasingly important, as some of these changes appear during storage rather than at the time of manufacture.

Subclinical mastitis can have a more drastic effect on the consumer of dairy products. A number of the mastitis causing bacteria and fungi are potentially pathogenic to humans, causing in many cases severe or even fatal infections or intoxications.

Staphylococcal food poisoning has been associated with mastitis. Olson *et al* ( 1970 ), found that 23 out of 157 strains of Staphylococcus aureus isolated from cases of mastitis produced enterotoxin. Staphylococcal enterotoxin causes a form of food poisoning characterized by vomiting and severe abdominal pains, lasting for 24 to 48 hours, following ingestion of the preformed toxin in food ( Lennette *et al*, 1980 ). Neither pasteurization of milk, nor the spray-drying procedure used in the manufac-

ure of milk powder is effective in destroying the toxin ( Read and Bradshaw, 1966 ).

If the storage conditions of raw milk support the multiplication of the staphylococci, the enterotoxin is likely to be found in the milk and products produced from it. Anderson and Stone ( 1955 ) investigated a number of outbreaks of food poisoning in school canteens in Surrey, England. The cause was traced to spray dried milk powder which came from a common source. During manufacture the raw milk had been kept warm for several hours before passing into the final drying chamber. A total of 1190 cases were involved in the outbreaks.

Streptococcus agalactiae has been associated with human disease, causing a variety of conditions, amongst which is a bacteraemia and meningitis of neonatal children ( Lennette et al, 1980 ). Wilkinson ( 1978 ) reviewed the involvement of group B streptococcal infection in humans. No firm conclusions regarding the origin of human infections were drawn but these organisms are part of the normal bacterial flora indigenous to humans, having been cultured from the vagina, urethra, rectum and pharynx of healthy adults, and from the umbilicus and body cavities of infants, who are usually contaminated during birth.

The human and bovine isolates of Lancefield's group B streptococci differ in many respects including haemolytic pattern, pigment production, bacitracin sensitivity and fermentation pattern and it has been concluded that organisms from the two sources form distinct populations ( Finch and Martin, 1984 ). This view is shared by Onile ( 1986 ), who reported differences in serotypes and in colonial morphology between human and bovine strains.

The advent of antibiotics have markedly reduced the incidence of Streptococcus agalactiae mastitis and its susceptibility to pasteurization further decreases the risk to humans.

Escherichia coli may cause mastitis in dairy cattle, particularly around parturition ( Eberhart, 1977 ). Escherichia coli has been associated with a variety of infections in humans including diarrhoea in young babies ( Lennette et al, 1980 ). It is not clear whether or not bovine serotypes of this species are ever involved in human disease, but young calves are susceptible to scouring due to Escherichia coli.

Many other organisms may be associated with sporadic cases of bovine mastitis and a number of these can cause severe infections or intoxications in humans. These include: Leptospira spp, Listeria monocytogenes, Pasteurella multocida, Actinomyces spp, Nocardia spp, Cryptococcus neoformans, Bacillus cereus and Clostridium perfringens, ( Cousins and Bramley, 1981 ).

With the exception of Bacillus cereus and Clostridium perfringens, both of which form endospores, the above species are all sensitive to heat and are destroyed by pasteurization of milk. Clostridium perfringens spores are unlikely to germinate in the conditions present in milk, while Bacillus cereus causes off flavours during growth in milk, and dairy products are not commonly incriminated as sources of food poisoning with this organism.

If milk is kept refrigerated after collection to reduce bacterial growth, and adequate pasteurization carried out, then mastitis presents little threat to public health. Should refrigeration fail, or raw milk be consumed or used for manufacture of dairy products, as in the case of certain cheeses, then the potential for outbreaks of human disease as a result of mastitis is very real.

### SUMMARY

Subclinical mastitis forms part of a greater disease complex - that of mastitis as a whole. This disease can cause problems for the producer of milk, and for the manufacturer and the consumer of dairy products. The cost of the disease is difficult to accurately estimate, but mastitis is generally regarded as being very important economically ( Janzen, 1969; Dobbins, 1977; Stevenson and McVeagh, 1983 ). Control of mastitis in dairy cattle relies on a number of factors which include regular maintenance of milking equipment, good hygiene, the use of teat sprays or dips, appropriate antibiotic therapy for clinically affected quarters, either selective or total dry cow therapy at the end of the season, accurate recording of the mastitis history of each cow, and culling of cows which do not respond to therapy and control measures.

Central to the control of mastitis is the identification of quarters which are subclinically infected. Milk taken from these quarters is, by definition, normal in appearance, and a number of cowside and laboratory tests have been advocated for detection of the subclinical state.

In the following chapters, the literature relating to these tests is reviewed, and some of the tests which have shown promise as indicators of udder infection are studied and the results presented, in an effort to evaluate their performance under local conditions of dairy farming.

## CHAPTER TWO

### THE DIAGNOSIS OF SUBCLINICAL MASTITIS

#### A REVIEW OF THE LITERATURE

For more than 100 years, researchers have been studying the problem of diagnosing bovine mastitis. During that time, many techniques have been suggested and evaluated, with varying degrees of success. The following chapter is a summary of the voluminous data which have accumulated as a result of this work.

#### THE DIAGNOSIS OF SUBCLINICAL MASTITIS BY BACTERIOLOGICAL METHODS

The infectious nature of mastitis was established by Nocard and Mollereau, who in 1887 isolated a streptococcus from a cow with mastitis and succeeded in producing the disease experimentally in both a cow and a goat ( cited by Merchant and Packer, 1971 ).

The use of bacteriology as a diagnostic tool for mastitis went hand in hand with the development of microbiology as a scientific discipline. Although much emphasis was at first placed on the demonstration of streptococci in milk, by 1905 case reports of mastitis caused by staphylococci, coliforms and corynebacteria had appeared in the literature ( Giesecke and van den Heever, 1974 ). The demonstration that milk was a potential source of human pathogens such as those responsible for tuberculosis, or scarlet fever ( Hamer and Jones, 1909 ) stimulated research in the area of milk microbiology.

Bacteria which are present in milk samples may be demonstrated in a number of ways:

The direct microscopic examination, proposed by Prescott and Breed ( 1910 ), can be used for the enumeration of chains of cocci in milk. However, Minett *et al* ( 1930 ) found the direct microscopic examination of stained smears to be unreliable, in that nearly half of the affected samples were misdiagnosed as being free of streptococci. The above problem is due to the relatively low numbers of bacteria which are present and an obvious way to overcome the problem is to incubate the sample prior to staining and examination of the smear ( Bryan, 1935; Bryan and Devereux, 1937 ). A major drawback of the preincubation method is the simultaneous growth of saprophytic organisms in the milk.

The preparation and examination of stained smears is tedious and time consuming. A more promising method of demonstrating the presence of bacteria in the milk is that of inoculating agar plates and examining the resultant growth. Bryan and Devereux ( 1937 ) compared the culturing of milk samples on blood agar plates, with the microscopic examination of preincubated samples and found that the culturing could detect 92.5 % of quarter samples, and 89 % of composite samples which were shown to be positive by microscopic examination.

The culturing of milk samples on agar can give rise to problems of contamination which may obscure the presence of streptococci or of other potential pathogens. A number of sampling techniques which largely overcome this problem have been reported. Roselle ( 1931 ), cited by Giesecke and van den Heever ( 1974 ) described an elaborate procedure which involves the washing of the flanks, tail and udder of each cow, before wiping the udder with antiseptic, the whole procedure being carried out in a dust-free environment.

A simplified procedure has been used by Bryan ( 1932 ) in which the udder and teats are wiped with a cloth moistened with antiseptic. The first two squirts of milk are discarded, after examination for physical signs of mastitis, and the next 10 millilitres of milk are collected in a sterile tube for transport to the laboratory.

Another method of overcoming sample contamination and subsequent overgrowth of pathogens is to use selective agar. Bryan ( 1932 ) added the dye gentian violet to liver-infusion blood agar and found that this prevented the growth of many contaminants. Brilliant green could also be used. A selective medium for streptococci which includes crystal violet as the inhibitor, and esculin, which is used to differentiate between species of streptococci, has been described by Edwards ( 1933 ).

The culturing of samples on agar is the most accurate method of demonstrating the presence of streptococci if centrifuge deposits are used to inoculate the plates ( Minett *et al*, 1930 ).

The use of gravity cream gives counts five to ten fold higher than those observed when whole milk is used as the inoculum ( Pullinger, 1935 ). Plating out the centrifuge deposit is clearly inferior to the use of gravity cream ( Pullinger, 1935; Edwards, 1938 ).

Use of a glucose broth to which the inhibitory substance sodium azide has been added may be more convenient for the detection of streptococci than is the use of solid media ( Edwards, 1938 ).

During the 1930s, the generally held belief was that relative to streptococci, staphylococci were not of great importance, in bovine mastitis, ( Minett, 1937 ). However, this author concluded that staphylococci are capable of causing several types

of mastitis including the gangrenous form, although it is generally reported to be of a chronic nature. The use of blood agar plates has been advocated. This would allow the demonstration of alpha toxin production, and would therefore give an indication of pathogenicity of the organism. Without a proper bacteriological examination of the milk, staphylococcal and streptococcal forms of the disease can be confused.

As microbiological techniques evolved, an increased number of different organisms were shown to be associated with mastitis. Gray ( 1935 ) reported a case of mastitis which was caused by an aerobic acid-fast actinomycete ( Nocardia sp. ). If contaminated materials are used, pseudomonads are capable of causing mastitis following the administration of antibiotics ( Tucker, 1950 ).

Corynebacterium bovis too was found to be capable of causing mastitis ( Cobb and Walley, 1962 ), while in the same year, mycoplasma were isolated from a serious herd outbreak of the disease ( Hale et al, 1962 ).

Over the years, there has been a considerable variation in the bacteriological methods used to diagnose subclinical mastitis. A need for standardization of both sampling methods and also of the isolation and identification of mastitis bacteria was answered by the Group A2 of the International Dairy Federation ( IDF ), ( Dodd, 1981 ). Standard methods for the collection of aseptic samples include recommendations on the types of sample vials, cleaning pads and disinfectant as well as the procedures involved in taking the samples. Standard methods for the isolation and identification of mastitic bacteria include recommendations on type of bacteriological media, cultural methods and the tests to be used for identification of bacteria to the species level.

Neave ( 1975 ) listed a number of conditions, which if fulfilled, would allow for the accurate diagnosis of subclinical mastitis by bacteriological means alone:

- (1) Mastitis of economic importance should be generally of bacteriological origin and caused by bacteria which are readily isolated by relatively simple methods.
- (2) The most common primary pathogens should be normally secreted in sufficient numbers from the subclinically infected quarters to be detected when a small volume of foremilk is examined.
- (3) The pathogens should be regularly secreted from infected quarters in detectable numbers so that failure to recover the pathogen from one, or two or more successive foremilk samples is unusual.
- (4) The infections detected from the affected quarter should produce a marked increase ( > 10 fold ) in the somatic cell count in milk.

- (5) The pathogens should be found infrequently in the milk from uninfected glands if samples are taken aseptically, or rarely found in two or more successive samples taken a week apart.

Evidence in support of each of these conditions was presented as follows:

- (1) The causes of about 90 % of mastitis cases of economic importance are the major pathogens Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae and Streptococcus uberis, all of which may be readily isolated and identified in the laboratory.
- (2) Approximately 95 % of samples from 1,039 cases of staphylococcal and streptococcal mastitis had colony counts of over 100 colony forming units per ml, ie > 5 organisms per plate when 0.05 ml of milk was plated. Comparable figures for coliforms were 87 % of samples with over 100 colony forming units per ml.
- (3) A total of 2,884 foremilk quarter samples from 206 cases of established Staphylococcus aureus infections were taken over a period of 29 weeks. Forty four percent of infected quarters showed one or more negative ( false negative ) samples. However, only 7 % and 0.5 % showed 2 and 3 successive false negative results respectively for Staphylococcus aureus. Comparable figures for streptococci were 37 %, 2 % and 1 % of samples respectively.
- (4) In 1466 subclinical Staphylococcus aureus infections, 81 % of samples had somatic cell counts above 300,000 cells per ml, although 11 % had, at some time, a cell count of below 100,000.
- (5) The frequency of finding pathogens in milk from uninfected glands ( false positives ) depends upon the skill of the sampler and on whether or not a teat dip is used. In 59 herds, the herds not using teat dips averaged 4 % false positives, while those herds using a teat dip gave a value of 1.3 %. Only 0.4 % and 0.07 % of uninfected samples yielded two successive false positive results in the two types of herd respectively.

The evidence presented indicates that all the criteria can be fulfilled within acceptable limits, and therefore bacteriology alone may be used to detect subclinical bovine mastitis.

There are few problems in using bacteriological methods for the diagnosis of mastitis in small scale experiments where sampling is frequent. Foremilk sampling can be backed up by techniques such as teat puncture and streak canal swabs if indecisive results are obtained ( Griffin et al 1977 ).

In larger trials, however, the frequency of sampling may be less, and the use of techniques such as teat puncture are not practical under normal farm conditions. It was considered important to establish a sampling system which would, if carried out with due care, give reliable results in these large scale experiments. If two foremilk samples were taken a week apart and the first sample contained a major pathogen, then in 95 % of cases, this pathogen was reisolated one week later. In the case of a negative result at the first sampling, 97 % of such quarters were negative at the second test. The authors concluded that two and if necessary three samples be taken at weekly intervals, and if two, or 2 out of 3 samples are found to contain a major pathogen, then the quarter should be considered infected. The frequency of sampling during the season is dependent on the experiment in question, but samples should be taken at the start of the experiment, when cows enter the herd, at calving, at herd tests, at drying off, when cows are sold and after antibiotic therapy ( Griffin et al, 1977 ).

Griffin et al ( 1987 ) carried out an international collaborative trial as directed by the I.D.F. Twelve laboratories from 10 countries took part in the trial which involved repeated sampling of quarters from a small number of herds over a three week period. A number of methods of diagnosing the infection status of the quarters were tried using either bacteriological results alone, or in combination with various somatic cell count thresholds. When bacteriological results were used alone it was found that 8 out of 12 of the laboratories were able to correctly classify more than 90 % of the milk samples using a single sample. When the diagnosis was based on two, or 2 out of 3 samples, 10 out of the 12 laboratories were able to correctly identify the samples in over 95 % of cases, while 5 out of the 12 laboratories had an accuracy of 99 %.

When sampling and laboratory work give consistent results, bacteriological tests alone on a single sample will give results of sufficient accuracy for most surveys and routine mastitis control work. If greater accuracy is required, bacteriological tests alone on two, or 2 out of 3 occasions, will give superior results.

While bacteriological analysis of samples, when carried out according to the recommended methods can be an accurate method of diagnosing subclinical mastitis in experimental situations, it is not particularly suited for use in larger surveys or national mastitis control schemes. Bacteriology is both time consuming and expensive, requiring skilled personnel both to take samples and to interpret the growth. Problems may also occur with overgrowth of pathogens during the transport of samples to the laboratory.

A number of indirect tests for the determination of cows or quarters which are infected have been suggested, and a summary of the literature relevant to these tests is given in the following sections of this chapter.

## THE DIAGNOSIS OF MASTITIS BY NON-BACTERIOLOGICAL INDIRECT METHODS

### (1) TESTS WHICH MEASURE THE SOMATIC CELL COUNT OF MILK

#### (A) THE MICROSCOPIC SOMATIC CELL COUNT

The somatic cell count measures the number of "somatic" ( body ) cells per ml of milk. Somatic cells include both cells derived from the mammary epithelium, and leukocytes which enter the mammary tissue in response to infection or other damage to the gland. With the aid of a light microscope, these cells can be stained and counted manually. Prescott and Breed ( 1910 ) described a new method for the counting of " body cells " in milk. This method, which became widely adopted, involves spreading a set volume of milk over a defined area on a glass microscope slide. The milk is allowed to dry, and the smear is stained using an alcoholic methylene blue solution. The number of cells in 100 fields are counted and multiplied by a working factor to give the number of cells per ml of milk. This procedure removes the need for centrifugation of milk and examination of the sediment.

Prescott and Breed ( 1910 ) noted that in samples with a somatic cell count of less than 250,000 cells per ml of milk, there was a very high error in the cell count, but these samples were considered as being well within the normal range, and hence this was not seen as being a major problem.

The counting of 100 fields is tedious and a number of modifications to the method have been described. Strynadka and Thornton ( 1937 ) recommended that only 60 fields be counted per slide. In replicate samples, there can be as much as a three fold difference between the highest and lowest counts

The selected microscopic fields are not necessarily representative of the whole smear ( Strynadka and Thornton, 1937 ). Schneider and Jasper ( 1966 ) also showed that the cells are not distributed evenly throughout the smear, there being more cells at the center than towards the edges as a result of the drying process.

Further modifications have been made to the staining and counting procedures over the years. Broadhurst and Paley ( 1939 ) developed a single dip staining method, which improves the differentiation of cells from background material. Similarly, Paape *et al* ( 1963a ) found that when using Wrights stain, many leukocytes tend to be masked by the background and that the use of pyronin Y - methyl green stain reduces the variance of the microscopic count.

Despite these modifications, the method continued to show low repeatability. Paape *et al* ( 1965 ) reported the coefficient of variation to be 19 %, while Thompson *et al* ( 1976 ) gave a figure of 15.6 %, this being higher than those of other somatic cell counting procedures ( see below ). The standard deviation for the microscopic somatic cell count is approximately four times that of electronic cell counting ( Read *et al*, 1969; Ginn *et al*, 1979 ).

The temperature at which the slide is dried does not affect the distribution of cells in the smear, but the area over which the set volume of milk is spread is an important source of error in the cell count ( Schneider and Jasper, 1966 ).

Other factors can affect the results of the microscopic count. Paape *et al* ( 1965 ) reported a significant effect (  $p < 0.01$  ) of the person performing the count, as well as a significant difference between smears from the same sample (  $p < 0.01$  ).

The storage of milk samples is likely to cause a difference in the somatic cell count as determined by the microscopic count ( Read *et al*, 1969 ).

None of the modifications made to the original Prescott Breed method have overcome the two paramount objections - the inaccuracy of the method, and the labour required ( Cullen, 1968 ), while Schultze ( 1970 ) reported the microscopic counting of somatic cells to be time consuming and visually fatiguing.

The Direct Microscopic Somatic Cell Count ( DMSCC ) was developed by the National Mastitis Council ( 1968 ). This is a modified Prescott - Breed technique in which the establishment of strict criteria for each aspect of the test enables both the determination of, and close control over the precision. Despite close control, the repeatability of the DMSCC is relatively low. A coefficient of variation of 15 % for the DMSCC has been reported by Smith ( 1969 ).

Similarly strict guidelines have been laid down by the International Dairy Federation ( Dodd, 1981 ) for the use of the microscopic somatic cell count as a reference method.

### (B) THE WHITESIDE MASTITIS TEST

An indirect method of estimating the somatic cell count has been developed in which sodium hydroxide is added to milk, with the formation of a viscid mass in milk samples from infected udders ( Whiteside, 1939 ). The test is known as the " Whiteside Mastitis Test " .

Many samples from infected quarters were subsequently shown to give negative results to the original test, and the method has been modified by Murphy and Hanson ( 1941).

The Modified Whiteside Test has been promoted as a simple test which closely parallels the leukocyte count and is able to detect the presence of udder infection. The Modified Whiteside Test is successful in the identification of infected cows using composite foremilk samples and bucket samples, but less effective when compared with the DMC in reflecting the infection status of the udder ( Murphy, 1942 ).

The correlation between the Modified Whiteside Test results and microscopic counts has been studied. Brazis *et al* ( 1967 ) found the agreement between Modified Whiteside Test and the DMC to be 84 % and 81 % for individual and pooled quarter samples respectively.

Other reports have shown the Modified Whiteside Test to be less efficient in identifying infected quarters. The percentage of milk samples having a Modified Whiteside Test score of 1 or greater, in quarters harbouring various mastitis pathogens ranged from 8 % to 75 %, coagulase positive staphylococci occurred at a low level of 24 % of samples ( Easterday *et al*, 1958 ). A wide range of cell counts were associated with each Modified Whiteside Test score, giving a high degree of overlap between scores.

### (C) THE CALIFORNIA MASTITIS TEST

An improved indirect test has been devised ( Schalm and Noorlander, 1957 ) to overcome the inconvenience of using glassware in the dairy shed, a problem encountered with the MWT. The California Mastitis Test ( CMT ) uses a reagent which reportedly does not involve milk fat as a part of the test, and the indicator bromocresol purple is added to detect pH changes. A plastic paddle with four cups is used, the cups simply being rinsed with water between uses. The results of a trial by Schalm and Noorlander ( 1957 ) are given in table 2.1. These authors concluded that the CMT is a sensitive indicator of the presence of inflammation of the udder.

The CMT is closely related to the microscopic somatic cell count. Brazis *et al* ( 1967 ) found the CMT to agree with the D.M.C. in 85 % and 90 % of cases for individual and pooled quarter samples respectively. Similarly, correlation coefficients between the CMT and DMC for quarter foremilk, bucket milk and for bulk tank milk samples have been determined ( Spencer and Simon, 1960; Daniel *et al*, 1966; and Postle and Blobel, 1965 ). Despite the correlation between tests, discrepancies have been found, and it is suggested that the CMT does not depend upon the number of body cells for its entire reaction ( Spencer and Simon, 1960 ). A disadvantage of the technique is the large proportion of doubtful scores, and a scoring system using three rather than five grades has been suggested. The CMT has a limited value as a screening test for mastitis, and it is not advisable to use the results of the test as a basis for therapy or for herd segregation ( Giesecke and Van den Heever, 1967 ).

TABLE 2.1

SUMMARY OF RESULTS FROM SCHALM AND NOORLANDER ( 1957 )

RELATIONSHIP BETWEEN CMT AND SOMATIC CELL COUNT

| SAMPLE TYPE                              | NUMBER | NEGATIVE | TRACE | 1     | 2    | 3     |
|------------------------------------------|--------|----------|-------|-------|------|-------|
| SOMATIC CELL COUNT ( x 10 <sup>6</sup> ) |        |          |       |       |      |       |
| QUARTER<br>FOREMILK                      | 481    | 0.081    | 0.431 | 1.16  | 3.25 | 11.40 |
| BUCKET<br>MILK                           | 113    | 0.129    | 0.310 | 0.690 | 1.97 | 8.50  |
| BULK<br>TANK MILK                        | 154    | 0.250    | 0.550 | 0.720 | 1.13 | 1.48  |

#### (D) THE WISCONSIN MASTITIS TEST

One criticism of the CMT is that it relies upon a subjective judgement of the amount of precipitate and thickening of the reagent-milk mixture. A more objective and quantitative test was required, and the Wisconsin Mastitis Test was developed by Thompson and Postle ( 1964 ) to meet these requirements.

The Wisconsin Mastitis Test ( WMT ) is based on the measurement of the viscosity of a milk-detergent mixture by allowing it to flow for 15 seconds through a narrow hole in the cap of a plastic tube, and measuring the amount of the mixture left in the tube.

A number of workers have estimated the relationship between the WMT and the DMC. A correlation coefficient of 0.91 between the WMT scores and the square root of the cell count in bulk tank milk samples was one estimate ( Thompson and Postle, 1964 ). Kroger and Jasper ( 1967a ) reported a correlation of 0.96 between the WMT and log cell count for their own data, and 0.93 for the log transformed data of Thompson and Postle ( 1964 ). Correlations ranging from 0.97 - 0.99 for untransformed data in bulk milk samples, and 0.91 for composite milk samples when the square root of the WMT score was plotted against log cell count, have been reported ( Janzen, 1969; Daniel *et al*, 1971 ). Daniel *et al* ( 1971 ) found that the best linear relationship is between the square root of the WMT and log (10) of cell counts. Using this regression, WMT scores of 2, 10, 17 and 35 corresponded to cell counts of 34,000 214,000 482,000 and 2,192,000 respectively.

While the WMT provides a reliable estimate of the cell count, a number of factors can affect the results of the test. The age of the sample, storage conditions and the temperature at which the test is carried out, may all affect the results ( Kroger and Jasper 1967a, Kroger and Jasper 1967b ). Small clots in the milk may cause exaggerated readings, due to the small size of the hole through which the mixture must pass ( Milne, 1971 unpublished ).

#### (E) THE ROLLING BALL VISCOMETER

An objective estimate of the somatic cell count is also given by the rolling ball viscometer ( RBV ), ( Milne and Smyth, 1976 ). This device measures the distance travelled by a stainless steel ball down an inclined tube in a set period of time. The correlation between the DMSCC and the RBV has been estimated as 0.92, and one technician can analyse 120 samples per hour ( Milne and Smyth, 1976 ).

#### (F) MEASUREMENT OF DNA IN MILK

The addition of deoxyribonuclease ( DNAase ) to the gel which forms during the California Mastitis Test, results in the disappearance of the gel, indicating that deoxyribonucleic acid ( DNA ) is responsible for gel formation ( Paape et al, 1962 ), and a system for the quantitative measurement of the DNA content of milk was sought.

The Feulgen reaction, which stains the DNA of the cell was modified for use with milk samples. This modified Feulgen-DNA test shows a very high correlation with the California Mastitis Test (  $r = 0.94$  ) with the standard deviation between duplicate samples being lower for the Feulgen-DNA test than for the California Mastitis Test. The coefficients of determination were 9.0 % and 19.8 % for the two tests respectively, ( Paape et al, 1962 ).

The correlation between the number of somatic cells, determined by direct microscopic somatic cell count, and the Feulgen-DNA test is 0.88 ( Paape et al, 1963b ).

Paape et al ( 1965 ) reported a within sample coefficient of variation for Feulgen-DNA of 13 %. However, when the colour was read with a spectrophotometer, rather than subjectively, the figure was reduced to 1 %. Although not considered to be suitable as a cowside test, the fact that the Feulgen-DNA test is not affected by sample age, has a low within sample coefficient of variation, high correlation with total somatic cells, and is both sensitive and objective, indicates that the spectrophotometric Feulgen-DNA test should be of value for the estimation of the somatic cell count.

While the Feulgen-DNA test is an improvement on the indirect measurements of milk DNA due to increased viscosity, problems have been found with the stability of reagents. This led Hutjens et al ( 1970 ) to use a diphenylamine-DNA method for an estimation of the cell count after concentration of the cells using filtration, and this filter DNA method accurately reflects the cell numbers. The coefficient of variation between duplicate samples was 3.5 %, while that for the direct microscopic somatic cell count was 29.6 %.

This filter DNA test is relatively inexpensive, with one technician being able to process approximately 100 milk samples per day. Unfortunately the method includes a 16 hour incubation step. Bremel et al ( 1977 ) evaluated this membrane-filter-DNA procedure and found a greater degree of precision than is obtainable with the direct microscopic count. The procedure is not subject to technician bias, and has a lower initial cost than does fluoro-optical counting. A method for standardizing the membrane-filter-DNA technique, both within and between laboratories has been reported ( Bremel et al, 1977 ).

Other chemical reactions have been used to measure the DNA content of milk. de Langen ( 1967 ) reported a routine method for the determination of DNA in milk, using an indole colour reaction. Although the method is acknowledged as being more time consuming than other DNA procedures, it is useful for the calibration of tests such as the California Mastitis Test, or the Feulgen-DNA test.

### (G) THE ELECTRONIC SOMATIC CELL COUNT

The variety of cells in a biological fluid may be counted by passing the sample through a narrow aperture between two electrodes and recording the impulses generated.

It was recognized during the early 1960s that the electronic cell counter offers the possibility of rapid and comparatively accurate somatic cell counting.

One problem which needed to be overcome, was the counting of non-cellular particles such as fat globules. Phipps and Newbould ( 1966 ) reported a method of sample preparation which includes dilution of the milk with physiological saline, centrifugation, removal of the fat layer, and the redispersion of the cells by gentle rocking followed by vortexing. A threshold setting of 7.5 micrometers diameter was used to minimize the influence of other particulate matter such as clumps of bacteria or red blood cells. A second count using a threshold of 15 micrometers was carried out to remove irrelevant large particulate matter, this value being subtracted from the first. The numbers of cells as determined by this electronic counting method correlates linearly with the direct microscopic cell counts up to 6,000,000 cells per ml.

A similar method was used by Cullen ( 1967a ), utilizing disposable glass tubes for centrifugation, the fat fraction being removed by breaking the tube at a weak point. Using a slightly lower threshold of 6.3 micrometers, variation in replicate samples ranged from 2.5 % to 5.0 %. The problem of some of the cells rising with the fat layer was largely overcome by the dilution step. The addition of a known number of cells showed a high absolute accuracy for this method, as well as a high repeatability. One operator can analyse 40 samples per hour using this method, which is a vast improvement over the figure claimed by Phipps and Newbould ( 1966 ) of 50 samples per day.

Provided that the somatic cell count is over 300,000 cells per ml, there are no significant differences between the results of the direct microscopic and those of the electronic somatic cell count although when the milk samples contain fewer than 300,000 cells per ml, the electronic cell count gives significantly higher counts than does the direct microscopic test ( Read et al., 1967 ). This was not regarded as a problem, since the figure of 300,000 cells per ml was well within the range of cell counts considered to be normal for milk.

Meanwhile, Tolle et al ( 1966 ) had described a chemical method for dispersal of the milkfat, which involves the treatment of cells with formalin, followed by the addition of a mixture of a surface active agent and alcohol.

The methods of Phipps and Newbould ( 1966 ) and Tolle et al ( 1966 ) were compared by Phipps ( 1968 ). The chemical method of sample preparation gave good results when used in conjunction with a 5.0 micrometer threshold setting. There was good agreement between the two methods, although the chemical method tended to give relatively higher values for low cell count samples. The chemical method is simple and capable of analysing 60 samples per hour.

A similar comparison was carried out by Pearson et al ( 1970 ) using a slightly lower threshold setting of 4.7 micrometers. The chemical method of sample preparation was found to give better precision than did the centrifugation method, the coefficients of variation being 1.75 % and 5.2 % respectively ( Pearson et al, 1970 ).

Using the centrifugation method of sample preparation, the correlation coefficient between the DMSCC and the electronic count was estimated to be 0.93. When the chemical method of sample preparation was used, this figure increased to 0.97 ( Pearson et al, 1970 ). These values are similar to those reported by other authors ( Read et al, 1967; Tolle et al, 1966; Phipps, 1968 ).

The chemical method of sample preparation is probably the preferred technique, as it can be more readily and economically designed to cope with large numbers of samples.

Other methods of sample preparation have been evaluated. Philpot and Pankey ( 1973 ), in addition to the two aforementioned methods of sample preparation also tested a Rapid Fixation Dispersion ( RFD ) method, and the somafix / somaton method. While the RFD method of sample preparation was unsatisfactory, the chemical, centrifugal and somafix / somaton methods of sample preparation enabled the electronic somatic cell count to show good agreement with the direct microscopic somatic cell count.

A number of factors have been considered likely to affect the reproducibility of the electronic somatic cell count. Among these factors are the storage time and temperature, the mixing and dilution of samples, holding times prior to counting, and the volume of milk used ( Pearson et al, 1974; Greer and Pearson, 1976 ).

While the problem of interference by fat globules has been overcome by chemical treatment, other concerns have arisen when the electronic counting of cells has been attempted. Small air bubbles, formed during mechanical milking, could cause an elevated reading ( Dijkman et al, 1979 ), this problem being overcome by preheating the sample prior to fixing with formalin.

#### (H) THE FLUORO-OPTICAL SOMATIC CELL COUNT

Although the electronic somatic cell count represents an improvement over the previous methods of estimating the somatic cell count, in terms of sample throughput and repeatability, problems remain in the areas of calibration and adjustment. The Coulter counter does not discriminate between somatic cells and other particles of similar dimensions. The need for an automated yet specific test for the estimation of the somatic cell count led to the development of the fluoro-optical count.

A preliminary report on the determination of milk somatic cells using fluorescence microscopy suggested that an automated opto-electronic method should be possible ( Schmidt Madsen, 1968 ).

A fully automated fluoro - optical counter ( Fossomatic - A/S N. Foss Electronic, Hillerod, Denmark ) was tested and found to be capable of analysing 180 samples per hour ( Schmidt Madsen, 1975 ). This instrument became available commercially, and the association between the Fossomatic somatic cell count and those of the other methods of cell count estimation have been studied by several workers.

Estimates of the correlation between the Fossomatic and the direct microscopic somatic cell count range from 0.92 to 0.99 ( Heeschen, 1975; Schmidt Madsen, 1975 ). The correlation between the Fossomatic and the Coulter counter ( electronic somatic cell counting ) has the same range ( Heeschen, 1975; Schmidt Madsen, 1975 ).

A high degree of repeatability is claimed for the Fossomatic, with the coefficient of variation ranging from 2 % to 5 % for replicate samples ( Schmidt Madsen, 1975 ). Slightly higher coefficients of variation have been reported by Heeschen ( 1975 ). The carry over between samples averages 2 % ( Schmidt Madsen 1975 ). This is not regarded as a problem unless a low cell count sample is measured after the processing of a very high cell count sample.

Both the Coulter counter and the Fossomatic give higher readings than those obtained by the direct microscopic count when the sample contains a low number of cells. But when the sample has a higher cell count ( 1,500,000 to 2,000,000 million cells per ml ), the results for the Fossomatic and the direct microscopic count are similar ( Schmidt Madsen, 1975 ). Miller *et al* ( 1986 ) found no difference between the cell counts of foremilk when the results of the Coulter counter and those of the Fossomatic were compared. For strippings samples, the Fossomatic gave lower counts. This may have been caused by the higher number of cell fragments present in the strippings ( Paape and Tucker, 1966 ). These fragments would be counted by the Coulter counter if their size range overlaps with that of the intact cells, while the Fossomatic, which stains and counts nuclear material would give a lower count.

Preincubation of the milk at 60 degrees celsius, rather than 40 degrees celsius gives a higher cell count reading for the Fossomatic ( Miller et al, 1986 ). This finding is attributed to greater dye penetration at the higher temperature with 99 % and 78 % of the cells fluorescing at the two temperatures respectively.

Several workers have studied the problem of sample storage. Schmidt Madsen ( 1975 ) found that formalin used for stabilizing the cells in the Coulter counter method is not suitable to be used with milk samples in the Fossomatic. When potassium dichromate ( 0.05 % w/v ) was used as a preservative there was no significant effect on the Fossomatic cell count. Cell counts do not differ significantly when sodium dichromate replaces the potassium salt ( Hodgson, 1978 ). Optimal results using potassium dichromate as a preservative are obtained when the chemical treatment is combined with storage at 4 degrees celsius, conditions under which the correlation between the direct microscopic somatic cell count and the Fossomatic at seven days is 0.96 ( Heald et al, 1977 ). No significant trend between the somatic cell count and storage time has been found for milk samples treated with potassium dichromate ( Hodgson, 1978 ).

Unfortunately, chromium salts are highly toxic to humans, causing skin irritation and damage to the nasal septum. Bronopol ( 2-bromo-2-nitro-1,3-propandiol ) has been tested as a milk preservative and this chemical compares favourably with dichromate for the preservation of samples for the Fossomatic counter ( Ardö, 1978 ). Bronopol has been used for several years in place of potassium dichromate for preservation of composite milk samples by the Livestock Improvement Corporation in New Zealand ( personal communication ).

The International Dairy Federation issued a set of recommendations for the use of the Fossomatic for somatic cell counting ( Dodd, 1981 ).

A method for the production of a set of reference samples, which may be used to calibrate both Coulter counter and Fossomatic machines, has been developed ( Heald, 1982 ), thereby increasing the agreement between laboratories in respect of automated somatic cell counting methods. A similar system devised by Szijarto and Barnum ( 1984 ) gave good agreement between four laboratories in Guelph, Canada.

## (I) FLOW-THROUGH CYTOPHOTOMETRY

The technique of flow-through cytophotometry may be used to measure the somatic cell count of milk ( Lutz et al, 1975 ). This method is similar to that of the Fossomatic, in that the dye ethidium bromide is used to stain the cells, the resulting fluorescence being measured as the sample flows past a microscope objective. This method offers similar benefits to those of the Fossomatic, in that fat globules, aggregated casein micelles and dirt particles are not counted. Correlation between the flow-through cytophotometry and the direct microscopic somatic cell count is very high,  $r = 0.99$  for quarter samples and  $r = 0.97$  for bulk milk samples ( Lutz et al, 1975 ).

Flow - through cytophotometry is a simple and a reliable procedure for determination of the somatic cell count in milk ( Breer et al, 1976 ).

A two colour fluorescence flow-through cytophotometry method has been used by Hageltorn ( 1982 ), cited by Emanuelson et al ( 1987 ) for the measurement of bovine milk leukocytes. Similarly, two colour fluorescence flow-through cytophotometry has been used to estimate the number of somatic cells as a predictor of infection in udder quarters and was found to be more accurate than other diagnostic tests ( Emanuelson et al, 1987 ).

Although flow-through cytophotometry shows promise in the area of mastitis diagnosis, it has not become widely used. This may be as a result of the similarities between the methods of flow-through cytophotometry and Fossomatic fluoro-optical cell counting. Equipment for the latter method has been produced commercially for some years.

## (2) TESTS WHICH MEASURE OTHER CHANGES IN MILK COMPOSITION

### (A) MAJOR IONS IN MILK

The ionic content of milk varies markedly from that of the extracellular fluid which bathes the acini of the mammary gland. Milk contains a high concentration of potassium relative to sodium, the latter being actively removed from the secretory cells by an energy dependent ATPase, which is located on the basolateral surface of the cell. The extracellular fluid / milk barrier is maintained by tight junctions between cells in the healthy udder. During mastitis, however, these tight junctions appear to leak, allowing the exchange of ions down their relative concentration gradients ( Peaker, 1978 ).

It has been well established that with the occurrence of mastitis there is usually an increase in the concentration of sodium ions and of chloride ions, while the concentration of potassium ions decreases ( Muldoon and Liska, 1971; Linzell and Peaker, 1972; Wegner and Stull, 1978; Fernando and Spahr, 1983; Corbellini, 1984; Fernando et al, 1985; Daniel and Pavithran, 1985 ).

The salty taste of mastitic milk was noted prior to 1890, and the chloride content of milk had been used for mastitis diagnosis by 1930 ( Giesecke and van den Heever 1974 ).

Early efforts were directed to the titration of chloride in milk using potassium chromate as the indicator ( Sharp and Struble, 1935 ). This method is subject to interference from milk proteins, notably casein, and is not suitable for use with sour milk. While the protein can be removed by ashing the sample, this method results in the loss of some of the chloride and a potentiometric titration method has been developed ( Herrington and Kleyn, 1960 ). This procedure is carried out at pH 2.0, a pure silver electrode being used to determine the endpoint.

Flame photometry has been used for the determination of both sodium and potassium ions ( Murthy and Whitney, 1956 ).

The use of atomic absorption spectrophotometry for the analysis of major cations in milk has been compared with flame photometry ( Murthy and Rhea, 1967 ). For sodium and potassium there are no significant differences between the results obtained by these two methods.

Brooks et al ( 1970 ) overcame the problem of interference from milk proteins by their precipitation using trichloroacetic acid, before using atomic absorption spectrophotometry to analyse for sodium and potassium. There were no appreciable differences noted between this precipitation method and that of the ashing method with regard to sodium, but the potassium values did differ between the two methods of sample preparation.

An ion selective electrode has been used to measure the chloride concentration of bulk milk samples ( Muldoon and Liska, 1971 ).

The simultaneous measurement of sodium, potassium and chloride, using a Technicon Autoanalyser, has been reported by Fleet et al ( 1972 ). The cations are determined by flame photometry and the chloride by a colourimetric procedure. This method is capable of analysing 40 samples per hour for these three ions ( as well as lactose concentration ) at a low cost per sample. The coefficient of variation is less than 1 % for each ion.

An automated ion selective electrode method for the simultaneous measurement of sodium, potassium and chloride has been used by Balulescu ( 1985 ) and this method has a high precision. There is excellent correlation between flame photometry and the ion selective electrode method for sodium and potassium, and between the titrimetric chloride method and the ion selective electrodes. The automated ion selective electrode system is capable of analysing approximately 40 samples per hour.

A number of workers have published figures for the correlation between these major ions and other measures of milk abnormality. A correlation of 0.53 has been reported between the somatic cell count ( Fossomatic ) and sodium concentration, while that between the somatic cell count and the sodium/potassium ratio has been estimated as 0.71 ( Kitchen *et al*, 1978b ).

Correlation coefficients between the direct microscopic somatic cell count and sodium, potassium or chloride have been estimated as 0.67, -0.39 and 0.95 respectively, all correlations being statistically significant at the 1 % level ( Wegner and Stull, 1978 ). Lower coefficients have been reported between log somatic cell counts ( Coulter counter ) and the major ions in strippings samples ( Fernando *et al*, 1985 ). These ions have also been shown to be correlated with each other:

|                          |                                          |
|--------------------------|------------------------------------------|
| For sodium - potassium   | $r = -0.35$                              |
| For sodium - chloride    | $r = 0.68$                               |
| For chloride - potassium | $r = -0.73$ ( Muldoon and Liska, 1971 ). |

Sample storage is less of a problem when the ionic content is used for mastitis diagnosis than is the case for the somatic cell count. The concentration of chloride ions remains constant in milk stored for six days, in contrast to the electronic somatic cell count which increases daily with sample age ( Muldoon and Liska, 1971 ). It was concluded that the determination of the chloride concentration shows some promise as a test for mastitis, and that the combination of chloride and sodium may also prove useful for diagnosing this disease.

The results of quarters within a cow over successive days have been used to calculate a Relative Milk Quotient for sodium, potassium or chloride. Using this type of analysis, milk sodium, potassium or chloride concentrations compare favourably with bacteriology or somatic cell counts for detecting abnormal milk samples ( Linzell and Peaker, 1972 ). The incorporation of a device capable of measuring sodium or chloride into the milking machine was suggested.

Both chloride and sodium concentrations have been shown to give lower percentages of false results when detecting abnormal milk than does the somatic cell count. The potassium concentration was found to be slightly more accurate for classifying abnormal samples, but less accurate for classifying normal milk samples, than was the somatic

cell count ( Renner, 1975 ) . However, the definition of normality used by this author relates to the results of indirect tests including the pH, conductivity, lactose concentration and the concentration of whey proteins in the milk, rather than to the bacteriological findings.

Kitchen et al ( 1978b ) reported that both the sodium concentration and the sodium/potassium ratio are similar to other tests such as somatic cell counts, NAGase activity, and electrical conductivity in their ability to diagnose animals producing milk of abnormal composition.

Given the easy and economic method of evaluation, the long keeping time of the samples and the close association with the somatic cell count, the use of the milk sodium concentration has been advocated for large scale campaigns for the diagnosis of subclinical mastitis ( Corbellini, 1984 ).

Fernando et al ( 1985 ) have reported that both the sodium and the chloride concentrations of foremilk samples have a greater discriminative power between infected and uninfected quarters than does the potassium concentration. These authors found that the accuracy of detection was greater in strippings samples than in foremilk, and the log somatic cell count proved to be inferior to sodium or chloride ions in terms of discriminative power.

While the major ions have shown some promise as indicators of subclinical mastitis, their use has not become widespread. The ease of measurement for the related method of electrical conductivity could have precluded their use.

#### (B) ELECTRICAL CONDUCTIVITY

Some attention has been paid to the use of the electrical conductivity of milk for mastitis detection.

The ions responsible for the electrical conductivity of milk include sodium, potassium and chloride, with ions such as calcium, magnesium, and phosphate playing a minor role.

As expected, the correlations between the electrical conductivity and major ions of milk are generally high, with coefficients of 0.84, 0.88 and -0.37 between conductivity and sodium, chloride, and potassium respectively ( Fernando et al, 1985 ).

The electrical conductivity and the somatic cell count of the milk are correlated . A correlation coefficient of 0.24 has been reported between conductivity and the direct microscopic cell count ( Postle and Blobel, 1965 ), while that between the conductivity

and the Wisconsin Mastitis Test has been estimated as 0.28 ( Gebre-Egziabher et al ( 1979 ). Fernando et al ( 1982 ) reported correlation coefficients of 0.49 and 0.54 between the filter - DNA method and electrical conductivity for foremilk and for strippings samples respectively. Estimates of the correlation between the conductivity and the somatic cell count by the Coulter counter range from 0.39 to 0.7 ( Little et al, 1968; Peaker, 1978; Mijnen et al, 1982; Chamings et al, 1984 ) while those between the conductivity and the Fossomatic cell counts vary from 0.30 to 0.82 between herds, the highest value being for the herd with the greatest number of abnormal quarters ( Okigbo et al 1984 ).

A between herd variation in correlation between electrical conductivity and the somatic cell count has been noted by Sheldrake et al ( 1983a ) while the correlation between conductivity and the somatic cell count estimated by flow cytometry is higher in infected, than in uninfected quarters ( Emanuelson et al, 1987 ).

The electrical conductivity of milk, with a coefficient of variation of 0.5 %, shows higher reproducibility than do a number of tests including the direct microscopic cell count, the California Mastitis Test, the Whiteside test, chloride concentration or catalase activity, ( Postle and Blobel, 1965 ). Little et al ( 1968 ) also reported considerably less variation between replicate measurements of conductivity than between replicate measurements of the somatic cell count using a Coulter counter.

A number of studies have shown the electrical conductivity to be higher in the milk of mastitic than of normal quarters or cows ( Davies 1938; Davis, 1947; Greatrix et al, 1968; Linzell and Peaker, 1971; Linzell and Peaker, 1972; Davis, 1975; Linzell and Peaker, 1975; Chamings et al, 1984; Fernando et al, 1985 ).

The stage of lactation, parity or lactation number, stage of milking process, estrus, antibiotic treatment and milking interval are among the physiological or environmental factors which have been shown to affect the conductivity of milk ( Linzell and Peaker, 1975; Woolford and Williamson, 1982; Sheldrake et al, 1983b; Batra and McAlister, 1984; Fernando et al, 1985 ). The temperature of milk has also been shown to affect the electrical conductivity, with an increase of approximately 2 % for every degree celsius rise in temperature ( Davis, 1975 ). Milk obtained prior to milk ejection can be up to 2 degrees celsius cooler than that obtained after ejection ( Woolford and Williamson, 1982 ). This variation due to physiological or environmental factors may explain the lack of success which has often been noted when the absolute value of the conductivity has been used for diagnosis.

In general, early efforts involving the use of electrical conductivity for the diagnosis of mastitis were unsuccessful when absolute levels of conductivity were measured ( Davis, 1975 ). Sheldrake and Hoare ( 1981 ) used two absolute thresholds of 6.0 and 6.8 mS/cm, but reported a low sensitivity, with only 39 % to 57 % of infected quarters

detected, while the specificity was higher, with 71 % to 91 % of uninfected quarters correctly classified ( see chapter seven for an explanation of the terms "sensitivity" and "specificity" ). The variation in conductivity between cows within a herd, and also between herds, was suggested as being responsible for the poor results which precluded the setting of one threshold for all herds.

Chamings *et al* ( 1984 ) found that the mean electrical conductivity of normal quarters within one herd was higher than the mean conductivity of infected quarters in another herd. Batra and McAlister ( 1984 ), using a slightly lower absolute threshold of 5.4 mS/cm, also found a low sensitivity, with 84.3 % of the infected cows being misdiagnosed ( false negatives ), while 9 % of the uninfected cows were classified as being infected ( false positives ). The electrical conductivity was not considered to be a good indicator of subclinical mastitis in comparison with the California mastitis test or the Fossomatic by these authors.

The level abnormality testing aid, a hand held device for measuring the conductivity of milk, manufactured by Agricultural Technical Aids ( Wrexham, England ), was tested using the International Dairy Federation ( IDF ) definitions of mastitis as the reference method. This reference method takes into account both the bacteriological status of the quarter, and the somatic cell count of the milk. In this case, all quarters which were classified as either latent infection, non-specific mastitis or mastitis were diagnosed correctly, but 99 % of the normal quarters were misdiagnosed as being abnormal and this device was not recommended for mastitis diagnosis ( Green and Middleton, 1984 ).

Other hand held devices have appeared on the market. Chamings *et al* ( 1984 ) tested the AHI mastitis detector ( AHI plastic moulding Co., Hamilton, New Zealand ) and found that 90.6 % of samples classified by the above IDF criteria as being mastitic, gave a positive reading, but that 32.7 % of the normal quarters were also positive. Better results were reported by Okigbo *et al* ( 1984 ), who used a Mas-D-Tec hand held device and found that this method of detection correctly classified a greater number of abnormal quarters than did the somatic cell count or the lactose concentration.

With respect to uninfected cows, the conductivities of the four quarters tend to move in parallel from day to day, whereas for infected quarters, there is usually a substantial departure from parallelism ( Linzell *et al*, 1974 ). This suggests that the effects of these physiological factors would tend to affect all four quarters of a cow equally, in the absence of infection and that a more accurate diagnosis might be achieved by comparing quarters within cows.

The difference conductivity value, which is the difference between the conductivity reading of a quarter and that of the quarter with the lowest conductivity reading within the cow, has been suggested as having merit ( Davis, 1947 ). However, the use of the difference conductivity value or related measurements did not gain wide acceptance for

another 20 years ( Davis, 1975 ). The use of differential methods for detecting abnormal quarters, using the somatic cell count as the reference method has been highly successful ( Greatrix *et al*, 1968 ). Using a somatic cell count threshold of 500,000 cells per ml to classify quarters as normal or abnormal, the electrical conductivity correctly identified 53 % to 76 % of abnormal, and 94 % to 100 % of normal samples.

Other within udder comparisons have been carried out with varying results. Differential conductivity readings were found to identify infections with a high degree of efficiency, with 82 % to 86 % of infected and 96 % to 97 % of uninfected samples correctly classified ( Woolford and Williamson, 1982 ). It was concluded during this study that the differential conductivity test is a rapid and efficient cowside test for the detection of subclinical mastitis, and that trends observed during the milking process could be used to identify infected quarters if the conductivity cell were to be incorporated into the milking machine.

Using frequency distributions for normal and for abnormal quarters, 95 % of normal quarters were found to fall below a quarter difference of 0.34 mS/cm, while 99 % fell below a quarter difference of 0.56 mS/cm ( Oshima, 1985 ).

Comparisons between the various detection systems have been conducted. Fernando *et al* ( 1982 ) compared the accuracy of detecting infected quarters, using absolute conductivity values, differential values and a combination of these two methods. The absolute values were generally similar to, and sometimes superior to, the differential values in terms of accuracy of classifying infected samples. The combination of absolute and differential values showed the greatest accuracy.

Rapidity and ease of measurement together with objectivity make the measurement of electrical conductivity advantageous as a cowside test, but the greatest potential for use of the conductivity has been seen as an in-line test, during the process of machine milking ( Fernando *et al*, 1982 ).

While Linzell and Peaker ( 1975 ) claim that the electrical conductivity is able to detect most cases of subclinical mastitis at a single visit providing both absolute and differential values are used, Sheldrake *et al* ( 1983a ) found little advantage in using quarter differences over the use of absolute levels of conductivity for diagnosis.

Gebre-Egziabher *et al* ( 1979 ) found an interquarter ratio of 1.2 to be the optimal ratio in terms of accuracy of discrimination, with 69 % of established cases of subclinical mastitis being detected using this method, giving an accuracy similar to that of other screening tests which were available at the time.

In another study, the absolute and differential values were compared with the out of balance method. The out of balance method compares the conductivities of all four

quarters which form four arms of a conductivity bridge, the arrangement of the quarters within the system making a difference to the value obtained. The out of balance system was the most effective providing that the various arrangements of quarters within the system were tested, as was the case with the three way out of balance method. The overall accuracy using this system was claimed to be 82 % of cows, and this is similar to the accuracy obtainable using the somatic cell count ( Peaker, 1978 ).

Linzell and Peaker ( 1971 ) overcame the physiological variation by using the relative milk quotient. This statistic takes into account not only the ratio of conductivity between quarters, but also between days. The relative milk quotient is a sensitive indicator of early stages of subclinical mastitis or of susceptibility to it. This idea was taken a step further by Linzell et al ( 1974 ) who used the quarter x day interaction mean square as a measure of parallelism. The quarter x day interaction takes into account changes in the ratio of conductivity between quarters, over a longer time period than is the case with the relative milk quotient which was used by Linzell and Peaker ( 1971 ). Using the interaction mean square, 19 out of 19 uninfected cows and 24 out of 25 infected cows were correctly classified. Absolute conductivity, differential conductivity, mixed conductivity using a mixed sample of foremilk from all four glands, and out of balance conductivity were all found to be less sensitive than the interaction mean square for parallelism. Differential, absolute and mixed conductivity measurements all detected severe cases of the disease, but missed the mild cases, while out of balance conductivity missed some of the severe cases.

The development of in-line methods of measuring and recording the electrical conductivity of milk are continuing.

### (C) LACTOSE CONCENTRATION

Lactose is synthesized in the secretory epithelial cells of the udder, from carbohydrate precursors supplied from the blood. This is an energy dependent process, the rate of which might be expected to decrease as a result of damage to the mammary gland. However, the control of the lactose concentration of milk is complex.

A number of researchers have shown the concentration of lactose to be lower in mastitic than in normal milk ( Wheelock et al, 1966; Kramer et al, 1980; Rottscheidt and Iben, 1981; Wiesner, 1985; Fernando et al, 1985; Daniel and Pavithran, 1985 ) and this property has been used for mastitis detection since the early 1920s ( Giesecke and van den Heever, 1974 ).

During the past 60 years, a number of techniques for the assay of lactose in milk have been described. Hinton and Macara ( 1927 ) developed a chloramine-T method for the

determination of lactose in milk and dairy products, replacing the earlier copper reduction technique. The procedure involves a preliminary clearing step to remove fat and protein, followed by a manual titration.

Lawrence ( 1968 ) described a colourimetric technique for the determination of lactose in milk and milk products.

Gas Liquid Chromatography ( GLC ) has been used for the determination of lactose in milk and was found to be satisfactory, although relatively complicated ( Reineccius et al, 1970 ). GLC gives similar results to those obtained with the chloramine-T method. The repeatability is high for the GLC method, the standard deviation between replicate samples having been estimated as 0.13 % ( Jaynes and Asan, 1973 ). The recovery of added lactose in the GLC method ranges from 87.5 % to 115 % and this method gives significantly lower (  $p < 0.05$  ) results than does the standard titrimetric method ( Adachi and Yamaji, 1978 ).

Up to this point, the available methods for the determination of lactose in milk were slow and tedious. Fleet et al ( 1972 ) developed an autoanalyser method capable of the simultaneous analysis of lactose plus the major milk ions ( sodium, potassium and chloride ). This fully automated method can analyse 40 samples per hour at a low cost, the lactose analysis being based on a colourimetric reaction using o-toluidine reagent.

Despite the availability of a rapid method for measurement of lactose, work continued on the development of chromatographical techniques. Euger and Brunner ( 1979 ) developed a high performance liquid chromatography ( HPLC ) method, involving deproteinization with trichloroacetic acid and the subsequent separation of the lactose from other components on a uBondapak/carbohydrate column. Although the recovery of the method is good ( 96.1 % to 101.1 % ) and the results do not differ significantly from the phenol-sulphuric acid method of Lawrence ( 1968 ), HPLC is time consuming and relatively expensive as a method of analysis.

The use of acetonitrile to precipitate fat and protein, prior to using HPLC to determine the lactose concentration has been tested. The average recovery of added lactose is 97.5 % and the analysis takes approximately 20 minutes, although a number of samples may be prepared at once thereby increasing the sample throughput ( Pirisino, 1983 ).

The determination of lactose by HPLC is highly sensitive, requires little sample preparation, and can be fully automated. The coefficient of variation ranges from 1.27 % to 3.26 % ( Kowalski and Giesecke, 1986 ), while the accuracy of the HPLC method compares favourably with that of the chloramine-T method ( Smit and Nels 1987 ).

Analysis of lactose in milk by infra-red spectrometry ( IR ) was assessed by Biggs ( 1972 ), this method being based on the absorption of infra-red energy at specific

wavelengths by OH groups in the lactose molecule. The precision of the IR analyser is slightly superior to that of the reference method and it was recommended that the Association of Official Analytical Chemists adopt the IR measurement of milk lactose as a standard technique.

The IR method has a high repeatability between duplicate samples and agrees well with the results of the HPLC ( Grappin *et al*, 1980; Packard *et al*, 1986 ).

Recently, a rapid colourimetric technique has been described by Abu-Lehia ( 1987 ). The standard curve is linear over the range of 2.5 % to 6.5 % lactose. Recovery is approximately 99 % and the repeatability is also high, the average difference between duplicate milk samples being 0.011 %.

Studies have shown the lactose concentration of milk to be useful for the diagnosis of subclinical mastitis. Renner ( 1975 ) classified cows as having a normal or an abnormal secretion based on the results of the majority of a battery of diagnostic tests which included the somatic cell count, pH, catalase activity, and concentrations of sodium, potassium and chloride ions. Frequency distributions using this classification showed less overlap between normal and abnormal samples for lactose than for other parameters including the somatic cell count. This resulted in the greater accuracy of diagnosis of subclinical mastitis for lactose ( false negatives 6.0 %, false positives 6.7 % ) compared with the somatic cell count ( false negatives 21.1 %, false positives 14.8 % ). However, the success noted by this author has been attributed to the method of classification which does not take bacteriology into account, but relies on indirect indicators ( Mijnen *et al*, 1982 ).

The percentage frequency of positive bacteriological results increases markedly below a lactose concentration of 4.6 %, and it is feasible that this level of lactose could replace the IDF standard of 500,000 cells per ml of milk for quarter samples without changing the distribution of diagnoses ( Stahlhut-Klipp, 1973 ). However, the lower variation of lactose in comparison to that of the somatic cell count makes measurement of lactose unsuitable for use in bulk ( herd ) milk samples.

Fernando *et al* ( 1985 ) found the milk lactose concentration to be superior to the somatic cell count, but inferior to the concentrations of sodium ions, chloride ions, or to the electrical conductivity of the milk, with respect to discriminating between infected and uninfected quarters. In other studies, the lactose concentration has been of less value in the discrimination between infected and uninfected animals. While the lactose concentration of whole udder samples is lower in infected than in uninfected cows, the somatic cell count gives a better differentiation of the two groups ( Kramer *et al*, 1980 ).

Rottscheidt and Iben ( 1981 ) found that the lactose concentration fell below 4.6 % in 65.8 % of cows with Staphylococcus aureus mastitis, but there was a wide variation in the lactose concentration between cows, with 30 % of healthy cows showing values below 4.6 %.

A number of factors other than the health of the gland have been shown to affect the lactose concentration of the milk. These include the stage of lactation, parity, month of test, and milk fraction ( Rook and Campling, 1965; Kramer et al, 1980; Mijnen et al, 1982; Fernando et al, 1985; Daniel and Pavithran, 1985 ). The stage of lactation, parity and month of test have a stronger influence on the lactose concentration than does the presence of pathogens and this results in a greater overlap between frequency distributions of infected versus uninfected samples for lactose than for the somatic cell count. As a result, the lactose concentration is of less value for the diagnosis of udder infection ( Kramer et al, 1980 ).

Even after adjustment of the results for physiological factors, the lactose concentration proves of only limited value for mastitis detection, being inferior to the cell count of the milk ( Mijnen et al, 1982 ).

Correlations between the lactose concentration and other suggested indicators of mastitis have been determined. Stahlhut-Klipp ( 1973 ) reported a correlation coefficient of -0.6 between lactose and the somatic cell count, while Fernando et al ( 1985 ) found a correlation coefficient of -0.27 between lactose and log somatic cell count in strippings samples. In general, lactose is well correlated with the major ions of milk, particularly sodium and chloride ( Wheelock et al, 1966; Oshima and Fuse, 1977; Fernando et al, 1985 ).

#### (D) THE pH OF MILK

The pH of normal milk is lower than that of the blood and is controlled by the concentration of ions, notably bicarbonate, citrate and phosphate, the ratios of which differ from those of blood or extracellular fluid. Damage to the mammary gland during periods of mastitis results in leakage between adjacent epithelial cells, with an alteration in the concentrations of various milk components, the nett result being that the pH of the milk tends to increase. In severe cases, the pH of the milk may approach that of the extracellular fluid with which the exchange of ions takes place.

The pH of milk was used as a screening test for mastitis in New Zealand during the 1930s. An indirect method, involving the use of pH indicators has been developed and a field outfit for the bromothymol blue test designed as a milking shed technique ( Hume, 1941 ).

Factors which can affect the results of the bromothymol blue test include the milk fraction, the time lag between sampling and testing, and the fat content of the milk ( McDowell, 1941 ).

Samples which test positive for the bromothymol blue test have a higher chloride concentration, but a lower lactose concentration than do bromothymol blue negative samples ( McDowell, 1945 ).

The pH of milk may be determined electronically. The quinhydrone electrode can be used, although it gives results which are different to those obtained with the glass electrode ( Steinsholt and Calbert, 1960 ). Labuschagne ( 1976 ) used calomel, glass and combined electrodes to measure milk pH.

Milk drawn from infected quarters shows a higher pH than does that from uninfected quarters within the same cow ( Feagen *et al.* 1966b ). Kiswa and Kruk ( 1970 ) reported the pH of normal and mastitic milk as 6.63 and 6.80 respectively, a relatively small difference.

That the pH test lacks sensitivity was noted by Fay *et al.* ( 1938 ) who reported that the bromothymol blue method detected only 21.1 % of samples taken from infected quarters ( false negatives 78.9 % ) while 98.4 % of uninfected samples were correctly classified ( false positives 1.6 % ). The problem of lack of sensitivity is not necessarily a function of the measurement technique. Mijnen *et al.* ( 1982 ) using a calomel-glass electrode system found pH of milk to be of little diagnostic value.

Factors which have been shown to affect the pH of milk include the lactation stage, lactation number, fat and protein content of the milk and temperature at which the measurement is carried out ( Steinsholt and Calbert, 1960; Kossila *et al.* 1967; Buchberger *et al.* 1986 ). Neither the time of sampling ( Buchberger *et al.* 1986 ) nor the milk fraction ( Shazly *et al.* 1974 ) have a significant effect on milk pH.

Despite the relatively poor history of the pH as a diagnostic test, the bromothymol blue method has recently been revived as an on-farm technique by Marschke and Kitchen ( 1981 ), the indicator being applied to blotting paper and the milk added. Using the somatic cell count as a reference method and a threshold of 500,000 cells per ml, the bromothymol blue test gave 30 % false negative results. However, in many of these samples, the somatic cell count exceeded 1,000,000 cells per ml with no colour change being noted in the bromothymol blue test. It was concluded that while the test is rapid and inexpensive, it is not sensitive enough to detect many cases of subclinical mastitis. Marschke and Kitchen ( 1985 ) used bacteriology as the reference method and again found that the sensitivity is low, with only 39 % of infected quarters being correctly classified. While the test lacks sensitivity, it has some attraction as a cowside test, being

more objective than the California Mastitis Test. The colour change is stable over time and may be kept for comparison at a later date.

The lack of sensitivity of the pH for detecting mastitis may be related to the low deflection of pH due to infection and to a high degree of overlap between the distributions of infected and of uninfected quarters.

## (F) ENZYME ACTIVITY

The activity of a number of enzymes have been measured in milk and in many cases they have been investigated as possible indicators of various pathological changes or of the inflammatory response of the secretory tissue. For convenience, these enzymes may be grouped. The E.C. number refers to the Enzyme Commission system of nomenclature ( International Union of Biochemistry, 1979 ).

### (1) OXIDATIVE ENZYMES

#### (i) XANTHINE OXIDASE E.C. 1.1.3.22

Xanthine oxidase has been investigated as an indicator of subclinical mastitis. Patterson *et al* ( 1969 ) reported a high activity of xanthine oxidase in milk and that there was a suggestion of a relationship between the level of this enzyme and the total cell count. However, there was no statistically significant correlation between the two parameters over a range of somatic cell counts from 117,000 to 13,200,000 cells per ml.

The xanthine oxidase activity is dependent on the fat concentration of the milk and is similar in normal and in mastitic milk samples ( Kitchen *et al*, 1970 ).

#### (ii) N,N:N',N'-TETRAMETHYL-p-PHENYLENE-DIAMINE OXIDASE

Patterson *et al*, ( 1969 ) reported that N,N:N',N'-tetramethyl-p-phenylene-diamine oxidase ( TPD oxidase ) was useful for monitoring the effect of acute experimental mastitis but there was no correlation between enzyme activity and the somatic cell count over a range of cell counts from 18,000 to 2,300,000 cells per ml.

Problems exist with the assay procedure for this enzyme.

(iii) CATALASE E.C. 1.11.1.6

By far the most promising of the oxidative enzymes studied thus far has been catalase.

The catalase test for the determination of mastitis was introduced in 1909 ( Ernst, 1909, cited by Giesecke and Van den Heever 1974 ). Monlux ( 1948 ), used a modified Lind method to determine the catalase content of foremilk samples, using a 2.0 ml free oxygen threshold to distinguish between infected and uninfected quarters. Thirty six infected quarters and 48 uninfected quarters had mean cell counts of 439,000 and 116,000 cells per ml respectively. Only one of the 36 infected quarters gave a catalase reading which was less than the chosen threshold, although a number of the uninfected quarters gave positive results. Higher catalase values occurred in early lactation and when the daily milk yield dropped in uninfected cows.

The catalase test has been used as an indirect method of estimating the number of somatic cells in the milk. Several authors have shown that under normal circumstances, the bacterial content of the milk has no significant effect on catalase levels, despite the fact that some of the bacteria which cause mastitis do produce the enzyme ( Monlux, 1948; Spencer and Simon 1960 ).

A number of factors have been shown to affect the results of the catalase test. These include: reaction temperature, type of preservative used, age of sample, and freezing of samples ( Monlux, 1948; Funk *et al*, 1967; Read *et al* 1969 ).

The early catalase tests suffered from a number of drawbacks including rather cumbersome glass reaction vessels, lengthy incubation periods and a difficulty in establishing the exact amount of oxygen produced due to bubbles in the cream layer of the milk ( Monlux, 1948; Spencer and Simon, 1960; Willits and Babel 1965 ). These drawbacks were largely overcome when Willits and Babel ( 1965 ) developed a simple catalase test in which milk is absorbed onto a piece of filter paper. The paper is placed in a solution of hydrogen peroxide and the time taken for paper to float to the surface is noted. This disc floatation test is suitable for the detection of catalase in bulk milk, composite samples or quarter samples, while being rapid, accurate and requiring little equipment.

The repeatability of the catalase test is relatively high. Spencer and Simon ( 1960 ) reported a standard deviation of 3.5 % to 5.0 % for catalase. Estimates of the coefficient of variation range from 8 % to 12.1 %, compared with those of the direct microscopic count, which range from 17.5 % to 19 % ( Postle and Blobel, 1965; Paape *et al*, 1965 ), suggesting that the catalase estimate has a higher repeatability than does the microscopic somatic cell count.

A number of studies have reported correlation coefficients between catalase and the direct microscopic count. The values range from 0.27 to 0.92 ( Paape et al, 1965; Willits and Babel, 1965; Postle and Blobel, 1965; Natzke et al, 1965; Funk et al, 1967; Read et al, 1969; Janzen, 1969; Kitchen, 1976 ).

Different methods of analysis for catalase, and the use of mathematical transformations prior to statistical analysis may explain some of the variation in correlation. The direct microscopic count identifies intact leukocytes only, whereas the catalase test measures the enzyme in both intact and lysed cells. Thus conditions of transport and storage prior to analyses may affect the number of intact cells without affecting the catalase activity, but altering correlations between the two tests. Kitchen ( 1976 ) measured the catalase activity in milk with > 1,500,000 cells per ml and found the activity to be 23 times that of milk with < 500,000 cells per ml.

While catalase has been shown to be repeatable, and to reflect the somatic cell count of milk, this enzyme is not a reliable indicator of the presence or absence of pathogenic bacteria ( Spencer and Simon 1960 ).

More elaborate systems of quantitating the catalase activity have been devised. Kitchen ( 1976 ) used an electronic oxygen analyser to measure catalase activity. This method is accurate, simple and capable of measuring 30 samples per hour, but the equipment used is expensive.

#### (iv) LACTATE DEHYDROGENASE E.C. 1.1.1.27

The activity of the enzyme lactate dehydrogenase ( LDH ) increases during mastitis. The infusion of bacterial endotoxin results in an 18 fold increase in the LDH activity of the milk ( Bogin and Ziv, 1973 ). This activity is higher than that observed for the blood serum, suggesting that the serum is not the sole source of LDH in milk. The determination of the activity of this enzyme has been suggested as an accurate and objective method of evaluating the extent of udder damage ( Bogin and Ziv, 1973 ). Similarly, a higher activity of LDH has been found in naturally occurring cases of mastitis, than in healthy cows ( Symons and Wright, 1974 ).

A number of isoenzymes of lactate dehydrogenase have been reported. Bogin et al ( 1977 ) examined isoenzyme patterns for LDH in normal and in inflamed udder tissue, in normal and in mastitic milk, and in blood serum. The isoenzymes are designated LDH1 to LDH5, according to their electrophoretic mobility. Lactose dehydrogenase isoenzyme 1 ( LDH1 ) is the most common isoenzyme in all tissues, but inflamed tissue and leukocytes from mastitic milk contain relatively higher proportions of LDH4 and of LDH5 ( Bogin et al, 1977 ). During mastitis there is an increase in the total LDH content and a marked increase in LDH5 relative to LDH1 in the milk. The results

suggest that the LDH in mastitic milk might originate both from the leukocytes and from the parenchymal cells of the udder. Kitchen et al ( 1980 ) reported that the LDH activity of mammary tissue is 15 times greater than that of the blood, while mastitic milk shows approximately nine times the activity of normal milk.

Correlation between the LDH activity and the somatic cell count has been reported to be 0.53 ( Kitchen et al, 1980 ).

Unfortunately, the described assay has a lengthy incubation time, and requires tedious sample preparation. The future development of this procedure into an automated assay system would make the LDH activity an attractive alternative to the somatic cell count for mastitis diagnosis ( Kitchen, 1981 )

#### (v) GLUTAMATE-OXALOACETATE TRANSAMINASE E.C. 2.6.1.1

Experimental infusion of endotoxin into the udder has been shown to cause an eight fold increase in the activity of glutamate-oxaloacetate transaminase ( GOT ) in milk, the blood serum being suggested as the principle source of the enzyme ( Bogin and Ziv, 1973 ). However, mammary gland tissue has been shown to have 50 times the GOT activity of blood serum and the mammary gland secretory cells has been suggested as the principle source of GOT in mastitic milk ( Kitchen et al, 1980 ).

Symons and Wright ( 1974 ) found that the GOT activity in the milk from clinically affected quarters was seven fold higher than that of normal quarters, while Kitchen et al ( 1980 ) reported that milk containing > 1,000,000 cells per ml showed three times the GOT activity of milk with < 500,000 cells per ml.

The correlation between GOT activity and the somatic cell count was estimated to be 0.55 ( Kitchen et al, 1980 ).

Tedious sample preparation and a lengthy incubation time are disadvantages associated with the assay of GOT, making this assay unattractive as a diagnostic method ( Kitchen et al, 1980 ).

#### (vi) ACID PHOSPHATASE E.C. 3.1.3.2

The presence of acid phosphatase in cow's milk was first shown by Mullen ( 1950 ) and the distribution within the milk has been studied further. Kitchen et al ( 1970 ) found a high specific activity for this enzyme in the cream fraction of the milk, while

Anderson *et al* ( 1974 ) reported a higher activity of acid phosphatase in the membrane material isolated from mastitic, than that from normal milk.

Normal milk contains a single acid phosphatase isoenzyme, while milk from cows with mastitis contains three isoenzymes, one being the same as that from normal milk, the other two being derived from leukocytes ( Andrews and Alichandis, 1975 ).

The increase in the activity of acid phosphatase during mastitis is relatively small. Anderson *et al* ( 1975 ) found that the infusion of bacterial endotoxin into the udder caused a 2 to 3 fold increase in the activity of acid phosphatase over the baseline levels in whole milk. Smaller increases were observed when staphylococci were infused into the udder quarters. The acid phosphatase activity in high cell count milk, ie milk with > 1,500,000 cells per ml, is only slightly higher than that of milk with fewer than 500,000 cells per ml ( Kitchen, 1976 ).

The correlation between the acid phosphatase activity and the somatic cell count has been reported as only 0.42 ( Kitchen, 1976 ), although more recently Obara ( 1985 ) reported a closer association with a coefficient of 0.68.

Acid phosphatase is unsuitable as an indicator of mastitis because of the variation in concentration of milkfat and therefore of milkfat globule membrane with which the enzyme is associated ( Kitchen, 1976 ).

#### (vii) ALKALINE PHOSPHATASE

The activity of alkaline phosphatase increases only slightly during mastitis. Kitchen *et al* ( 1970 ) reported little difference in alkaline phosphatase activity between normal and mastitic milk, while Bogin and Ziv ( 1973 ) found that the infusion of bacterial endotoxin caused a six fold increase over baseline levels.

The distribution of the enzyme within milk fractions is similar to that of acid phosphatase ( Kitchen *et al*, 1970 ). Normal milk shows a higher alkaline phosphatase activity than does the blood serum, suggesting that leukocytes or possibly areas of regenerating mammary tissue are the chief sources of alkaline phosphatase in cow's milk ( Bogin and Ziv, 1973 ).

#### (viii) A-ESTERASE E.C. 3.1.1.2

Milk from mastitic quarters shows a 10 fold higher A-esterase activity than does that from healthy quarters ( Forster *et al*, 1961 ). Kitchen *et al* ( 1980 ) reported that milk

from quarters with a cell count > 1,000,000 cells per ml, had an A-esterase activity three times that of milk from quarters with < 500,000 cells per ml.

The infusion of streptococci ( Booth et al, 1965 ) or of staphylococci ( Marquardt et al, 1966 ) into the udder causes a modest elevation of A-esterase activity.

The A-esterase activity is related to the severity of the mastitis. Estimates of the correlation coefficient between the somatic cell count and the A-esterase activity range from 0.66 to 0.81 ( Marquardt and Forster, 1964; Marquardt et al, 1966; Kitchen et al, 1980 ).

The average blood plasma to milk ratio for A-esterase is 1800:1, suggesting that the plasma is the major source of this enzyme in the milk, and that this assay could be used as an indicator of leakage between blood and milk compartments ( Marquardt and Forster, 1966; Marquardt et al, 1966 ). This suggestion is supported by the finding that A-esterase is highly correlated with bovine serum albumin ( Kitchen et al, 1980 ). Mammary gland tissue is a poor source of A-esterase in comparison with the blood serum ( Kitchen et al, 1980 ).

The enzyme has been purified and characterized by Kitchen et al ( 1973 ) who found it to occur in milk as a lipoprotein with a molecular weight of approximately 440,000 daltons. This would suggest that the diffusion of A-esterase from the blood to milk might be slower than that of smaller proteins, thus limiting its value as an indicator of altered permeability.

The activity of A-esterase in milk is affected by the stage of lactation and by the breed of cow, although the activity does not vary significantly between milk fractions ( Forster et al, 1961; Marquardt and Forster, 1966 ). Although this assay is time consuming, because of the need for an ultracentrifugation step, an improved assay procedure could make the determination of A-esterase a useful diagnostic test for subclinical mastitis ( Kitchen, 1981 ).

#### (ix) CARBOXYLESTERASE E.C. 3.1.1.1

Although little attention has been paid to the activity of the enzyme carboxylesterase in response to mastitis, a higher activity has been associated with the disease. Fitzgerald et al ( 1981 ) reported marked increases in the enzyme carboxylesterase following the infusion of bacterial endotoxin. The activity is also elevated during naturally occurring mastitis. The correlation between carboxylesterase and the somatic cell count has been estimated as 0.77 ( Fitzgerald et al, 1981 ).

#### (x) LIPASE E.C. 3.1.1.3

Conflicting reports on the effect of mastitis on the milk lipase activity have been published. In an early study, little difference was observed between the lipase activity of normal and of mastitic milk ( Forster et al, 1961 ).

Tallamy and Randolph ( 1969 ) reported that milk with a Wisconsin Mastitis Test score of > 20 mm showed a small but significantly higher lipase activity than did milk with a Wisconsin Mastitis Test score of < 10 mm, with similar findings being reported by Randolph and Erwin ( 1974 ). However, Deeth and Fitzgerald ( 1975 ) reported a lower lipase activity in mastitic than in normal milk. The conflicting results have been attributed to the presence of inhibitors and of activators of this enzyme in the milk ( Kitchen, 1981 ).

#### (xi) PROTEINASE ENZYMES

Indirect evidence for the existence of proteinase enzymes in mastitic milk has been provided by several studies. High cell count milk contains a lower concentration of both alpha and beta caseins than does normal milk, and this is associated with an increase in the concentration of kappa casein ( Haenlein et al, 1973 ). Anderson and Andrews ( 1977 ) confirmed the presence of kappa casein in milk from mastitic cows and suggested that this might be due to the high levels of proteolytic enzymes which are present in the milk.

The proteolysis of casein in mastitic milk has been attributed to a plasmin-like enzyme which probably originates from the blood plasma ( Barry and Donnelly, 1981 ). De Rham and Andrews ( 1982 ), who had found the proteolytic activity of mastitic milk to be 5 to 10 fold higher than that of normal milk, reported that plasmin accounted for approximately one third of the total proteinase activity of the milk following infusion of udders with endotoxin.

Different proteinases are associated with various milk fractions ( De Rham and Andrews, 1982 ).

Cows with clinical mastitis or those receiving endotoxin infusions have a very high proteinase activity ( Andrews, 1983 ). The plasmin is thought to originate from the blood serum, while the remaining proteinases may come from the leukocytes.

The electrophoretic techniques used to demonstrate these enzymes are not conducive to large scale monitoring for subclinical mastitis.

(xii)  $\beta$ -GLUCURONIDASE E.C. 3.2.1.31

The activity of the enzyme  $\beta$ -glucuronidase is higher in mastitic than in normal milk. Kitchen ( 1976 ) reported that the  $\beta$ -glucuronidase activity of milk with a cell count of  $> 1,500,000$  cells per ml was approximately four fold higher than that of milk containing  $< 500,000$  cells per ml.

The activity of  $\beta$ -glucuronidase correlates well with the milk somatic cell count (  $r = 0.71$  ) suggesting that this enzyme could be useful for estimating the somatic cell count ( Kitchen, 1976 ). Obara ( 1985 ) reported a correlation coefficient of only 0.59 between  $\beta$ -glucuronidase activity and somatic cell count, but when the variables were transformed to log derivatives this figure increased to 0.72. Nagahata et al ( 1987 ) reported a very close association between an indirect indicator of the somatic cell count ( the California Mastitis Test ) and the  $\beta$ -glucuronidase activity (  $r = 0.92$  ).

Milk macrophages are a rich source of  $\beta$ -glucuronidase ( Nagahata et al, 1987 ).

The  $\beta$ -glucuronidase activity is related to the presence of both major and of minor pathogens, as well as to the somatic cell count ( Peridigon et al, 1986 ). These authors suggested that  $\beta$ -glucuronidase might be a reliable indicator of the inflammatory process, since it is specific to leukocyte lysosomes.

The activity of  $\beta$ -glucuronidase may be determined by spectrophotometry, although this method includes a four hour incubation period ( Kitchen, 1976 ). A more rapid method involving an incubation period of only 10 minutes, using the fluorimetric determination of  $\beta$ -glucuronidase, has been described ( Nagahata et al, 1987 ).

(xiii)  $\alpha$ -MANNOSIDASE E.C. 3.2.1.24

The primary source of milk  $\alpha$ -mannosidase activity is unclear. Mellors and Harwalkar ( 1968 ) showed that the  $\alpha$ -mannosidase activity is associated with casein micelles in milk, and suggested that it is released from the lysosomes of damaged leukocytes. However, the cell debris fraction was not found to contain significant activity of this enzyme. This finding is reflected in a low correlation between the somatic cell count and  $\alpha$ -mannosidase activity ( Kitchen, 1976; Obara et al, 1983 ). Kitchen ( 1976 ) reported a similar activity for  $\alpha$ -mannosidase in milk from normal and from mastitic quarters.

The activity of  $\alpha$ -mannosidase would appear to be of little value in the detection of cows with subclinical mastitis.

(xiv) N-ACETYL- $\beta$ -D-GLUCOSAMINIDASE E.C. 3.2.1.30

Perhaps the most widely studied milk enzyme, with respect to mastitis diagnosis, has been that of N-acetyl- $\beta$ -D-glucosaminidase ( NAGase ), whose presence and distribution in milk was noted by Mellors ( 1968 ). It was suggested that the leukocytes or epithelial cells might be the source of this enzyme and that should the leukocytes prove to be the principal source, then the NAGase activity could be a convenient indicator of mammary gland infection.

Kitchen et al ( 1980 ) reported that the NAGase activity of milk containing > 1,000,000 cells per ml was five fold higher than that of milk which contained < 500,000 cells per ml.

Natural infection has been shown to affect the NAGase activity. Kitchen et al ( 1984a ) reported that minor pathogens cause a marginal increase in milk NAGase activity, while major pathogens cause a greater increase, the mean NAGase activity being twice that of milk from uninfected quarters, with similar findings being reported by Miller and Paape ( 1988 ). The difference between the NAGase activity in milk from infected and from uninfected quarters is statistically significant (  $p < 0.01$  ), ( Mattila and Sandholm, 1985 ).

Clinical mastitis causes a 20 fold increase in milk NAGase activity ( Nagahata et al, 1987 ), while a marked increase in activity follows infusion of bacterial endotoxin into the udder ( Fitzgerald et al, 1981 ).

The NAGase activity is highly correlated with the somatic cell count, estimates ranging from 0.62 to 0.87 ( Kitchen, 1976; Kitchen and Middleton, 1976a; Kitchen et al, 1978a; Kitchen et al, 1978b; Kitchen et al, 1980; Obara et al, 1983; Callieri et al, 1987; Miller and Paape, 1988 ). Emanuelson et al ( 1987 ) reported that the correlation between the two variables is slightly higher in infected, than in uninfected quarters.

A number of assay procedures for the determination of milk NAGase activity have been described. A spectrophotometric method for the determination of NAGase was used by Kitchen ( 1976 ). This technique is relatively simple and capable of analysing up to 60 samples per hour. A more rapid colourimetric method was used by Kitchen and Middleton ( 1976a ), the colour being read in a Lovibond Comparator. This test is simple, inexpensive and has a higher throughput than does the former spectrophotometric technique.

Kitchen et al ( 1978a ) have since developed a fluorimetric assay which is associated with increased sensitivity, as well as overcoming the problems of sample turbidity which can affect the colourimetric methods. This fluorimetric assay is claimed to be more suited to conversion to an automated system than are the earlier methods. The

fluorimetric method has been adapted to a microtitration plate system by Mattila and Sandholm ( 1985 ) thereby increasing the rate of measurement of the activity.

Bacterial growth in milk does not affect the NAGase activity ( Kitchen, 1976 ). Kitchen and Middleton ( 1976a ) showed that the storage of milk at room temperature or at 5 degrees celsius, with or without a preservative for up to 10 days, does not affect the NAGase activity. These findings were confirmed by Kitchen and Middleton ( 1976b ) who showed that either formalin or somafix may be used to preserve the samples, although potassium dichromate and mercuric chloride inactivate the enzyme.

It was concluded that the NAGase activity would serve as an excellent marker for the somatic cells in milk ( Kitchen, 1976 ). However, it was later shown that within the milk, the NAGase is located mainly in the soluble whey protein fraction, while NAGase activity of the somatic cell fraction ranged from 5 % of the total in normal quarters to 12 % in milk from mastitic quarters ( Kitchen et al, 1978a ). The main source of NAGase in healthy quarters is considered to be the cytosol of intact secretory cells, the enzyme being released as a consequence of normal milk secretion, while in infected quarters, the damage to the tissue would result in a greater release of NAGase from these cells.

Mammary gland tissue has been shown to have a NAGase activity much higher than that of the blood plasma, milk somatic cells or blood leukocytes ( Kitchen et al, 1978a ). These authors concluded that the NAGase activity should not be used as an estimate of the somatic cell count per se, but rather as a measure of the integrity of the mammary secretory tissue. Additional evidence for the secretory cell origin of NAGase comes from the very high correlation between NAGase activity and the protein lactoferrin ( Obara et al, 1983 ).

Nevertheless, the contribution of the leukocytes to the NAGase activity cannot be discounted. Dulin et al ( 1984 ) reported that intact neutrophils could contribute 16 % of the total NAGase activity, while lysed neutrophils would contribute 31 %. Furthermore, neutrophils themselves may damage the mammary secretory tissue, thereby causing the release of NAGase into the milk, the greatest damage being caused by neutrophils which are actively phagocytosing ( Capuco et al, 1986 ).

The effect of factors other than the presence of bacterial infection on the NAGase activity of milk have been reported. NAGase activity is affected by stage of lactation, with high levels being found at parturition and towards the end of the lactation period ( Timms et al, 1984; Mattila and Sandholm, 1985; Mattila et al, 1986b; Miller and Paape, 1988 ).

The parity or lactation number has also been shown to affect the NAGase activity, with a small increase in activity being seen in older cows ( Mattila and Sandholm, 1985; Mattila et al, 1986b ). Miller and Paape ( 1988 ) however, found no such effect.

The effect of estrus on the NAGase activity of milk is unclear. Mattila et al ( 1986a ) reported that estrus affects the milk NAGase activity. However, Berning et al ( 1987a ) found no effect of estrus, nor of administered estradiol benzoate.

A number of authors have reported the NAGase activity to vary during the milking process, with higher activity being found in the strippings milk than in earlier fractions. In general, this variation is greater for infected than for uninfected quarters ( Obara, 1985; Nagahata et al, 1987; Marschke et al 1987; Berning et al, 1987b ).

The time of sampling has been examined as a source of variation. Marschke et al ( 1987 ) reported that uninfected quarters show no differences in NAGase activity between morning or evening samples, but for infected quarters, higher values are obtained at the morning milking. No effect with regard to the position of the quarter within the udder, the age of the cow or the season is associated with the NAGase activity ( Miller and Paape, 1988 ).

The reproducibility of the fluorimetric NAGase assay is relatively high, the coefficient of variation having been estimated as 9 % ( Mattila and Sandholm, 1986a ).

The NAGase activity is generally an accurate indicator of subclinical mastitis. Kitchen et al ( 1978b ) reported that the NAGase activity is very similar to the somatic cell count in its ability to diagnose animals producing milk of abnormal composition, while Kitchen et al ( 1980 ) concluded that NAGase is the best alternative to the somatic cell count. The NAGase activity may be successfully used on composite milk samples to diagnose mastitis in cows as well as at the individual quarter level ( Kitchen et al, 1984a ). Using bulk milk samples, the NAGase activity is a useful means of monitoring the infection status of dairy herds ( Kitchen et al, 1984b ).

Differentiation of infected and uninfected quarters is enhanced by interquarter evaluation over that of the absolute activity of NAGase, while best results have been achieved using a combination of interquarter evaluation plus absolute values ( Mattila and Sandholm, 1985; Mattila et al, 1986a ). Mattila et al ( 1986a ) reported the measurement of NAGase activity to be superior to other diagnostic tests including the somatic cell count, for the diagnosis of udder infection.

Emanuelson et al ( 1987 ) included the effects of physiological factors such as the lactation stage and lactation number in the logistic regression model. The predictive ability of NAGase, and of other parameters, using this method of adjustment was superior to that of within-udder evaluation. In the above study, the NAGase showed a

higher predictive ability for quarter infection status than did other parameters including the somatic cell count.

The suggestion that the NAGase activity could be used in combination with the somatic cell count to improve the diagnosis of pathological or physiological changes in the udder has been made ( Kitchen et al, 1980 ). However Emanuelson et al ( 1987 ) reported that the combination of NAGase with other indicators of mastitis gives only a marginal improvement of diagnostic accuracy over the use of NAGase activity alone.

#### (xv) MISCELLANEOUS ENZYMES

Other enzymes have been studied and have been found to be of little value in the diagnosis of subclinical bovine mastitis. These include: peroxidase, NADH-tetrazolium reductase and hexose monophosphate dehydrogenase ( Patterson et al, 1969 ), aldolase, ribonuclease ( RNAase ) and carbonic anhydrase ( Kitchen et al, 1970 ), and arylsulphatase ( Kitchen, 1976 ).

#### (G) PROTEINS IN MILK

The proteins which occur in the milk may be manufactured in the mammary gland itself, or may be derived from the blood. Disruption of the normal tissue during mastitis can result in a decrease in the rate of biosynthesis.

A feature of inflammation is the increase in vascular permeability, which allows large molecules such as proteins to escape from the local capillaries, into the extracellular fluid. In the mastitic gland, proteins may pass between cells of the mammary epithelium and thus be found in the milk. The measurement of the concentration of proteins from either source may serve as an indication of mastitis.

#### (i) BOVINE SERUM ALBUMIN

Bovine serum albumin ( BSA ) is present in high concentration in the blood and a number of techniques for the measurement of this protein in milk have been described. Mancini et al ( 1965 ) developed a radial immunodiffusion technique for the measurement of milk BSA. This method has a high repeatability and is able to detect a low concentration of BSA. Laurell rocket electrophoresis has been used to quantify BSA in milk for the diagnosis of mastitis ( Smith et al, 1979 ).

The concentration of BSA in the milk increases as a result of mastitis. Kostov and Dzurov ( 1968 ) found a higher concentration of BSA in quarters with subclinical

mastitis than in normal quarters. The BSA concentration increases early in experimental coliform mastitis, then declines rapidly after the acute phase ( Harmon et al, 1976 ). Similar changes occur during natural coliform mastitis. The results suggest that during acute inflammation there is destruction of the blood-milk permeability barrier, with leakage of BSA from the blood into the milk.

A similar sharp increase in BSA has been observed following the infusion of Staphylococcus aureus or of purified alpha toxin into the udder ( Mackenzie and Lascelles, 1968 ). Anderson and Andrews ( 1977 ) found a 20 fold increase in BSA following the infusion of bacterial endotoxin, while experimental infection with streptococci caused a 10 fold increase. Kitchen et al ( 1980 ) reported that milk with a somatic cell count of > 1,000,000 cells per ml contains 2.5 to 3 times the BSA concentration of milk with fewer than 500,000 cells per ml.

A small but statistically significant difference (  $p < 0.01$  ) has been detected between the BSA content of milk from infected quarters and that from uninfected quarters ( Poutrel et al, 1983 ). Similar results have been reported by Sheldrake et al ( 1983a ), but later Sheldrake et al ( 1983b ) found the difference to be nonsignificant.

The reported correlation between the BSA concentration and the somatic cell count of the milk varies from 0.53 to 0.87 ( Haenlein et al, 1973; Kitchen et al, 1980; Honkanen-Buzalski and Sandholm, 1981; Poutrel et al, 1983 ). The correlation between the two variables is higher within infected than in uninfected quarters ( Mattila et al, 1986a; Emanuelson et al, 1987 ). The milk BSA concentration is also correlated with the antitrypsin concentration of the milk ( Honkanen-Buzalski and Sandholm, 1981; Mattila et al, 1986a; Emanuelson et al, 1987 ).

The accuracy with which the BSA has been reported to detect subclinical mastitis has generally been lower than that of some other parameters. Thus Sheldrake et al ( 1983a ) reported the BSA concentration to be less accurate for classifying quarters as infected or uninfected than is the somatic cell count or the electrical conductivity, with the probability of misclassification ranging from 15 % to 48 % in the three herds studied.

While infected and uninfected quarters differ with respect to their BSA content, there is considerable overlap, making the setting of a threshold level difficult ( Poutrel et al, 1983 ). Thus 46.5 % of infected quarters were misdiagnosed ( false negatives ), while 39.9 % of healthy quarters were misdiagnosed ( false positives ). Fernando et al ( 1985 ) concluded that in comparison with other parameters which were tested, the BSA is ineffective for classifying quarters according to bacteriological status. Similar results have been found by Mattila et al ( 1986a ) while Emanuelson et al ( 1987 ) have reported the predictive ability of BSA to be lower than that of the somatic cell count or of NAGase activity, but slightly superior to that of the electrical conductivity. The use

of interquarter evaluation improves the diagnostic accuracy, but the number of quarters which are misclassified is still relatively high ( Mattila et al, 1986a ).

Epithelial damage resulting from subclinical mastitis is often not great enough to cause an increase in the capillary permeability in the udder ( Poutrel et al, 1983 ).

Physiological factors might affect the BSA concentration in the milk. Stage of lactation has been shown to influence the BSA concentration, but the lactation number is not a significant cause of variation ( Poutrel et al, 1983; Sheldrake et al 1983b ). Individual cows show significant variations with respect to BSA content but there is no effect due to the position of the quarter within the udder ( Poutrel et al, 1983 ). A significant effect of the herd on the BSA concentration of milk has been demonstrated ( Sheldrake et al, 1983a ).

Within uninfected quarters, the BSA concentration remains similar throughout the milking process, but infected quarters show an increase in BSA concentration as the milking proceeds, ( Meyer and Senft, 1979 ).

The assay for BSA is time consuming and tedious, making this technique impractical for mastitis diagnosis on a large scale ( Kitchen et al, 1980 ).

## (ii) LACTOFERRIN

The development of an assay technique for the protein lactoferrin allows the examination of the concentration of this protein in milk from healthy and from diseased glands.

Harmon et al ( 1975 ) used an electroimmunodiffusion technique to measure the lactoferrin concentration in milk. During naturally occurring mastitis, the peak lactoferrin content of the milk increased approximately three fold, although coliforms caused a greater increase than did other major pathogens. This increase due to infection was statistically significant at the 1 % level.

A 30 fold increase in the lactoferrin concentration 90 hours after inoculation of the mammary gland with coliforms has been reported ( Harmon et al, 1976 ).

The changes in lactoferrin do not mirror those of BSA, suggesting a different source. It has been suggested that the increase in lactoferrin might be a response on the part of the gland to the infection ( Harmon et al, 1976 ). While neutrophils do contain lactoferrin, the major source of this protein is thought to be the secretory cells of the udder. Support for this source comes from the very high correlation which has been

observed between the lactoferrin and NAGase activity of milk ( Obara and Komatsu, 1984 ).

Harmon and Newbould ( 1980 ) found the mean lactoferrin concentration for blood neutrophils and for milk neutrophils to be 8.63 and 2.98 micrograms per million cells respectively. The difference might be due to degranulation of the neutrophils in the milk as phagocytosis takes place, the lactoferrin being released into the milk. The contribution of neutrophils to the total lactoferrin concentration of mastitic milk was calculated to be only 4.9 %.

The lactoferrin content of milk from mastitic quarters varies during milking, whereas that of healthy quarters remains relatively constant ( Meyer and Senft, 1979 ).

Despite the large changes in lactoferrin concentration which have been shown to occur as a result of mastitis, this protein has received little attention as a potential diagnostic aid for the disease. This may be due to the nature of the assay.

### (iii) IMMUNOGLOBULINS

Mastitis affects the concentration of the various immunoglobulin fractions in the milk. Kostov and Dzhurov ( 1968 ) and Haenlein et al ( 1973 ) reported higher immunoglobulin levels in mastitic than in normal milk, while Mackenzie and Lascelles ( 1968 ) showed that the infusion of virulent Staphylococcus aureus or of purified alpha haemolysin causes a sharp rise in IgG2 in comparison with the level of IgG1. The latter authors suggested that a selective mechanism for the transfer of IgG1 continues during the lactation period, and that inflammation inhibits this selective mechanism.

The mechanism of entry of immunoglobulins into the milk has been examined. Harmon et al ( 1976 ) reported that during experimental coliform infection, the levels of IgG and of BSA increased together, but that the IgG concentration remained elevated for longer than did that of the BSA. The slower recovery of the IgG was suggested as being due to either local production of IgG, or to increased activity of the selective transfer mechanism in response to infection.

The finding that infusion of toxins or of pathogens into the udder causes an increase in the immunoglobulin levels has been confirmed, and it has been shown that the passive transfer of proteins from the blood can account for the observed changes in IgG1 and IgG2, but not those of IgA or IgM ( Anderson and Andrews, 1977 ). Local production of IgA and IgM has been suggested as an explanation of the difference by these authors.

While the concentration of these proteins in the milk has been shown to change both markedly and rapidly in response to infection, little interest has been shown in using them for mastitis diagnosis. As with other non-enzymic proteins, this may be due to the relatively tedious assays involved in their quantification.

#### (iv) $\alpha$ 1-ANTITRYPSIN

Several techniques have been devised for the measurement of the protein  $\alpha$ 1-antitrypsin ( antitrypsin ) in cow's milk. Honkanen-Buzalski and Sandholm ( 1981 ) used a method involving the diffusion of trypsin in agar containing rennet-precipitated casein to demonstrate the presence of antitrypsin in milk. Gel-filtration chromatography showed that the molecular weight is approximately 70,000 daltons which is similar to that of the protein bovine serum albumin.

A colourimetric procedure for the measurement of antitrypsin has been developed by Sandholm et al ( 1984 ) and is suitable for large scale monitoring.

Infection causes a statistically significant increase (  $p < 0.01$  ) in the milk antitrypsin concentration ( Mattila et al, 1986a ).

The origin of the milk antitrypsin is thought to be the blood plasma. Sandholm et al ( 1984 ) established that the serum to milk ratio for antitrypsin is approximately 200:1 in normal milk. Antitrypsin is classed as an acute phase reaction protein, the concentration in the blood increasing rapidly during periods of inflammation, including clinical mastitis in the cow ( Conner et al, 1986 ). Figures for the effect of subclinical mastitis on the level of antitrypsin in the blood are not available.

The reported correlation between antitrypsin and another blood derived protein ( BSA ) has varied from 0.64 to 0.89 ( Honkanen-Buzalski and Sandholm, 1981; Mattila et al, 1986a ), while the correlation between the two variables is higher within infected than in healthy quarters ( Emanuelson et al, 1987 ).

The success of antitrypsin in discriminating between infected and uninfected glands has varied. Mattila et al ( 1986a ) found that when using absolute values for diagnosis, antitrypsin was slightly superior to the somatic cell count for differentiating between infected and uninfected quarters. However, Nelson et al ( 1985 ) reported a comparatively low predictability of infection for antitrypsin, this finding being confirmed by Emanuelson et al ( 1987 ).

Better differentiation between infection statuses is achieved when interquarter analysis is used, while for optimal results, the absolute values and interquarter values must be combined ( Mattila and Sandholm, 1985 ). Similar results were reported by Mattila et al

( 1985 ) and by Mattila et al ( 1986a ), the latter authors claiming that the diagnostic accuracy of antitrypsin is markedly better than that of the somatic cell count when interquarter values are used.

The antitrypsin concentration is less useful than some other parameters for differentiating between infected and uninfected cows, as opposed to quarters because of the lower degree of deflection of the antitrypsin concentration as a result of infection ( Mattila et al, 1986a ).

There is a greater difference in antitrypsin levels between cows, than between quarters within a cow ( Mattila and Sandholm, 1985 ).

Physiological factors affect the antitrypsin concentration of the milk. The stage of lactation affects the antitrypsin concentration ( Sandholm et al, 1984; Mattila and Sandholm, 1985; Mattila et al, 1986b; Emanuelson et al, 1988 ). Mattila and Sandholm ( 1985 ) and Mattila et al ( 1986b ) reported that the lactation number does not have a significant effect on the milk antitrypsin concentration, while Sandholm et al ( 1984 ) found only a slight effect. Emanuelson et al ( 1988 ) reported a significant effect of lactation number on antitrypsin, although the effect on antitrypsin was less than that upon some other parameters tested.

Estrus does not have a significant effect on the milk antitrypsin concentration ( Mattila et al, 1986a ). The freezing of samples prior to analysis does not affect the results of the assay ( Sandholm et al, 1984 ).

The assay for antitrypsin has a high repeatability, with a coefficient of variation of 6 %, and the throughput of samples is high, although this depends largely on the prehandling and the sample preparation, because reading of the reaction is very rapid ( Mattila et al, 1986a ). Sandholm et al ( 1984 ) reported that one technician can analyse 1000 samples per day.

#### (v) MISCELLANEOUS PROTEINS

The concentrations of many proteins in the milk change during the occurrence of mastitis. Haenlein et al ( 1973 ) reported that increasing degrees of subclinical mastitis, as evidenced by an increase in the somatic cell count are accompanied by significant decreases in total casein,  $\alpha$ s1-casein,  $\beta$ -casein,  $\alpha$ -lactalbumin,  $\beta$ -lactoglobulin and a lower ratio of casein to total protein. At the same time, total whey proteins and k-casein increase in concentration. Other workers have found similar results ( Kostov and Dzheurov, 1968 ). Similar changes have been induced by experimental infection with Staphylococcus aureus or Streptococcus agalactiae ( Kiddy et al, 1968 ) and with Escherichia coli ( Harmon et al, 1976 ).

While these changes have been well documented, little interest has been shown in using the changes to detect subclinical mastitis. This may be due to the rather tedious electrophoretic or immunological techniques which are required to demonstrate or quantitate these proteins.

#### (H) MISCELLANEOUS TESTS

##### ADENOSINE TRIPHOSPHATE ( ATP ) IN MILK

The determination of adenosine triphosphate ( ATP ) in milk can be used as a screening test for the presence of mastitis ( Anon, 1985 ). This assay uses firefly luciferase, which catalyses the reaction between luciferin and ATP, the resulting light being measured by luminometry. This method has a high repeatability with estimates of the coefficient of variation ranging from 2.58 % to 7.25 %, but it has been noted that sampling and storage conditions need to be standardized if ATP is to be used for the diagnosis of subclinical mastitis ( Bossuyt, 1978 ).

The assay is simple, and gives high yields of ATP from the somatic cells without interference from bacterial ATP ( Olsson et al, 1986 ).

The ATP is present in the nondialysable portion of the skim milk, being localized within the calcium phosphate-citrate-casein micelle fraction of the milk ( Richardson et al, 1980 ). These authors found little correlation between the somatic cell count and the ATP content of the milk, and when the bacteria and the somatic cells were removed by centrifugation, the total milk ATP content decreased by only 20 %. In the study cited, however, the cell counts in the small number of samples varied from 5,500 to 49,000 cells per ml only. Other researchers have found a stronger relationship between the ATP content and the somatic cell count of milk, the correlation ranging from 0.73 to 0.91 ( Bossuyt, 1978; Anon, 1985; Nelson et al, 1985; Emanuelson et al, 1987 ). Nelson et al ( 1985 ) reported that the correlation between the two variables is greater within infected than in uninfected quarters.

The ATP content of milk has a high predictability of the infection status, showing a greater discriminative power than the somatic cell count, NAGase activity, electrical conductivity, BSA concentration or the antitrypsin concentration of milk ( Nelson et al, 1985; Emanuelson et al, 1987 ).

Both the lactation stage and the lactation number have been shown to exert a significant effect on the ATP content of milk ( Emanuelson et al, 1988 ).

### SUMMARY

During the past 100 years, a number of methods have been suggested for diagnosing subclinical mastitis. In many cases an ability to discriminate between infected and uninfected cows and or quarters has been demonstrated. However, for a particular test to be of practical use in the screening of large numbers of milk samples for abnormality, it must be:

- (1) Sensitive ( able to detect a large proportion of infected samples ).
- (2) Specific ( able to correctly classify a large proportion of uninfected samples ).
- (3) Repeatable ( have a low coefficient of variation ).
- (4) Rapid ( have a high throughput ).
- (5) Inexpensive ( have low capital costs and low running costs ).

Few, if any of the procedures which have been proposed for mastitis diagnosis meet all of these requirements. A summary of the performance of each test under the above headings is given in table 2.2.

New techniques are constantly being described particularly in the field of protein chemistry. Thus a number of the tests which have in the past been rejected, may in the future prove to be worthy of further study.

Currently, the somatic cell count, as determined by the Fossomatic fluoro-optical counter, is used in large scale screening programmes for mastitis in New Zealand and in many other dairying countries. For this method to be displaced, the alternative test should show an improvement in those factors listed above, with emphasis on the accurate classification of milk samples. A rapid and inexpensive test which gives misleading results will not benefit the dairy farmer, nor the dairy industry as a whole.

It is often difficult to compare the results of diagnostic tests which have been reported in the literature. There may be wide variations in factors such as definitions of infection or of mastitis, sampling techniques, milk fractions, breed of cow, age of cow, stage of lactation or conditions under which the animals are kept. This must also be borne in mind when comparing the results of the present trial with those of other workers cited above.

TABLE 2.2

COMPARISON OF TESTS WITH RESPECT TO SENSITIVITY, SPECIFICITY,  
REPEATABILITY, RAPIDITY AND OPERATING COSTS

| TEST                    | SENSITIVITY | SPECIFICITY | REPEATABILITY | RAPIDITY        | OPERATING COSTS |        |
|-------------------------|-------------|-------------|---------------|-----------------|-----------------|--------|
| S.C.C. <sup>1</sup>     | MEDIUM      | —           | HIGH          | MEDIUM          | HIGH            | LOW    |
| IONS <sup>2</sup>       | MEDIUM      |             | HIGH          | HIGH            | HIGH            | LOW    |
| ELECTRICAL CONDUCTIVITY | MEDIUM      |             | HIGH          | HIGH            | HIGH            | LOW    |
| LACTOSE <sup>3</sup>    | MEDIUM      |             | MEDIUM        | HIGH            | HIGH            | LOW    |
| pH                      | LOW         |             | HIGH          | ND <sup>6</sup> | HIGH            | LOW    |
| ENZYMES <sup>4</sup>    | MEDIUM      |             | HIGH          | ND              | MEDIUM          | MEDIUM |
| PROTEINS <sup>5</sup>   | MEDIUM      |             | HIGH          | ND              | MEDIUM          | MEDIUM |

1 Somatic cells counted by fluoro-optical counter.

2 Ions, eg sodium or chloride, measured by ion-selective electrodes .

3 Lactose measured by infra-red spectrometry.

4 Enzyme activity, eg NAGase activity, measured by fluorimetry.

5 Protein concentration, eg Antitrypsin, measured by inhibition of trypsin using microplate technology.

6 No data available.

## CHAPTER THREE

### MATERIALS AND METHODS

#### MAIN EXPERIMENT

The present study was carried out between January 1986 and May 1988. The main experiment involved the regular sampling of milk from selected cows from three dairy herds in the Manawatu region of New Zealand.

During the 1986 to 1987 dairy season, samples were taken from the Massey University Dairy Cattle Research Unit ( No 3 Dairy farm ), which is situated adjacent to the university, on the outskirts of Palmerston North. This farm is designated farm C. During the following season ( 1987 to 1988 ) samples were taken from each of two dairy farms, designated farms A and B. Farm A is situated near the town of Bunnythorpe, whilst herd B is situated on the edge of the township of Bulls. A brief description of the three farms is given in table 3.1.

Sixty four cows were randomly selected from farm C, the age range being from 3 to 11 years. No two year old cows were included, the animals being in their second or later lactation.

A slightly different approach was taken in selecting animals from farms A and B. The cows were sorted by age, and then they were randomly selected within age groups to give equal numbers of 2, 3, and 4 year old cows. A fourth age group consisted of cows aged either 5 or 6 years since low numbers precluded restricting this age group to five year old cows.

During each season, milk samples were taken from each of the selected cows at four-weekly intervals from within the first month post-partum, to the time at which drying-off occurred.

#### SMALLER EXPERIMENT - CHANGES IN PARAMETERS DURING MILKING

A second small scale trial was carried out using five cows from herd C to determine the effect of the milk fraction, ie the stage of the milking process at which the sample is taken, on the composition of milk. The five cows were selected on the basis of their infection status which had been determined during the main trial. This stage of milking trial was carried out during mid-lactation, the cows being sampled at one afternoon milking.

TABLE 3.1A DESCRIPTION OF THE THREE SELECTED FARMS

| FARM | BREED                    | TEATSPRAY            | DRY COW<br>THERAPY | BMSCC <sup>1</sup> | MILKING<br>SHED |
|------|--------------------------|----------------------|--------------------|--------------------|-----------------|
| A    | Friesian /<br>Friesian x | Throughout<br>season | Selective          | < 150,000          | Herringbone     |
| B    | Friesian                 | Intermittant         | Selective          | > 400,000          | Rotary          |
| C    | Mixed                    | Throughout<br>season | Selective          | > 300,000          | Walk-through    |

1 BMSCC is the mean bulk milk somatic cell count during the season prior to that during which the current trial was conducted.

### SAMPLING PROCEDURE

At each visit, both individual quarter foremilk samples and whole udder ( composite ) samples were taken. Sampling was carried out at the afternoon milking, which typically took place between 3.00 p.m. and 5.00 p.m., approximately nine hours after the preceeding morning milking.

The foremilk samples were collected using an aseptic technique as follows:

Firstly, the teats were washed by hand, using running water to remove any gross contamination. Each teat was then dried with an individual disposable paper towel ( Hygenex ). The teats were then rubbed vigorously with an individual cotton wool pledget which had been moistened with 70 % alcohol. The first three squirts of milk were discarded, and the next 20 ml collected in a sterile glass screw capped universal bottle ( United Glass, Great Britain ). A second 20 ml sample was collected into a clean plastic screw capped bottle.

The composite samples were taken using a milk meter ( Tru-Test Milk Meters, Hamilton, New Zealand ). The samples collected by the meter were proportionately representative of all four quarters, and of all stages of the milking, with the exception of the strict foremilk which had been removed by hand.

The samples were transported to the laboratory and processed within two hours of sampling.

### BACTERIOLOGICAL ANALYSIS

In the laboratory, the samples were well mixed by inversion, and a 0.05 ml aliquot transferred to a blood agar plate ( Tryptose Blood Agar, Difco Laboratories, to which 5 % v/v sheep blood was added ) using aseptic technique. The milk was spread over the plate using a glass spreader, and the plates were incubated at 37 degrees celsius for 24 hours. The type and number of colonies present was noted and any suspected pathogens were picked off to be purified. The plates were then reincubated for a further 24 hours and any additional colonies were noted.

The mastitis pathogens, which may be tentatively differentiated by their appearance on blood agar were identified as follows:

### (1) STAPHYLOCOCCI

The staphylococci were recorded as being either coagulase positive or coagulase negative on the basis of the tube coagulase test, which was carried out according to the method of Cruikshank *et al* ( 1975 ) with known control organisms being included with each batch of isolates ( Staphylococcus aureus, Oxford strain, NCTC 6571, and Staphylococcus epidermidis NCTC 11047 - National Health Institute, Porirua, New Zealand ). The plasma used in the coagulase test was oxalated pig plasma, prepared in the laboratory.

Each isolate was tested for the production of the enzyme deoxyribonuclease ( DNAase ), ( Difco Laboratories ) and the pattern of haemolysis on sheep erythrocytes was noted. In the case of a negative coagulase test, if either the DNAase was positive, or the isolate strongly haemolytic, the coagulase test was repeated.

No attempt was made to identify the staphylococci to the species level.

### (2) STREPTOCOCCI

Streptococci were differentiated by a number of biochemical tests:

The ability to hydrolyse esculin was tested using a Heart Infusion Agar ( Difco Laboratories ) to which esculin ( Sigma Chemical Company product number E-8250 ) was added to give a final concentration of esculin of 0.1 %.

The ability to ferment a number of sugars was tested using Phenol Red Carbohydrate medium ( MacFaddin, 1985 ). The following carbohydrates ( each at a final concentration of 1 % ) were tested:

|           |                                                          |
|-----------|----------------------------------------------------------|
| Lactose   | ( Difco Laboratories, product number 0156-17 )           |
| Salicin   | ( Difco Laboratories, product number 0177-13 )           |
| Trehalose | ( Difco Laboratories, product number 0180-12 )           |
| Inulin    | ( Difco Laboratories, product number 0165-15 )           |
| Mannitol  | ( Sigma Chemical Company, product number M-4125 )        |
| Maltose   | ( BBL product number 04-213 )                            |
| Raffinose | ( I.T. Baker Chemical Company Ltd, product number U825 ) |

The ability to hydrolyse sodium hippurate was tested using the rapid method of Hwang and Ederer ( 1975 ). The sodium hippurate and the ninhydrin used in the procedure were both obtained from Sigma Chemical Company, product numbers H-9380 and N-4876 respectively.

The CAMP test ( Christie et al, 1944 ), was carried out on sheep blood agar ( see above ), using a beta haemolysin producing strain of Staphylococcus aureus ( RARC 803, National Health Institute, Porirua, New Zealand ). A positive control organism ( Streptococcus agalactiae, NCTC 910, National Health Institute, Porirua, New Zealand ) was included on each plate.

### (3) COLIFORMS

Coliforms were identified using the API 20E system ( Analytical Profile Index, France ).

### (4) CORYNEBACTERIA

Corynebacteria were identified biochemically using a range of tests as specified by Cowan and Steel ( 1974 ). These tests included the carbohydrate fermentation pattern ( see section on streptococci ), but with the addition of horse serum to the Phenol Red Carbohydrate medium to improve growth. Additional tests included:

- (i) Vogues-Proskauer test - this was carried out as described by Cowan and Steel ( 1974 ) using alpha naphthol from BDH Chemicals Ltd, product number 10162. The organism was grown in Methyl Red Vogues-Proskauer Broth (Merck Chemicals).
- (ii) Urease production ( Christensen, 1946 ) was tested for using a commercial Urea Agar Base ( Difco Laboratories, product number 0283-01-7 ).
- (iii) Nitrate reduction was tested for in Heart Infusion Broth ( Difco Laboratories, product number 0038-01-5 ), to which had been added 2 grams per litre of potassium nitrate ( May and Baker Ltd ).
- (iv) Catalase determination was as described by Cowan and Steel ( 1974 ). A Corynebacterium bovis isolate ( NCTC 3224 - National Health Institute, Porirua, New Zealand ) was used as a control.

No bacteriological analyses of composite milk samples were performed, since the method of collection was subject to contamination. All other tests which are described below were carried out on both the individual quarter foremilk samples, and on the composite samples.

### SOMATIC CELL COUNTING

Following removal of the inoculum for the bacteriology, the two foremilk samples from each quarter were mixed and approximately 20 ml was transferred to a plastic container to which a preservative had been added. The composite milk samples were treated in a similar manner.

During the 1986-1987 season the preservative used was potassium dichromate. During the following season this was changed to Bronopol ( 2-bromo-2-nitro-1,3-propanediol ).

The samples were transported to the Livestock Improvement Corporation laboratory ( at Palmerston North during 1986-1987, and at Hillcrest, Hamilton during the 1987-1988 dairy season ), where the somatic cell counts were determined using a Fossomatic Fluor-optical Counter ( A/S N. Foss Electric, Hillerod, Denmark ). The testing procedure involves the automated preparation of a thin smear of the milk sample on a rotating disc. The smear is stained and illuminated with short wavelength light from a Xenon arc lamp. The light causes the nuclei of the somatic cells, which have been stained by the ethidium bromide stain to fluoresce, the emitted longer wavelength light being detected by a photomultiplier. The samples were prewarmed to 40 degrees celsius and the machine operated according to the manufacturer's instructions. Calibration was by direct microscopic somatic cell count and was carried out at regular intervals by the staff at the testing centre. The somatic cell count was given to the closest thousand cells per ml of milk.

The coefficient of variation, measured on 65 replicate subsamples of a bulk milk sample with a mean somatic cell count of 540,000 cells per ml was 7.3 %.

The somatic cell count of the milk was usually determined within three days of sampling. However, over the Christmas period, there was some delay. The effect of storage time on the somatic cell count was determined:

Ten composite milk samples were obtained from cows in mid-lactation. These samples were well mixed and split into three subsamples which were stored in glass bottles to which preservative had been added. One batch of samples was analysed on day two after the collection of samples, while the other two batches were stored at 4 degrees celsius and analysed on day 10 and day 16 after collection. An analysis of variance, using the ANOVAR procedure ( Statistical Analytical Systems, 1985 ), was carried out in order to determine whether storage of the milk causes any change in the cell count. Because of the marked skewness of the distribution, the data were transformed prior to analysis ( log 10 transformation ).

TABLE 3.2

RESULTS OF STORAGE TRIAL  
FOR THE SOMATIC CELL COUNT OF MILK

| DAY | MEAN LOG 10 SCC ( X 1000 ) | STANDARD DEVIATION |
|-----|----------------------------|--------------------|
| 2   | 2.555                      | 0.527              |
| 10  | 2.549                      | 0.560              |
| 16  | 2.558                      | 0.545              |

The mean value and standard deviation at each date are given in table 3.2. The effect of storage of preserved samples at 4 degrees celsius on the somatic cell count is not significant at the 5 % level.

#### STORAGE OF MILK FOR OTHER PARAMETERS

The remaining milk, after removal of aliquots for bacteriology and somatic cell counting, was frozen at -20 degrees celsius, and the remaining analyses were carried out in batches.

Preliminary experiments showed freezing to have no significant effect on the levels of these parameters in the milk ( unpublished data ).

Analyses were generally carried out within the week following sample collection.

#### SODIUM AND POTASSIUM MEASUREMENT

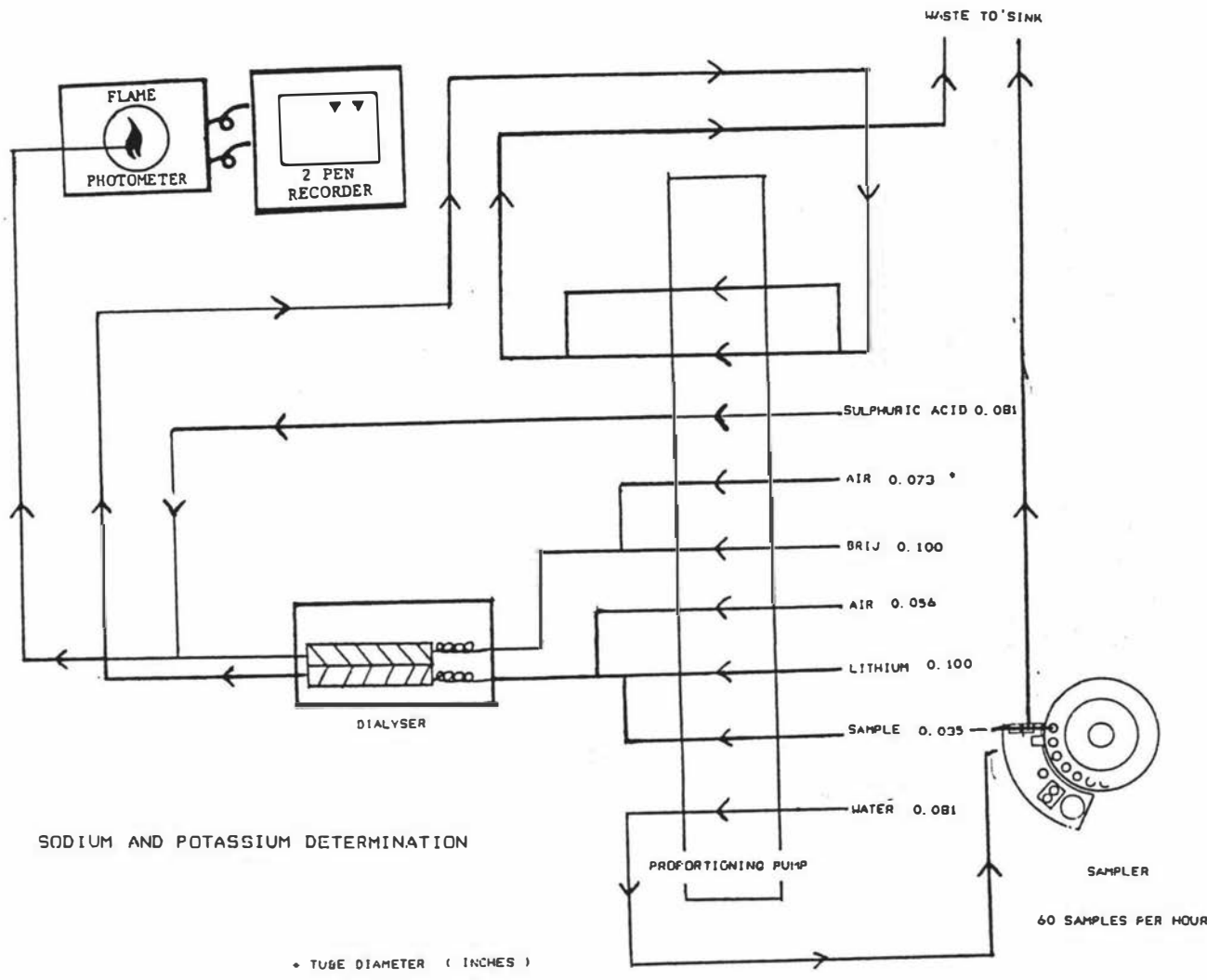
Sodium and potassium levels were measured by automated flame photometry, using a modified version of the method of Fleet et al ( 1972 ), see diagram 3.1 on page 68.

A Technicon Autoanalyser system was used (Technicon Instruments, Basingstoke, England), as shown in the flow diagram. This consisted of a sampler, proportioning pump, dialyzer, flame photometer III and a chart recorder. The sample was aspirated, mixed with 0.125 M lithium sulphate diluent and dialyzed against a solution of 1 % brij-35 ( Technicon Instruments, Basingstoke, England ). The dialysate was acidified with 0.15 M sulphuric acid ( BDH 10276 7Y, Poole, England ) and pumped to the flame photometer. Acidification must be carried out after the dialysis step to prevent coagulation of the milk proteins which may cause blockage of the tubing and rupture of the dialysis membrane.

Sodium and potassium were measured in one photocell, while lithium, as an internal standard, was measured in the other cell. No neutral density filters were required for sodium or potassium, but a 0.8 filter was used for lithium.

A standard curve was run before and after each batch of 20 samples using sodium chloride and potassium chloride ( May and Baker Ltd, Dagenham, England ) solutions made using double glass distilled water. A computer ( Z-2D System Two, Cromemco Inc, Mountain View, California, U.S.A. ) was used to interpolate results using both standard curves, the relative weighting being proportional to the position of the sample within the batch. Results were expressed as millimoles per litre ( mM ).

MANIFOLD DIAGRAM FOR THE MEASUREMENT OF MILK SODIUM  
AND POTASSIUM USING FLAME PHOTOMETRY



The coefficient of variation for sodium was 3.3 %, while that for the potassium was 1.0 %.

### pH MEASUREMENT

The pH of the milk samples was measured using an Orion Research Digital Ionalyser ( Orion Research Incorporated, Cambridge, Massachusetts, U.S.A. ), model no. 501, with a combination pH electrode ( model no. 910400 ). Measurements were carried out on samples which had been prewarmed to 37 degrees celsius. The instrument was operated in accordance with the manufacturer's instructions and was calibrated using a commercial buffer solution at pH 7.00 ( product no. 19240, BDH Ltd, Poole, England ). The electrode was rinsed with distilled water, and blotted dry with tissue paper between measurements.

The coefficient of variation for the pH was 2.1 %.

### ELECTRICAL CONDUCTIVITY

The electrical conductivity of the milk was measured with a Radiometer CDM 3 conductivity meter ( Radiometer, Copenhagen, Denmark ). The conductivity cell was type CDC 314, with a nominal cell constant of 0.316 cm. A standard solution of potassium chloride ( May and Baker Ltd, Dagenham, England ) at 0.01 M was used for calibration. Measurements were carried out at 37 degrees celsius and the results were expressed in millisiemens/cm.

The coefficient of variation for the electrical conductivity was 3.6 %.

### LACTOSE CONCENTRATION

The lactose concentration of the milk was determined using a Milko Scan 104 A/B semiautomated analyser ( A/S N. Foss Electric, Hillerod, Denmark ).

Measurement is based on infrared spectrometry using a single beam and cuvette. The different optical filters, two for each component measured, are presented to the beam in turn. The infrared energy which remains after the beam has passed through the cuvette is detected, amplified and converted to digital form. The analyser was operated in accordance with the manufacturer's instructions. Samples were prewarmed to 40 degrees celsius and mixed gently prior to aspiration. The machine was calibrated regularly by staff in the Animal Science Department, Massey University, using a

standardized milk sample. Before each batch of samples, the machine was zeroed using a solution of Triton X-100 ( A/S N. Foss Electric, Hillerod, Denmark ). The results were expressed as a percentage and were manually recorded.

The method used was capable of analysing for fat, protein and lactose concentrations at a rate of approximately 120 samples per hour. Although the fat and protein levels were recorded, the results were not analysed with respect to mastitis diagnosis, since neither the concentration of fat, nor of total protein have been shown to be of any practical use in the detection of infected animals in previous studies ( see chapter two ).

The coefficient of variation for the analysis of lactose was 0.5 %.

#### N-ACETYL- $\beta$ -D-GLUCOSAMINIDASE ( NAGase ) ACTIVITY

The NAGase assay used was that described by Kitchen ( 1976 ).

A 0.2 ml sample of milk was pipetted into a 100 x 16 mm glass, screw capped tube ( Wheaton No 358606, Millville, New Jersey, U.S.A. ). The tubes were placed in a waterbath at 37 degrees celsius and a 0.3 ml volume of the prewarmed substrate was added ( 2.0 mM p-nitrophenyl-N-acetyl-D-glucosaminide ( N 9376, Sigma Chemical Company, St. Louis Missouri, U.S.A. ) in 0.2 M citrate buffer, pH 4.5. The tubes were incubated for 15 minutes, after which the reaction was stopped by adding 1.0 ml of 1M glycine ( G 7126, Sigma Chemical Company, St. Louis Missouri, U.S.A. ) which had been adjusted to pH 10.0 with NaOH.

The milkfat was removed by the addition of 1.0 ml of chloroform ( Ajax Chemicals, Auburn, New South Wales, Australia ). This was followed by vigorous shaking and centrifugation at 2000 rpm for five minutes using a Sorvall GLC-1 centrifuge ( Dupont Instruments, Wilmington, Delaware, U.S.A. ). A 0.2 ml aliquot of the cleared supernatant was transferred to a well of a 96 well plastic flat-bottomed microtitre plate ( Nuc 269620, Nunc, Denmark ).

The absorbance was read using an automated multi-plate photometer ( SLT 210, Labinstrumente Gesellschaft m.b.H., A-5082 Grodig/Salzburg, Austria ), with a dual wavelength program ( 405 nm and 620 nm ) to compensate for any remaining turbidity in the assay supernatant.

A standard curve was prepared with each batch of samples using a stock solution of 4-nitrophenol GPR ( 29379, BDH Ltd, Poole, England ), made up in 0.2 M citrate buffer at pH 4.5.

Results were interpolated from the standard curve using a computer ( Z-2D System Two, Cromemco Inc. Mountain View, California U.S.A ) and the results expressed as nanomoles of product formed per ml of milk per minute.

The coefficient of variation for NAGase activity was 5.6 %.

### ANTITRYPSIN DETERMINATION

The  $\alpha$ 1-antitrypsin content ( relative values, not absolute values ) of the milk was determined using the method of Mattila et al ( 1985 ).

A 0.75 ml sample of milk was pipetted into a 16 x 75 mm polyallomer centrifuge tube ( Beckman Instruments Inc, Palo Alto, California ). Three volumes ( 2.25 ml ) of a clearing solution consisting of 16.7 % polyethylene glycol ( BDH, product number 44271 ) in a 0.1M tris ( BDH product number 10315 ), 0.02M calcium chloride ( BDH, product number 27583 ) buffer, pH 8.2. The tubes were centrifuged at 10,000 x gravity for one minute in a Sorvall RC-5 Superspeed Refrigerated Centrifuge ( DuPont Instruments, Wilmington, Delaware ).

A 0.5 ml aliquot of the resulting clear supernatant was diluted 1:2 with the Tris/calcium chloride buffer. A 0.1 ml volume of the diluted supernatant was transferred to a well in a microtitre plate ( Nunc, Denmark, product number NUC 269620 ). An equal volume of a 0.0025 mg/ml trypsin solution ( Sigma Chemical Company, product number A-4525 ), diluted from a stock of 0.5 mg/ml with 1mM hydrochloric acid was added to the well. To this was added 0.1 ml of a 1.0 mg/ml solution of the reagent Benzoyl-DL-Arginine-p-Nitroanilide hydrochloride (BAPNA) ( BDH, product number 40009 ).

The microtitre plate was incubated at 27 degrees celsius for a period of 3.5 hours. The resulting colour was measured with an automated multiplate photometer ( SLT 210, Lab Instruments, Gesellschaft m.b.H, Salzburg, Austria ), using a dual wavelength program at 405 nm and 610 nm to correct for any residual turbidity.

A standard curve was prepared and included with each assay, using a freeze dried milk sample, prepared from bulk milk obtained from the Milk Processing Plant in Palmerston North. This standard milk sample was reconstituted and clearing solution added to remove the fat, casein and  $\alpha$ 2-macroglobulin, as described for the samples above. The resulting supernate was then serially diluted using two fold dilutions in Tris/calcium chloride buffer ( see above ). A 0.1 ml aliquot of the dilutions were placed in each of two wells in the microtitre plate, and trypsin and BAPNA added, as for the milk samples which were being tested.

The results were fed into a computer ( Z-2D System Two, Cromemco Inc, Mountain View, California ), and a standard curve drawn using Healey's Sigmoid Curve Fit Program ( R.M. Greenway - personal communication ), the results for the unknown values being interpolated from this curve. The results were expressed as relative values, a value of 1.000 being the same as that of the standard milk sample. The cost of purified  $\alpha$ 1-antitrypsin precluded its use as a standard.

The coefficient of variation for antitrypsin was 10.2 %.

### ANALYSIS OF RESULTS

The results of the analyses were stored on the Massey University Prime computer ( model 9955 ). SAS ( Statistical Analytical Systems, 1985 ) packages were used for all statistical procedures, the particular packages used are outlined in the materials and methods section of each chapter.

Graphs were prepared using SPSSx graphics ( Statistical Procedures for the Social Scientist, Inc ) and were plotted on a Hewlett-Packard plotter ( model 7550 ).

### THE EFFECTS OF STAGE OF MILKING ( MILK FRACTION ) ON THE LEVEL OF SELECTED PARAMETERS IN MILK

#### INTRODUCTION

The diagnosis of subclinical mastitis involves the analysis of milk samples, taken either from individual quarters, or from all four quarters combined into one composite sample.

Typically, the sample used for the analysis is very small in comparison with the total volume of milk which is removed from the quarter or the udder during machine milking.

A large number of studies have shown that the composition of milk does not remain constant throughout the duration of the milking process, ( Paape and Tucker, 1966; Schalm and Lasmanis, 1968; Schalm and Ziv-Silberman, 1968; Linzell and Peaker, 1975; Fernando *et al*, 1982; Mijnen *et al*, 1982; Woolford and Williamson, 1982; Fernando and Spahr, 1983; Obara, 1985; Fernando *et al*, 1985; Yamamoto *et al*, 1985; Marschke *et al*, 1987; Berning *et al*, 1987b; and Fox *et al*, 1988 ). Therefore the results of the analyses used to diagnose subclinical mastitis are likely to be affected by the stage, during milk removal, at which the samples are taken.

Indeed, a number of studies have shown that the accuracy with which subclinical mastitis can be diagnosed is affected by the type of milk sample used ( Schalm and Ziv-Silberman, 1968; Fernando *et al*, 1982; and Fernando *et al*, 1985 ).

The New Zealand Dairy Board Livestock Improvement Corporation somatic cell counting service measures the somatic cell count in composite milk samples taken from the whole udder throughout the entire period of milk removal. On the other hand, individual quarter samples are generally taken manually, before milking begins.

These two procedures were adopted in the main body of the present study ( chapters five to nine ). However, a smaller experiment was also carried out to measure the concentration of the eight selected parameters in:

- (1) Strict foremilk samples, ie the first 10 to 15 ml of milk removed
- (2) Foremilk samples, ie the second 20 ml of milk removed

- (3) Midmilk samples, ie a 20 ml fraction taken at the midpoint of the milking
- (4) Strippings samples, a 20 ml sample taken following completion of machine milking
- (5) Composite samples, a representative sample taken throughout the process of machine milking.

This study will be referred to as the " Stage of Milking Trial ".

### STAGE OF MILKING TRIAL

### MATERIALS AND METHODS

Five cows from within herd C were selected on the basis of the results obtained during the course of the main trial. The bacteriological status of each quarter at the time of sampling is given in table 4.1. All tables are located at the end of the chapter. Consequently, nine quarters were uninfected, six quarters were infected with a major pathogen and five quarters were infected with a minor pathogen at the time of sampling.

Samples were taken at one afternoon milking during late February, at which stage the cows had been lactating for approximately six months. Quarter samples were taken at four stages during the milking process:

Strict foremilk samples were taken using aseptic methods ( see chapter three ), but prior to discarding the first squirts of milk. This consisted of approximately 10 to 15 ml of milk, but followed washing and drying of the udder and consequently milk letdown is likely to have taken place.

Foremilk samples were collected immediately following the strict foremilk. These samples corresponded to the foremilk samples which were taken during the main trial.

Midmilking samples were taken by interrupting the milking and removing the teatcups at the midpoint in milk yield, this being determined from the milk yield at the previous afternoon milking.

Strippings samples were taken by hand following cessation of machine milking. The endpoint of milking was determined by visual inspection of the milk flow rate in the sight glass.

Composite milk samples were also taken at the same milking using milk meters ( see chapter three ).

Bacteriological analyses were carried out on both strict foremilk and on foremilk samples. The somatic cell count, the sodium concentration, the potassium concentration, the electrical conductivity, the pH, the lactose concentration, the NAGase activity and the antitrypsin concentration were all measured in each sample from each quarter. In 3 of the 20 quarters the volume of strict foremilk was insufficient for the measurement of lactose concentration. Sufficient milk was obtained for the analyses of all other parameters.

Mean values for the overall results were obtained using the MEANS procedure ( Statistical Analytical Systems, 1985 ).

For the purposes of the analysis, quarters were regarded as being either uninfected or infected with a pathogen, either major or minor, there being an insufficient number of samples to support analyses of major and minor pathogens separately. Mean values were determined for each parameter within the two infection statuses, uninfected or infected.

The General Linear Models ( GLM ) procedure ( Statistical Analytical Systems, 1985 ) was used to determine the effects of milk fraction on the level of each parameter. Included in the model were the main effects of milk fraction, and of infection status, plus the two way interaction between the two effects. Since the distribution of the raw data was markedly skewed, all statistical analyses were carried out on data transformed to log 10 values. Where effects of milk fraction were shown to be significant, differences between individual fractions were determined using the CONTRAST option of the GLM procedure ( Statistical Analytical Systems, 1985 ) as the multiple comparison technique.

The mean values of the parameters, along with the standard error of each mean and the number of observations contributing to the calculations are given in tables 4.2 to 4.9. The results of the analyses of variance are given in table 4.10.

## RESULTS AND DISCUSSION

### (1) SOMATIC CELL COUNT

Table 4.2 gives the mean values for the somatic cell count of milk fractions within the total data set, and also by infection status.

The infected quarters showed significantly higher somatic cell counts (  $p < 0.01$  ) than did the uninfected quarters ( table 4.10 ).

The effect of milk fraction on the somatic cell count was also highly significant (  $p < 0.01$  ). Statistical analysis showed no significant differences between strict foremilk, foremilk or midmilking samples at the 5 % level. However, strippings samples showed significantly higher somatic cell counts than those of the other fractions (  $p < 0.05$  or less ).

A number of studies have shown variations in the somatic cell count between various milk fractions. All showed increased cell counts in strippings compared with other samples ( Paape and Tucker, 1966; Fernando and Spahr, 1983; Obara, 1985; Berning *et al.*, 1987b; ). The greatest increase in cell count occurs towards the end of the milking process ( Obara, 1985 ). These studies agree well with the results of the present stage of milking trial.

The reason for the increase in cell count may be that sloughed epithelial cells and leukocytes are retained in the alveoli as the intramammary pressure increases with the accumulation of milk. With the fall in pressure as the milk is removed, the cells are released into the remaining milk ( Schalm and Lasmanis, 1968 ). Furthermore, it has been suggested that the removal of the accumulated cells from the alveoli might lead to an increase in the exudation of leukocytes into the newly formed milk. This would explain the observation that the newly formed milk drawn 3 to 4 hours after routine milking contains the greatest number of cells of any milk fraction ( Schalm and Lasmanis, 1968 ).

The milk in the teat cistern may show a higher somatic cell count than that of the gland cistern due to the settling of the cells and the unequal dilution by newly formed milk ( Schalm and Ziv-Silberman, 1968 ). On the occasions at which foremilk samples give stronger reactions, it is conjectured that the inflammatory reaction is in the teat wall and the lining of the teat sinus, and that the exuding leukocytes enter the milk at this region. The comparison of the somatic cell counts in the various milk fractions might therefore give an indication of the site of inflammation. Where all fractions show a high cell count, it may be supposed that the inflammation is widespread ( Schalm and Ziv-Silberman, 1968 ).

More recently, the changes in somatic cell count during the milking process within infected and within uninfected quarters have been compared. Marschke *et al.* ( 1987 ) reported that in healthy quarters, the somatic cell count increased slightly during the first half of milking, but more rapidly during the second half, the greatest effect being seen in the final 20 %. Overall there was a fourfold increase in the somatic cell count during the milking process. Within the infected quarters, the cell count also increased, but the increase was more apparent during the first half of the milking.

In the present stage of milking trial, a similar situation occurred, with the difference in cell counts between foremilk and midmilk being greater for infected quarters than for uninfected quarters. The somatic cell count of uninfected quarters more than doubled between midmilking and strippings samples whereas the infected samples showed only a 25 % increase.

If the changes which occur in the somatic cell count, or of any other parameter, during the milking process differ between uninfected and infected quarters, then this may have implications for which type of sample to use for the diagnosis of subclinical mastitis, since the discrimination between healthy and infected quarters depends upon the degree of deflection of the test concerned. Fernando *et al.* ( 1985 ) reported higher log (10) somatic cell counts in strippings than in foremilk, but found little difference in the accuracy of mastitis detection using the two types of sample, false positives being 25 % and 27 %, false negatives 59.2 % and 59.7 % for the foremilk and strippings samples respectively. The results for foremilk samples obtained by Fernando *et al.* ( 1985 ) are less accurate than those obtained earlier by Fernando *et al.* ( 1982 ) who reported 8.2 % false positives and 18.9 % false negatives using foremilk samples for diagnosis.

Other workers have shown samples taken after cessation of machine milking to be superior to foremilk samples for the diagnosis of mastitis. Schalm and Ziv-Silberman ( 1968 ) used the California Mastitis Test to estimate the somatic cell count. These authors found that the foremilk samples detected 33.1 % of mastitic quarters, while strippings samples and residual milk samples detected 42.2 % and 60 % of such quarters respectively. In the same year, Schalm and Lasmanis ( 1968 ) found that mammary quarters with well advanced mastitis are positive to the California Mastitis Test on all milk fractions, but that milder cases of the disease, and early mastitis is detected to a much greater extent with strippings than with foremilk.

The published evidence points to a slightly better accuracy for detecting infected quarters when strippings samples are used than is the case with foremilk samples. It was not possible to test the accuracy of detection during the present stage of milking trial, due to the low number of quarters which were included. However, changes in the composition of the milk during the milking process may affect the accuracy of the composite milk sample for the detection of mastitis.

Prolonged milking tends to increase the somatic cell count of healthy cows and therefore give misleading results ( Marschke et al, 1987 ). Under the current conditions of mastitis diagnosis in New Zealand, the stage at which the cups are removed is left to the discretion of the milker and this may be rather haphazard. It would appear that some method of standardization of cup removal could be beneficial. One possibility would be the use of automatic cup removers, which operate on milk flow rate.

Results of the present stage of milking trial confirm those of previous workers in that the somatic cell count does change during milking , and that these changes are not always parallel for infected and for uninfected quarters. The failure of the interaction effect between bacteriological status and milk fraction on the somatic cell count of milk to reach statistical significance ( table 4.10 ) may have been due to the small number of samples which were included in the trial.

## (2) SODIUM CONCENTRATION

Table 4.3 gives the mean values for sodium concentration of milk fractions within the total data set, and also by infection status.

The infected quarters showed a significantly higher mean sodium concentration (  $p < 0.01$  ) than did the uninfected quarters ( table 4.10 ).

The effect of milk fraction on sodium concentration was also highly significant (  $p < 0.01$  ). Statistical analysis showed no differences between strict foremilk, foremilk or midmilking samples at the 5 % level. However, the sodium concentrations of strippings samples were higher than were those of any of the other samples (  $p < 0.01$  or less ).

Other workers have shown the sodium concentration to change during the milking process. Fernando and Spahr ( 1983 ) reported the effect of milk fraction on milk sodium concentration to be highly significant (  $p < 0.01$  ). The highest concentration of sodium was found in the strippings samples, lower levels were found in primary milk, taken after the foremilk and representing milk from the remainder of the milking process, and lower levels still in foremilk samples. The higher sodium concentration in strippings than in foremilk samples has been confirmed, but the sodium content of bucket milk, or composite milk, is similar to that of the foremilk ( Fernando et al, 1985 ). The results of the present stage of milking trial confirm those of Fernando and Spahr ( 1983 ), and of Fernando et al ( 1985 ) in relation to the overall changes in sodium concentration during the milking process.

The concentration of sodium ions in the milk is controlled by the energy dependent sodium/potassium pump, situated on the basolateral border of the secretory cell, with

sodium being pumped out of the cell into the extracellular fluid, and potassium entering the cell from the extracellular fluid ( Linzell and Peaker, 1975 ). The passage of ions through the apical membrane into the milk is governed by the electrochemical gradient across the membrane. Leakage between adjacent secretory cells is usually prevented in the healthy gland by the formation of tight junctions.

The high concentration of the sodium in strippings milk may be due to the effects of the hormone oxytocin, which causes milk ejection from the alveolar spaces. The injection of physiological amounts of oxytocin into a goat causes leakage of the extracellular fluid into the milk ( Linzell and Peaker, 1971 ). This communication between the milk and extracellular fluid allows the sodium ions to pass down their concentration gradient from extracellular fluid to the milk.

The change in sodium concentration during the milking process is greater within infected than within uninfected quarters ( Fernando and Spahr, 1983 ). This finding has been confirmed by Fernando *et al* ( 1985 ), and similar effects were seen during the present stage of milking trial. It is likely that the above changes are due to the effects of oxytocin exacerbating the already damaged secretory epithelium, causing further leakage of extracellular fluid and therefore of sodium ions into the milk of infected quarters.

The greater differences between uninfected and infected quarters in strippings than in foremilk has implications for the diagnosis of mastitis. Thus Fernando *et al* ( 1985 ) reported 19.6 % and 10.4 % false positives, and 41.9 % and 35.2 % false negatives using foremilk and strippings samples respectively for diagnosing subclinical mastitis.

In the present stage of milking trial, both healthy and infected quarters exhibited larger increases in sodium concentration between midmilking and strippings samples, than between foremilk and midmilking samples. Nevertheless, the increase observed with the infected samples was proportionately greater, therefore it may be predicted that had strippings samples been used in the main trial, instead of foremilk samples, then the accuracy of mastitis detection would have been enhanced, and also, that overmilking would tend to increase the accuracy of sodium concentration as a diagnostic test for mastitis using composite milk samples.

### (3) POTASSIUM CONCENTRATION

Table 4.4 gives the mean values for the potassium concentration of milk fractions within the total data set, and also by infection status.

The infected quarters showed slightly lower potassium concentrations for all milk fractions, although the effect of infection status on milk potassium did not reach significance at the 5 % level ( table 4.10 ).

The effect of milk fraction did exert a significant effect on the potassium concentration (  $p < 0.01$  ) while the interaction between infection status and milk fraction was not significant. Statistical analysis showed that as was the case with both the somatic cell count, and with the sodium concentration, there were no significant differences between the strict foremilk, foremilk or midmilking samples with respect to potassium concentration. Strippings samples showed significantly lower potassium levels than did any other milk fraction (  $p < 0.05$  or less ).

Other workers have recorded similar findings. Fernando and Spahr ( 1983 ) reported a lower potassium concentration in strippings than in foremilk. Bucket milk samples tended to be only slightly lower than foremilk samples in this respect.

The potassium concentration of milk is normally controlled by a sodium potassium pump ( see discussion for sodium concentration above ). The potassium to sodium ratio is usually held at approximately 3:1 in the milk, whereas that of the extracellular fluid is approximately 1:3.

The fall in potassium concentration which is evident in the strippings milk is likely to be due to the effects of oxytocin causing milk ejection, at which time gaps may appear between secretory cells, allowing the potassium to leak down the concentration gradient, out of the milk and into the extracellular fluid.

As is the case for the sodium concentration, the difference between the potassium concentration in the foremilk and the strippings samples is greater for infected than for healthy quarters ( Fernando and Spahr, 1983; Fernando et al., 1985 ). The accuracy of mastitis diagnosis is greater when the potassium concentration of strippings milk is used, than is the case when that of the foremilk is used ( Fernando et al., 1985 ).

In the present study, the difference in potassium concentration between foremilk and strippings samples was similar for healthy and infected quarters. This would suggest that had the strippings samples been used rather than the foremilk samples during the main trial, then similar results would have been obtained in terms of accuracy of diagnosis of subclinical mastitis. Furthermore, the changes between foremilk and midmilk, and between midmilk and strippings were similar for infected and for healthy

quarters. Therefore, overmilking would have little effect on mastitis detection if the potassium concentration of composite milk samples were to be used for diagnosis.

#### (4) ELECTRICAL CONDUCTIVITY

Table 4.5 gives the mean values for electrical conductivity of milk fractions both for the total data set, and by infection status.

The infection status exerted a highly significant effect on electrical conductivity (  $p < 0.01$  ), ( table 4.10 ). Infected quarters showed higher conductivity values than did uninfected quarters.

The effect of milk fraction on electrical conductivity did not reach statistical significance at the 5 % level. In the overall data set, the electrical conductivity values for the various milk fractions were remarkably similar.

The interaction between infection status and milk fraction did reach significance (  $p < 0.05$  ). In quarters of cows in both infection statuses the strict foremilk and foremilk samples showed similar conductivity readings. However, while the electrical conductivity of the strippings milk was lower than that of the earlier milk samples for healthy quarters, the reverse was true for infected quarters, which exhibited greater electrical conductivity than did samples taken before or during the milking process.

The electrical conductivity of milk from healthy quarters decreases slightly following milk ejection, and decreases further during the milking process, the overall decrease being 20 % ( Woolford and Williamson, 1982 ). However the conductivity of infected samples does not show this decline during milking, but often remains similar to, or increases relative to premilking values.

In the present stage of milking trial, foremilk samples were taken after milk ejection had occurred, but a decrease in the electrical conductivity of approximately 7 % occurred between the foremilk and strippings samples in uninfected quarters, while the increase in the infected quarters was of the same order.

Strippings samples have higher conductivity readings than either primary or foremilk samples ( Fernando and Spahr, 1983 ).

Uninfected quarters show little difference in conductivity between milk fractions, while a large increase in conductivity occurs between foremilk and strippings samples from infected quarters when the milking interval is six hours or longer ( Fernando and Spahr, 1983 ). Thus the higher electrical conductivity values in strippings milk are due

almost entirely to the effect of infected quarters, which in the case of Fernando and Spahr ( 1983 ) were not balanced by a decrease in conductivity of uninfected quarters.

The electrical conductivity of healthy quarters is lower for strippings than for foremilk samples, while in quarters which are infected by either a secondary pathogen or a primary pathogen, the strippings show higher values than are found in the foremilk ( Fernando *et al*, 1985 ). Yamamoto *et al* ( 1985 ) collected foremilk, strippings and eight fractions during machine milking. In 71 % of quarters, there was little change in the electrical conductivity during the milking process, although there was a small decrease seen in the strippings. A further 7.5 % of quarters showed a gradual increase in conductivity towards the end of milking, while in 5.4 % of quarters, this increase was rapid. In 6.5 % of quarters there was an increase during the final stage of milking. A further 6.5 % showed a gradual decrease towards the end of the milking process, while the remaining 3.1 % showed an initial decrease, followed by an increase during the final stage. A wider variation in conductivity during milking was observed in quarters which had high cell count and were likely to be infected, than in those with lower cell counts.

The electrical conductivity of milk is determined largely by the three ions: sodium, potassium and chloride, while calcium, magnesium and phosphate play a minor role. The changes in the concentration of sodium and of potassium during the milking process have been dealt with earlier in the present chapter. Analysis for chloride ions was not undertaken during the present trial.

The effect of milk fraction on the concentration of chloride ions is reported to be highly significant (  $p < 0.01$  ), with the highest concentration of chloride ions being found in the strippings samples and the lowest concentration in the foremilk, whilst primary milk shows intermediate values ( Fernando and Spahr, 1983 ).

The chloride ion concentration increases in the strippings in both healthy and in infected quarters, but the increase in the infected quarters is much the larger ( Fernando *et al*, 1985 ). Therefore in this respect, chloride ions behave in a similar manner to sodium ions. Thus using the figures of Fernando *et al* ( 1985 ) sodium concentration increases by 71 %, chloride by 31 % and potassium decreases by 18 %, between foremilk and strippings samples in infected quarters, while for healthy quarters the figures are 26 %, 9 % and 11 % respectively.

The mean concentrations of sodium, chloride and potassium have been recorded as 43.0, 15.0, and 24.0 mmols per litre respectively in bovine milk ( Peaker 1977 ). Therefore, even allowing for the greater concentration of potassium than of sodium plus chloride ions, the electrical conductivity could be expected to be greater in the strippings than in foremilk samples for both healthy and infected quarters. That this does not hold true for healthy quarters may be attributed to the fat concentration of the

milk. When fat is added to a milk sample from which the fat has been removed previously, there is a decrease in the electrical conductivity of the milk, although the ionic content of the milk remains the same ( Prentice, 1962 ). The inhibitory effect of fat on the electrical conductivity of milk has been confirmed by Fernando et al ( 1981 ).

The higher concentration of fat in milk taken towards the end of the milking process is well established, and during the present stage of milking trial, the mean concentration of fat in strippings samples was 5.63 % for infected quarters and 10.74 % for uninfected quarters. The corresponding mean concentrations of fat in foremilk samples were 4.15 % and 3.70 % for infected and for uninfected quarters respectively. Thus the increase in ionic content of the milk from healthy cows may be compensated by the increasing fat content, with the result that the conductivity may remain at similar levels, or as in the case in some trials, actually decrease.

Within the present stage of milking trial, the increase in fat % during milking was smaller for infected than for uninfected quarters. For infected quarters, the ratio of fat % strippings / fat % foremilk was 1.36, while the corresponding figure for uninfected quarters was 2.75. Obviously this smaller increase in fat concentration will have a less inhibitory effect on the increased ionic content of infected quarters.

The fact that the difference in the electrical conductivity between samples from quarters which were infected and those which were uninfected, were greater within the strippings than within the foremilk, suggests that the use of the strippings might give more reliable results with regard to the diagnosis of mastitis than would the use of foremilk samples. As noted earlier, the small size of the stage of milking trial precluded an assessment of the accuracy of diagnosis.

Other workers have shown the electrical conductivity of strippings to be superior to that of the foremilk for discriminating between infected and uninfected quarters. The proportion of quarters which are misclassified as being infected or uninfected is lower for strippings samples than for foremilk samples ( Fernando et al, 1982 ). Similar findings were reported by Fernando et al ( 1985 ), although it was noted that a greater diagnostic accuracy might have been achieved in respect of foremilk samples, had they been taken prior to, rather than after, milk ejection.

During the main experiment, as distinct from the stage of milking trial, the electrical conductivity of the composite sample from each cow tended to be lower than the readings from the individual quarters which contributed to that composite sample. This finding is in keeping with the changes in electrical conductivity which occur during the milking process. The individual quarter samples were taken before the milking process, while the composite samples were representative of milk from the entire milking process.

Since the majority of quarters in the main experiment were not infected ( see chapter five for details ), it may be predicted from the results of the stage of milking trial that the electrical conductivity of the composite milk samples should be lower than that of the corresponding quarter foremilk samples.

The contribution of milk from the latter stages of the milking to the electrical conductivity of the composite milk sample might be expected to improve the diagnostic ability of the composite sample relative to that of the foremilk. The results of the accuracy of mastitis detection using quarter foremilk samples, and using composite samples are given in chapters seven and nine respectively.

### (5) pH READING

Table 4.6 gives the mean pH of the various milk fractions both within the total data set, and by infection status.

The infection status of the quarter exerted a highly significant effect (  $p < 0.01$  ) on the pH ( table 4.10 ). The pH readings of healthy quarters were lower than are those of the infected quarters.

The effect of milk fraction was also highly significant (  $p < 0.01$  ). Statistical analysis revealed that strippings samples showed higher pH readings than did strict foremilk or foremilk samples.

The interaction between infection status and milk fraction was not significant at the 5 % level.

Little information has been published regarding changes in the milk pH during the milking process. Shazly et al ( 1974 ) found no significant effects of milk fraction on the pH in quarter samples from six cows in an Egyptian study.

The pH of milk is largely controlled by the free acidic groups of casein, citrate and phosphate as well as the carbon dioxide, carbonic acid, and bicarbonate ion system ( Schalm et al, 1971 ). It is thought that while the un-ionized forms, ie carbonic acid and carbon dioxide, can diffuse freely across membranes, the bicarbonate ions may be exchanged for chloride, with bicarbonate being pumped out of the cell both at the apical and the basal membranes ( Peaker, 1978 ). This system maintains a ratio of bicarbonate in extracellular fluid to bicarbonate in milk of approximately 8:1.

It was seen earlier that the injection of oxytocin causes increases in the concentration of sodium ions in the milk by allowing free communication between extracellular fluid

and milk compartments via the paracellular pathway. The intravenous injection of oxytocin causes an increase in the pH of milk ( Mielke, 1968 ), suggesting that a similar situation may occur with the bicarbonate ions, with an influx of these ions from the extracellular fluid. At the same time, other ions such as citrate and phosphate would tend to flow in the opposite direction. These changes would explain the increase in pH readings which occurred during the milking process in the present stage of milking trial.

As with other parameters, the increase in pH between foremilk and strippings was greater for infected than for uninfected quarters. Again, this suggests that the accuracy of mastitis diagnosis should be greater using strippings samples. Little recent information is available on the relative accuracies of using pH in foremilk against that in strippings for mastitis diagnosis.

The results achieved using foremilk samples have generally not been encouraging. Marschke and Kitchen ( 1985 ) used a bromothymol blue test to measure the pH of foremilk samples, but found this test to be relatively inaccurate. These results were similar to those of the earlier study of Fay *et al* ( 1938 ) who concluded that the pH, again measured indirectly with the bromothymol blue indicator, rarely gives a false reading on cows which are free of infection, ie few false positive results occur, but that the percentage of false negative results is very high.

Only foremilk samples were taken during the main phase of the present trial, therefore the accuracy of various milk fractions in the diagnosis of udder infection cannot be assessed.

#### (6) LACTOSE

Table 4.7 gives the mean lactose concentrations of the various milk fractions overall, and by infection status.

The effect of the infection status on the lactose concentration was highly significant (  $p < 0.01$  ), ( table 4.10 ), with infected quarters showing a lower mean lactose concentration than did uninfected quarters for all milk fractions.

The effect of milk fraction was also highly significant for this parameter ( table 4.10 ). Statistical analysis showed that the first three milk fractions, ie the strict foremilk, foremilk and midmilk, did not differ significantly from each other at the 5 % level, while the strippings milk showed significantly lower lactose levels than did any other milk fraction (  $p < 0.01$  ).

The interaction between infection status and milk fraction just failed to reach significance at the 5 % level, with the decrease in lactose concentration during milking being greater for uninfected than for infected quarters. The reason for this trend is unclear.

Other workers have reported changes in the concentration of lactose during the milking process. Fernando and Spahr ( 1983 ) found that milk fraction has a highly significant effect on the lactose content of milk, the lowest concentration of lactose being found in the strippings, higher levels in the primary milk, and highest levels in the foremilk samples. At a nine hour milking interval, there was a greater difference between the lactose concentration in foremilk as compared to strippings in infected quarters than in healthy quarters. Similar results have been reported by Fernando et al ( 1985 ), and by Ryniewicz and Wojcik, ( 1985 ).

The decrease in lactose tends to mirror the increase which is seen in the electrical conductivity, with quarters varying in the degree to which the lactose concentration decreases ( Yamamoto et al, 1985 ). The results of the present stage of milking trial do not agree with reports which appear in the literature and have been cited above. During the present trial, the difference between lactose in foremilk and strippings was actually greater for uninfected quarters than for infected quarters, although the interaction between infection status and milk fraction was not statistically significant at the 5 % level.

The lactose is formed in the Golgi apparatus of the secretory cell and secreted into the alveolar lumen by the process of exocytosis, thereby increasing the osmotic pressure, which in turn causes water to flow across the apical membrane to preserve the osmolarity ( Peaker, 1978 ). Thus the amount of lactose produced controls the final volume of the milk.

The lower concentration of lactose in the strippings milk may once again be due to the effects of oxytocin. Operation of the paracellular pathway would allow the lactose to flow down the high concentration gradient between milk and extracellular fluid.

In light of the discussion on other parameters, it might be expected that the concentration of lactose would be proportionately lower in the strippings samples of infected quarters compared with uninfected quarters. This occurred in several of the studies cited above, the operation of the paracellular pathway apparently exacerbating the damage caused by bacterial toxins.

Why this effect was not seen in the present stage of milking trial is not clear. The lactose concentration was markedly lower in milk from infected quarters than in milk from uninfected quarters at all stages of the milking procedure, but the absolute decrease in lactose concentration during the milking procedure was greater for

uninfected quarters than for infected quarters. It is possible that bacterial fermentation of the lactose in the milk of the gland cistern occurs to a greater extent in infected quarters, due to the presence of large numbers of lactose fermenting bacteria associated with the lesions. The newly formed milk which is contained in the alveoli, many of which are presumably not infected, could be expected to have a higher lactose concentration relative to the milk which has undergone fermentation. This would tend to balance the expected decrease in lactose concentration due to the operation of the paracellular pathway.

If this departure from the expected situation holds true for the present main trial, then the diagnosis of subclinical mastitis using the lactose concentration of composite milk would be rather less accurate than would be that using quarter foremilk samples.

#### (7) NAGase ACTIVITY

Table 4.8 gives the mean values for the NAGase activities of milk fractions within the total data set, and by infection status.

The effect of infection status was highly significant (  $p < 0.01$  ) on the NAGase activity ( table 4.10 ), the activity of this enzyme being greater in infected than in uninfected quarters.

The milk fraction also exerted a significant effect on NAGase activity (  $p < 0.05$  ). As was the case with many of the other parameters, the strict foremilk, foremilk and midmilk samples did not differ significantly from each other, but all three fractions showed significantly lower NAGase activities than did the strippings samples (  $p < 0.05$  or less ).

The interaction between infection status and milk fraction was not significant at the 5 % level.

A number of authors have reported changes in the milk NAGase activity during the milking process. Differing patterns of change occur during milking in individual quarters, most quarters showing only a small increase in activity in the strippings milk. However, other quarters show large increases during the milking process ( Yamamoto *et al*, 1985 ).

While the NAGase activity increases throughout the milking process, regardless of the state of health of the udder, the increases are greater within infected than within uninfected quarters ( Marschke *et al*, 1987 ).

Berning *et al* ( 1987b ) found that the NAGase activity differed significantly (  $p < 0.05$  ) between strict foremilk, composite milk, strippings, and residual milk, the latter being obtained by injection of oxytocin. The activity was higher in the strict foremilk than in composite milk, but was higher still in strippings samples. However, only uninfected cows were studied by these authors.

Although the interaction between the infection status and the milk fraction did not reach significance during the present stage of milking trial, the respective increases in the NAGase activity between the foremilk and the strippings were 22 % for uninfected quarters and 62 % for infected quarters. To understand the changes in NAGase activity during the milking process, the source of NAGase in the milk must be determined. Although NAGase is used as a marker of damage to the udder tissue ( Kitchen *et al*, 1980 ), Capuco *et al* ( 1986 ) reported that the polymorphonuclear neutrophils ( PMNs ) which migrate from the blood, may be an important source of this enzyme. If the PMNs were intact, viable and were not actively phagocytosing, then the contribution to the total NAGase activity was approximately 12 %, while PMNs which were undergoing lysis contributed 22 % of the total activity. A large increase in the contribution of PMNs to the NAGase activity occurred when the cells were actively phagocytosing. Thus the explanation for changes in NAGase activity during the milking process must take into account the effects of tissue damage, as well as the number, viability and activity of the blood derived PMNs.

The higher NAGase activity of the strict foremilk than of later fractions which was reported by Berning *et al* ( 1987b ), may have been due to the settling of the somatic cells into the teat cistern during the milking interval. These cells would have been in the milk for a period of some hours, and are likely to be senescent and therefore undergoing lysis. Thus the increase in NAGase activity may be due to the higher number of cells, as well as to their age.

The somatic cell count of the strippings milk has been shown to be higher than that of foremilk or midmilk fractions ( see earlier this chapter ). A reason for this difference might be the decrease in intramammary pressure as the milk is removed, enabling a greater number of PMNs to migrate through the walls of the alveoli and into the milk. Also, these incoming cells are likely to be more active in terms of phagocytosis and would release more NAGase into the milk, although they would be less likely to be undergoing lysis.

The reasons for the higher NAGase activity in infected rather than in healthy quarters are likely to be the direct damage caused by bacterial toxins and metabolites on the secretory tissue of the mammary gland, the greater number of PMNs which have migrated in response to the infection, the greater degree of phagocytosis exhibited by these leukocytes, and the greater degree of senescence or lysis of the PMNs.

The same factors which cause changes to the NAGase activity of uninfected quarters during the milking process also are likely to apply to infected quarters. The reason for the greater difference between NAGase activity in foremilk and strippings milk in infected quarters than in uninfected quarters could be related to the comparatively higher number of migrating PMNs which may enter the gland towards the end of the milking process.

Whether the milk ejection process itself causes a change in the NAGase activity is not clear. Injection of oxytocin intravenously, after machine milking has ceased and strippings milk has been taken, causes a decrease in the NAGase activity of the residual milk, over that of the strippings milk ( Berning *et al*, 1987b ). This suggests that in uninfected cows, the milk obtained by milk ejection dilutes that which is in the udder at the time.

The NAGase activity of bovine serum from uninfected cows is approximately ten times that of the milk ( Nagahata *et al*, 1987 ). In quarters which are infected, the resulting inflammation is likely to cause increased vascular permeability, which in turn causes the leakage of serum proteins into the milk ( see antitrypsin results later this chapter ). Thus in infected quarters, the effect of oxytocin might allow a greater proportion of this plasma derived NAGase to enter the milk compartment from the extracellular fluid via the paracellular pathway. This may help explain the greater difference in NAGase activity between the foremilk and strippings of infected than of uninfected udder quarters.

The greater difference in NAGase activity between strippings and foremilk samples in infected quarters than in healthy quarters, suggests that the strippings milk might be a better indicator of subclinical mastitis than would samples from earlier in the milking process.

In uninfected quarters, increases in the milk NAGase activity have been reported to occur more towards the end of the milking process, whilst within infected quarters, changes are seen earlier in the milking ( Marschke *et al*, 1987 ). The observations are similar to the findings for the somatic cell count and have similar implications for using composite milk samples for the diagnosis of mastitis in that any degree of overmilking is likely to decrease the ability of the NAGase activity to detect infected cows. However, during the present trial, the increase in the NAGase activity was greater during the second half of the milking process than during the first half, both for infected and for uninfected quarters. Thus in this respect the results of the present trial and those of Marschke *et al* ( 1987 ) do not agree.

### (8) ANTITRYPSIN CONCENTRATION

Table 4.9 gives the mean values for the antitrypsin concentration of the various milk fractions for the overall data set, and by infection status.

The effect of infection status on antitrypsin concentration was highly significant (  $p < 0.01$  ), ( table 4.10 ).

Neither the main effect of the milk fraction, nor the interaction of infection status with milk fraction reached significance at the 5 % level.

There is little information available in the literature regarding changes in the concentration of antitrypsin in milk during the milking process.

Antitrypsin is a blood derived protein, which has a similar molecular weight to that of bovine serum albumin ( BSA ), ( Honkanen-Buzalski and Sandholm, 1981 ). The transfer of low molecular weight proteins is thought to occur by diffusion from the blood to the alveolar lumen through the separated tight junctions. During mastitis, BSA and antitrypsin levels increase concurrently in the milk, and a similar transfer method for the two proteins has been proposed ( Honkanen-Buzalski and Sandholm, 1981 ). Thus in the absence of information on antitrypsin, changes in the BSA may serve as a model for the concentration during the milking process.

Studies have shown the concentration of BSA to change during the milking process. The BSA concentration remains constant during the milking process within uninfected quarters, but within infected quarters there is an increase in the BSA concentration as the milking proceeds ( Meyer and Senft, 1979 ). This suggests that in infected quarters, milk ejection allows the leakage of BSA through the tight junctions between cells. In the case of uninfected quarters, the concentration of BSA in the extracellular fluid would probably be lower due to the absence of increased permeability which is associated with inflammation.

In a later study, changes were found in the BSA concentration of milk from both infected and uninfected quarters, the BSA content of milk decreasing slightly during the milking process in uninfected quarters. Within infected quarters, the BSA concentration increased by approximately 27 % during the milking. This variation may have contributed to the difference in accuracy with which the BSA in foremilk and strippings could be used to identify infected quarters, the accuracy being greater for the strippings samples ( Fernando *et al*, 1985 ).

The intravenous injection of 40 IU of oxytocin following milking results in an increase in the BSA content of the milk ( Dill *et al*, 1972 ).

During the present stage of milking trial, the overall effects of milk fraction on the antitrypsin concentration were not significant. The uninfected quarters showed a decrease in the antitrypsin concentration between foremilk and strippings although the reverse was true for infected quarters. Thus the infected quarters showed a similar effect due to milk fraction with respect to antitrypsin concentration, as described in the references cited above for BSA.

While uninfected quarters have been reported to show little change in BSA concentration, in the present stage of milking trial, uninfected quarters showed a decrease in antitrypsin concentration during the milking process. This decrease in antitrypsin may be due to the increase in fat concentration in the strippings samples ( see chapter four, section 4 ). The antitrypsin assay employed in this study required the removal of fat from the assay mixture. This was achieved using the clearing solution ( see chapter three ). It is possible that the clearing procedure was incomplete for the high fat content strippings samples, and that the remaining fat interfered with the reading of the colourimetric reaction.

If on the other hand, the decrease in the antitrypsin concentration was not merely due to interference by milkfat, then the strippings samples might have proved to be the more accurate sample type for diagnosing mastitis, since the absolute difference between the mean antitrypsin concentration for healthy and for infected quarters was greater in strippings than in foremilk samples.

### SUMMARY AND CONCLUSIONS

- (1) In general, the parameters used to indicate the presence of mastitis and whose concentrations are increased by infection of the mammary gland, including the somatic cell count, sodium concentration, the pH, and the NAGase activity also exhibited increases in concentration during the milking process, both within infected and within uninfected quarters.

The opposite was true for those parameters whose concentrations are decreased by infection, ie the potassium concentration or the lactose concentration of the milk. For these parameters, the concentration was lower in the strippings than in the foremilk.

- (2) For a number of parameters these changes suggest that subclinical mastitis could be diagnosed with greatest sensitivity and accuracy if strippings samples were to be used rather than foremilk samples. However, a number of practical difficulties are associated with the use of stripping samples. Firstly there is the problem of

determination of the endpoint of milking, for the concentration of the parameter in question is likely to vary with the amount of milk remaining in the udder after machine milking. Secondly, there is the problem of physically obtaining the sample, as at this stage of the milking process, the cow is often eager to get out of the shed. As a result, the animal tends to kick, making aseptic sampling difficult to carry out.

The International Dairy Federation has recommended the use of foremilk samples for the diagnosis of subclinical mastitis. Clearly, the use of a standard sample is required if the results of surveys and experiments are to be compared.

- (3) The changes in the concentration of a parameter during the milking process partly explain the discrepancy between the results for composite samples, and those of the contributing quarter foremilk samples for a particular cow.
- (4) Since composite samples are representative of the whole milking process, and since milk from the latter stages of the milking process have a greater ability to discriminate between infected and uninfected quarters, the composite sample may prove to be of greater diagnostic accuracy than would otherwise have been the case.

TABLE 4.1STAGE OF MILKING TRIALBACTERIOLOGICAL STATUS OF EACH QUARTER AT TIME OF SAMPLING

| COW | QUARTER OF THE COW           |                  |                  |                                 |
|-----|------------------------------|------------------|------------------|---------------------------------|
|     | LEFT<br>FRONT                | LEFT<br>REAR     | RIGHT<br>FRONT   | RIGHT<br>REAR                   |
| A   | <u>C. bovis</u> <sup>1</sup> | <u>C. bovis</u>  | Negative         | <u>S. aureus</u> <sup>2</sup>   |
| B   | <u>S. aureus</u>             | <u>S. aureus</u> | <u>S. aureus</u> | <u>C. bovis</u>                 |
| C   | <u>C. bovis</u>              | <u>S. aureus</u> | <u>C. bovis</u>  | <u>Str. uberis</u> <sup>3</sup> |
| D   | Negative                     | Negative         | Negative         | Negative                        |
| E   | Negative                     | Negative         | Negative         | Negative                        |

1 C. bovis = Corynebacterium bovis

2 S. aureus = Staphylococcus aureus

3 Str. uberis = Streptococcus uberis

TABLE 4.2MEAN SOMATIC CELL COUNT ( CELLS PER ML,  $\times 10^3$  )STAGE OF MILKING TRIAL

|                     |           | STRICT<br>FOREMILK | FOREMILK | MID<br>MILK | STRIPPINGS | COMPOSITE |
|---------------------|-----------|--------------------|----------|-------------|------------|-----------|
| TOTAL DATA SET      |           |                    |          |             |            |           |
| SCC                 | $\bar{X}$ | 1212               | 1136     | 1401        | 1759       | 1406      |
|                     | SE        | 493                | 598      | 525         | 539        | 690       |
|                     | N         | 20                 | 20       | 20          | 20         | 5         |
| LOGSCC              | $\bar{X}$ | 2.538              | 2.521    | 2.637       | 2.884      | 2.755     |
|                     | SE        | 0.172              | 0.161    | 0.164       | 0.136      | 0.345     |
|                     | N         | 20                 | 20       | 20          | 20         | 5         |
| UNINFECTED QUARTERS |           |                    |          |             |            |           |
| SCC                 | $\bar{X}$ | 128                | 124      | 139         | 314        | 89        |
|                     | SE        | 65                 | 59       | 58          | 107        | 11        |
|                     | N         | 9                  | 9        | 9           | 9          | 2         |
| LOGSCC              | $\bar{X}$ | 1.865              | 1.884    | 1.971       | 2.358      | 1.946     |
|                     | SE        | 0.145              | 0.131    | 0.120       | 0.111      | 0.054     |
|                     | N         | 9                  | 9        | 9           | 9          | 2         |
| INFECTED QUARTERS   |           |                    |          |             |            |           |
| SCC                 | $\bar{X}$ | 2098               | 1963     | 2433        | 2940       | 2285      |
|                     | SE        | 814                | 858      | 844         | 828        | 789       |
|                     | N         | 11                 | 11       | 11          | 11         | 3         |
| LOGSCC              | $\bar{X}$ | 3.088              | 3.042    | 3.181       | 3.315      | 3.294     |
|                     | SE        | 0.145              | 0.135    | 0.135       | 0.121      | 0.177     |
|                     | N         | 11                 | 11       | 11          | 11         | 3         |

$\bar{X}$  = mean      SE = standard error of mean      N = number of observations

**TABLE 4.3**  
**MEAN SODIUM CONCENTRATION ( MILLIMOLES PER LITRE )**  
**STAGE OF MILKING TRIAL**

|                     |           | STRICT<br>FOREMILK | FOREMILK | MID<br>MILK | STRIPPINGS | COMPOSITE |
|---------------------|-----------|--------------------|----------|-------------|------------|-----------|
| TOTAL DATA SET      |           |                    |          |             |            |           |
| SOD                 | $\bar{X}$ | 23.12              | 21.00    | 22.77       | 29.34      | 21.09     |
|                     | SE        | 1.75               | 1.34     | 1.75        | 2.80       | 2.77      |
|                     | N         | 20                 | 20       | 20          | 20         | 5         |
| LOGSOD              | $\bar{X}$ | 1.344              | 1.307    | 1.335       | 1.432      | 1.309     |
|                     | SE        | 0.030              | 0.026    | 0.031       | 0.040      | 0.058     |
|                     | N         | 20                 | 20       | 20          | 20         | 5         |
| UNINFECTED QUARTERS |           |                    |          |             |            |           |
| SOD                 | $\bar{X}$ | 17.62              | 16.13    | 16.24       | 18.70      | 15.05     |
|                     | SE        | 0.94               | 0.35     | 0.33        | 0.89       | 0.20      |
|                     | N         | 9                  | 9        | 9           | 9          | 2         |
| LOGSOD              | $\bar{X}$ | 1.242              | 1.207    | 1.210       | 1.268      | 1.177     |
|                     | SE        | 0.022              | 0.009    | 0.009       | 0.021      | 0.006     |
|                     | N         | 9                  | 9        | 9           | 9          | 2         |
| INFECTED QUARTERS   |           |                    |          |             |            |           |
| SOD                 | $\bar{X}$ | 27.62              | 24.98    | 28.10       | 38.04      | 25.11     |
|                     | SE        | 2.34               | 1.61     | 2.05        | 3.10       | 2.28      |
|                     | N         | 11                 | 11       | 11          | 11         | 3         |
| LOGSOD              | $\bar{X}$ | 1.427              | 1.389    | 1.437       | 1.567      | 1.396     |
|                     | SE        | 0.034              | 0.027    | 0.032       | 0.033      | 0.042     |
|                     | N         | 11                 | 11       | 11          | 11         | 3         |

$\bar{X}$  = mean      SE = standard error of mean      N = number of observations

**TABLE 4.4**  
**MEAN POTASSIUM CONCENTRATION ( MILLIMOLES PER LITRE )**  
**STAGE OF MILKING TRIAL**

|                     |           | STRICT<br>FOREMILK | FOREMILK | MID<br>MILK | STRIPPINGS | COMPOSITE |
|---------------------|-----------|--------------------|----------|-------------|------------|-----------|
| TOTAL DATA SET      |           |                    |          |             |            |           |
| POT                 | $\bar{X}$ | 40.09              | 41.30    | 40.44       | 37.04      | 41.08     |
|                     | SE        | 0.91               | 0.74     | 0.76        | 0.97       | 1.96      |
|                     | N         | 20                 | 20       | 20          | 20         | 5         |
| LOGPOT              | $\bar{X}$ | 1.601              | 1.615    | 1.605       | 1.566      | 1.612     |
|                     | SE        | 0.010              | 0.008    | 0.008       | 0.012      | 0.021     |
|                     | N         | 20                 | 20       | 20          | 20         | 5         |
| UNINFECTED QUARTERS |           |                    |          |             |            |           |
| POT                 | $\bar{X}$ | 40.95              | 41.79    | 41.10       | 37.56      | 40.28     |
|                     | SE        | 1.46               | 1.17     | 1.16        | 1.29       | 4.40      |
|                     | N         | 9                  | 9        | 9           | 9          | 2         |
| LOGPOT              | $\bar{X}$ | 1.611              | 1.620    | 1.612       | 1.573      | 1.602     |
|                     | SE        | 0.019              | 0.012    | 0.012       | 0.015      | 0.048     |
|                     | N         | 9                  | 9        | 9           | 9          | 2         |
| INFECTED QUARTERS   |           |                    |          |             |            |           |
| POT                 | $\bar{X}$ | 39.28              | 40.89    | 39.90       | 36.61      | 41.62     |
|                     | SE        | 0.92               | 0.96     | 1.04        | 1.46       | 2.46      |
|                     | N         | 11                 | 11       | 11          | 11         | 3         |
| LOGPOT              | $\bar{X}$ | 1.593              | 1.610    | 1.599       | 1.560      | 1.618     |
|                     | SE        | 0.011              | 0.010    | 0.011       | 0.019      | 0.026     |
|                     | N         | 11                 | 11       | 11          | 11         | 3         |

$\bar{X}$  = mean      SE = standard error of mean      N = number of observations

**TABLE 4.5**  
**MEAN ELECTRICAL CONDUCTIVITY ( MILLISIEMENS PER CENTIMETER )**  
**STAGE OF MILKING TRIAL**

|                     |           | STRICT<br>FOREMILK | FOREMILK | MID<br>MILK | STRIPPINGS | COMPOSITE |
|---------------------|-----------|--------------------|----------|-------------|------------|-----------|
| TOTAL DATA SET      |           |                    |          |             |            |           |
| CON                 | $\bar{X}$ | 6.425              | 6.420    | 6.493       | 6.505      | 6.330     |
|                     | SE        | 0.163              | 0.147    | 0.160       | 0.263      | 0.299     |
|                     | N         | 20                 | 20       | 20          | 20         | 5         |
| LOGCON              | $\bar{X}$ | 0.805              | 0.805    | 0.810       | 0.806      | 0.799     |
|                     | SE        | 0.011              | 0.010    | 0.011       | 0.019      | 0.021     |
|                     | N         | 20                 | 20       | 20          | 20         | 5         |
| UNINFECTED QUARTERS |           |                    |          |             |            |           |
| CON                 | $\bar{X}$ | 5.856              | 5.872    | 5.850       | 5.456      | 5.650     |
|                     | SE        | 0.177              | 0.161    | 0.151       | 0.273      | 0.300     |
|                     | N         | 9                  | 9        | 9           | 9          | 2         |
| LOGCON              | $\bar{X}$ | 0.766              | 0.768    | 0.766       | 0.733      | 0.751     |
|                     | SE        | 0.013              | 0.012    | 0.011       | 0.022      | 0.023     |
|                     | N         | 9                  | 9        | 9           | 9          | 2         |
| INFECTED QUARTERS   |           |                    |          |             |            |           |
| CON                 | $\bar{X}$ | 6.891              | 6.868    | 7.018       | 7.364      | 6.783     |
|                     | SE        | 0.152              | 0.113    | 0.112       | 0.164      | 0.101     |
|                     | N         | 11                 | 11       | 11          | 11         | 3         |
| LOGCON              | $\bar{X}$ | 0.837              | 0.836    | 0.846       | 0.866      | 0.831     |
|                     | SE        | 0.010              | 0.007    | 0.007       | 0.010      | 0.007     |
|                     | N         | 11                 | 11       | 11          | 11         | 3         |

$\bar{X}$  = mean      SE = standard error of mean      N = number of observations

**TABLE 4.6**  
**MEAN pH READING ( pH UNITS )**  
**STAGE OF MILKING TRIAL**

|                     |           | STRICT<br>FOREMILK | FOREMILK | MID<br>MILK | STRIPPINGS | COMPOSITE |
|---------------------|-----------|--------------------|----------|-------------|------------|-----------|
| TOTAL DATA SET      |           |                    |          |             |            |           |
| pH                  | $\bar{X}$ | 6.761              | 6.684    | 6.710       | 6.795      | 6.738     |
|                     | SE        | 0.016              | 0.012    | 0.015       | 0.021      | 0.026     |
|                     | N         | 20                 | 20       | 20          | 20         | 5         |
| LOGpH               | $\bar{X}$ | 0.830              | 0.825    | 0.827       | 0.832      | 0.829     |
|                     | SE        | 0.0010             | 0.0008   | 0.0010      | 0.0014     | 0.0017    |
|                     | N         | 20                 | 20       | 20          | 20         | 5         |
| UNINFECTED QUARTERS |           |                    |          |             |            |           |
| pH                  | $\bar{X}$ | 6.709              | 6.643    | 6.658       | 6.721      | 6.675     |
|                     | SE        | 0.013              | 0.010    | 0.011       | 0.014      | 0.005     |
|                     | N         | 9                  | 9        | 9           | 9          | 2         |
| LOGpH               | $\bar{X}$ | 0.827              | 0.822    | 0.823       | 0.827      | 0.825     |
|                     | SE        | 0.0009             | 0.0006   | 0.0007      | 0.0009     | 0.0003    |
|                     | N         | 9                  | 9        | 9           | 9          | 2         |
| INFECTED QUARTERS   |           |                    |          |             |            |           |
| pH                  | $\bar{X}$ | 6.804              | 6.717    | 6.753       | 6.855      | 6.780     |
|                     | SE        | 0.0195             | 0.0139   | 0.0174      | 0.0256     | 0.0058    |
|                     | N         | 11                 | 11       | 11          | 11         | 3         |
| LOGpH               | $\bar{X}$ | 0.833              | 0.827    | 0.830       | 0.836      | 0.831     |
|                     | SE        | 0.0012             | 0.0009   | 0.0011      | 0.0016     | 0.0004    |
|                     | N         | 11                 | 11       | 11          | 11         | 3         |

$\bar{X}$  = mean      SE = standard error of mean      N = number of observations

TABLE 4.7  
MEAN LACTOSE CONCENTRATION ( % )  
STAGE OF MILKING TRIAL

|                     |           | STRICT<br>FOREMILK | FOREMILK | MID<br>MILK | STRIPPINGS | COMPOSITE |
|---------------------|-----------|--------------------|----------|-------------|------------|-----------|
| TOTAL DATA SET      |           |                    |          |             |            |           |
| LAC                 | $\bar{X}$ | 4.538              | 4.576    | 4.464       | 4.072      | 4.484     |
|                     | SE        | 0.079              | 0.063    | 0.072       | 0.054      | 0.120     |
|                     | N         | 17                 | 20       | 20          | 20         | 5         |
| LOGLAC              | $\bar{X}$ | 0.656              | 0.660    | 0.649       | 0.609      | 0.651     |
|                     | SE        | 0.008              | 0.006    | 0.007       | 0.006      | 0.012     |
|                     | N         | 17                 | 20       | 20          | 20         | 5         |
| UNINFECTED QUARTERS |           |                    |          |             |            |           |
| LAC                 | $\bar{X}$ | 4.819              | 4.843    | 4.758       | 4.191      | 4.755     |
|                     | SE        | 0.070              | 0.042    | 0.056       | 0.039      | 0.045     |
|                     | N         | 8                  | 9        | 9           | 9          | 2         |
| LOGLAC              | $\bar{X}$ | 0.683              | 0.685    | 0.677       | 0.622      | 0.677     |
|                     | SE        | 0.006              | 0.004    | 0.005       | 0.004      | 0.004     |
|                     | N         | 8                  | 9        | 9           | 9          | 2         |
| INFECTED QUARTERS   |           |                    |          |             |            |           |
| LAC                 | $\bar{X}$ | 4.288              | 4.357    | 4.224       | 3.974      | 4.303     |
|                     | SE        | 0.056              | 0.043    | 0.055       | 0.083      | 0.079     |
|                     | N         | 9                  | 11       | 11          | 11         | 3         |
| LOGLAC              | $\bar{X}$ | 0.632              | 0.639    | 0.625       | 0.598      | 0.634     |
|                     | SE        | 0.006              | 0.004    | 0.006       | 0.009      | 0.008     |
|                     | N         | 9                  | 11       | 11          | 11         | 3         |

$\bar{X}$  = mean      SE = standard error of mean      N = number of observations

**TABLE 4.8****MEAN NAGase ACTIVITY ( NANOMOLES PER ML PER MINUTE )****STAGE OF MILKING TRIAL**

|                     |           | STRICT<br>FOREMILK | FOREMILK | MID<br>MILK | STRIPPINGS | COMPOSITE |
|---------------------|-----------|--------------------|----------|-------------|------------|-----------|
| TOTAL DATA SET      |           |                    |          |             |            |           |
| NAG                 | $\bar{X}$ | 9.179              | 8.369    | 8.966       | 12.667     | 9.048     |
|                     | SE        | 1.233              | 1.156    | 1.051       | 2.406      | 2.136     |
|                     | N         | 20                 | 20       | 20          | 20         | 5         |
| LOGNAG              | $\bar{X}$ | 0.902              | 0.865    | 0.902       | 1.006      | 0.899     |
|                     | SE        | 0.050              | 0.048    | 0.047       | 0.061      | 0.116     |
|                     | N         | 20                 | 20       | 20          | 20         | 5         |
| UNINFECTED QUARTERS |           |                    |          |             |            |           |
| NAG                 | $\bar{X}$ | 5.361              | 4.992    | 5.270       | 6.107      | 4.210     |
|                     | SE        | 0.306              | 0.166    | 0.205       | 0.333      | 0.200     |
|                     | N         | 9                  | 9        | 9           | 9          | 2         |
| LOGNAG              | $\bar{X}$ | 0.724              | 0.697    | 0.720       | 0.781      | 0.624     |
|                     | SE        | 0.023              | 0.013    | 0.015       | 0.022      | 0.021     |
|                     | N         | 9                  | 9        | 9           | 9          | 2         |
| INFECTED QUARTERS   |           |                    |          |             |            |           |
| NAG                 | $\bar{X}$ | 12.303             | 11.132   | 11.990      | 18.035     | 12.273    |
|                     | SE        | 1.739              | 1.703    | 1.324       | 3.678      | 1.479     |
|                     | N         | 11                 | 11       | 11          | 11         | 3         |
| LOGNAG              | $\bar{X}$ | 1.048              | 1.002    | 1.051       | 1.190      | 1.083     |
|                     | SE        | 0.060              | 0.061    | 0.050       | 0.072      | 0.053     |
|                     | N         | 11                 | 11       | 11          | 11         | 3         |

 $\bar{X}$  = mean

SE = standard error of mean

N = number of observations

TABLE 4.9MEAN ANTITRYPSIN CONCENTRATION ( RELATIVE UNITS )STAGE OF MILKING TRIAL

|                     |           | STRICT<br>FOREMILK | FOREMILK | MID<br>MILK | STRIPPINGS | COMPOSITE |
|---------------------|-----------|--------------------|----------|-------------|------------|-----------|
| TOTAL DATA SET      |           |                    |          |             |            |           |
| ANT                 | $\bar{X}$ | 0.8587             | 0.8593   | 0.7967      | 0.8693     | 0.8400    |
|                     | SE        | 0.0566             | 0.0549   | 0.0414      | 0.0877     | 0.0678    |
|                     | N         | 20                 | 20       | 20          | 20         | 5         |
| LOGANT              | $\bar{X}$ | -0.0799            | -0.0786  | -0.1093     | -0.0915    | -0.0811   |
|                     | SE        | 0.0238             | 0.0227   | 0.0219      | 0.0352     | 0.0338    |
|                     | N         | 20                 | 20       | 20          | 20         | 5         |
| UNINFECTED QUARTERS |           |                    |          |             |            |           |
| ANT                 | $\bar{X}$ | 0.7591             | 0.7747   | 0.6969      | 0.6273     | 0.7240    |
|                     | SE        | 0.0342             | 0.0255   | 0.0295      | 0.0316     | 0.0540    |
|                     | N         | 9                  | 9        | 9           | 9          | 2         |
| LOGANT              | $\bar{X}$ | -0.1233            | -0.1129  | -0.1602     | -0.2070    | -0.1415   |
|                     | SE        | 0.0198             | 0.0149   | 0.0193      | 0.0316     | 0.0325    |
|                     | N         | 9                  | 9        | 9           | 9          | 2         |
| INFECTED QUARTERS   |           |                    |          |             |            |           |
| ANT                 | $\bar{X}$ | 0.9402             | 0.9285   | 0.8784      | 1.0673     | 0.9173    |
|                     | SE        | 0.0939             | 0.0944   | 0.0621      | 0.1313     | 0.0829    |
|                     | N         | 11                 | 11       | 11          | 11         | 3         |
| LOGANT              | $\bar{X}$ | -0.0445            | -0.0505  | -0.0678     | 0.0030     | -0.0409   |
|                     | SE        | 0.0376             | 0.0383   | 0.0320      | 0.0444     | 0.0379    |
|                     | N         | 11                 | 11       | 11          | 11         | 3         |

$\bar{X}$  = mean      SE = standard error of mean      N = number of observations

TABLE 4.10RESULTS OF ANALYSIS OF VARIANCE - STAGE OF MILKING TRIAL

| PARAMETER | SOURCE            | DF | F      | Pr > F   |    |
|-----------|-------------------|----|--------|----------|----|
| LOGSCC    | STATUS            | 1  | 67.84  | p < 0.01 | ** |
|           | FRACTION          | 3  | 16.26  | p < 0.01 | ** |
|           | STATUS x FRACTION | 3  | 1.00   | p < 0.40 | NS |
| LOGSOD    | STATUS            | 1  | 139.46 | p < 0.01 | ** |
|           | FRACTION          | 3  | 7.46   | p < 0.01 | ** |
|           | STATUS x FRACTION | 3  | 2.06   | p < 0.12 | NS |
| LOGPOT    | STATUS            | 1  | 1.77   | p < 0.19 | NS |
|           | FRACTION          | 3  | 4.66   | p < 0.01 | ** |
|           | STATUS x FRACTION | 3  | 0.03   | p < 0.99 | NS |
| LOGCON    | STATUS            | 1  | 114.31 | p < 0.01 | ** |
|           | FRACTION          | 3  | 0.01   | p < 0.96 | NS |
|           | STATUS x FRACTION | 3  | 3.42   | p < 0.03 | *  |
| LOGpH     | STATUS            | 1  | 67.84  | p < 0.01 | ** |
|           | FRACTION          | 3  | 16.26  | p < 0.01 | ** |
|           | STATUS x FRACTION | 3  | 1.00   | p < 0.40 | NS |
| LOGLAC    | STATUS            | 1  | 102.81 | p < 0.01 | ** |
|           | FRACTION          | 3  | 32.03  | p < 0.01 | ** |
|           | STATUS x FRACTION | 3  | 2.40   | p < 0.08 | NS |
| LOGNAG    | STATUS            | 1  | 95.99  | p < 0.01 | ** |
|           | FRACTION          | 3  | 2.80   | p < 0.05 | *  |
|           | STATUS x FRACTION | 3  | 0.42   | p < 0.74 | NS |
| LOGANT    | STATUS            | 1  | 23.27  | p < 0.01 | ** |
|           | FRACTION          | 3  | 0.45   | p < 0.73 | NS |
|           | STATUS x FRACTION | 3  | 2.14   | p < 0.11 | NS |

NS = not significant

\* = significant

\*\* = highly significant

DF = degrees of freedom

F = F statistic

## CHAPTER FIVE

### A DESCRIPTION OF THE BACTERIOLOGICAL STATUS OF QUARTERS AND OF COWS DURING THE TRIAL PERIOD

#### INTRODUCTION

The bacteriological species which are commonly associated with infection of the bovine mammary gland include:

- (1)        Staphylococcus aureus
- (2)        Staphylococcus epidermidis
- (3)        Streptococcus agalactiae
- (4)        Streptococcus dysgalactiae
- (5)        Streptococcus uberis
- (6)        Coliform bacteria including Escherichia coli and Klebsiella sp.

Over 90 % of cases of intramammary infection are caused by Staphylococcus aureus, Streptococcus agalactiae, Streptococcus uberis and Streptococcus dysgalactiae ( Dodd, 1971 ).

Less common pathogens include:

- |     |                                 |                                           |
|-----|---------------------------------|-------------------------------------------|
| (1) | <u>Pseudomonas aeruginosa</u>   | ( Packer, 1977a )                         |
| (2) | <u>Corynebacterium pyogenes</u> | ( Packer, 1977b )                         |
| (3) | <u>Corynebacterium ulcerans</u> | ( Packer, 1977b )                         |
| (4) | <u>Mycoplasma sp</u>            | ( Jasper, 1977 )                          |
| (5) | <u>Nocardia sp</u>              | ( Orchard, 1979 )                         |
| (6) | Anaerobic organisms             | ( Du Preez <u>et al</u> , 1981 )          |
| (7) | <u>Prototheca zopfii</u>        | ( Hodges <u>et al</u> , 1985 )            |
| (8) | <u>Corynebacterium bovis</u>    | ( Honkanen-Buzalski <u>et al</u> , 1984 ) |

This is by no means an exhaustive list, with sporadic cases being caused by a wide variety of microorganisms.

The infections caused by these organisms vary widely with respect to source, frequency, duration and severity, this variation occurring both within and between species.

This chapter provides a comprehensive description of the bacteriological status for each of the three herds which were selected for the main experiment of the present trial. The bacterial species and their frequency of isolation are presented in a number of ways in order to provide a clear picture of the bacteriological status of the sampled quarters and cows within the three herds, and to highlight differences between the herds with respect to bacteriological findings.

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## MATERIALS AND METHODS

The material included for analysis in this chapter consists of aseptically taken quarter foremilk samples from cows within each of three herds. A description of the herds, and of the sampling methods which were used is given in chapter three of this thesis.

Table 5.1 shows the number of quarters and of cows which were sampled within each herd. All tables and graphs are located at the end of the chapter.

Any difference between herds, and between age groups were tested for statistical significance using the SAS procedure CATMOD ( Statistical Analytical Systems, 1985 ).

## DEFINITION OF TERMS USED

The terms which are applied in describing the mastitis status of a herd over a period of time need to be defined. For the current chapter, and for the remainder of this thesis, the following definitions apply:

- (1) Infection: the term " infection " is used to describe the isolation of a pathogenic, or of a potentially pathogenic species from an aseptically taken quarter foremilk sample on one occasion only.
- (2) Mixed infection: the term " mixed infection " describes the isolation of more than one species of pathogen from the same single sample of milk.
- (3) Incidence: the term " incidence " of an infection refers to the number of quarters within the herd from which the organism concerned was isolated on at least one occasion during the period of sampling, and is expressed as the percentage of the total number of quarters which were sampled from that herd ( Brolund, 1985 ).
- (4) Average duration of infection: the term " average duration of infection " refers to the length of time, on average, that the infection status persisted during the sampling period, and is expressed as a percentage of the lactation period, corrected for unequal numbers of observations ( Dodd, 1971 ).
- (5) Average level of infection: the term " average level of infection " refers to the product of the incidence and of the average duration of the infection ( Dodd, 1971 ). Therefore the average level of infection will

approximate the bacteriological findings at a random sampling, taken during the period of interest.

- (6) Prevalence of infection: the term " prevalence of infection " refers to the number of quarters, or of cows within a herd which are infected by a specific pathogen at a particular point in time. Such figures are reported in surveys in which each cow is sampled on one occasion only.

In past studies, a variety of names have been applied to bacterial species or to the groups of bacteria which are associated with bovine mastitis. This lack of standardization leads to confusion, and makes a comparison between studies difficult. The following definitions are used throughout this thesis:

- (1) Coagulase positive staphylococci

This term refers to all the isolates of staphylococci which were positive to the tube coagulase test ( this test has been described in chapter three ). These isolates were usually, but not invariably, positive to the DNAase test, and usually displayed haemolysis on sheep blood agar. The coagulase test has traditionally been used to split the staphylococci into coagulase positive and coagulase negative organisms. The coagulase positive staphylococci may then be further split, according to the results of a number of tests, into two species, Staphylococcus aureus, and Staphylococcus intermedius ( Schleifer, 1986 ).

Of 831 coagulase positive staphylococcal isolates from milk samples, 97 % were classified as Staphylococcus aureus, while the remaining 3 % were Staphylococcus intermedius ( Hodges et al, 1984 ).

During the present trial no attempt was made to split the coagulase positive staphylococci into separate species, the group simply being referred to as " coagulase positive staphylococci ".

For purposes of comparison, both the terms " Staphylococcus aureus ", and " haemolytic staphylococci ", which have been used by other authors ( Stabenfeldt and Spencer, 1965; Lee and Frost, 1970; Schmidt Madsen et al, 1974; Wilson and Richards, 1980; Brooks et al, 1982 ), may be considered to be equivalent to the grouping of " coagulase positive staphylococci " which is used in this thesis.

## (2) Coagulase negative staphylococci

The staphylococcal isolates which were negative to the tube coagulase test are referred to as " coagulase negative staphylococci ". This group of organisms may be split into at least 17 species on the basis of a number of biochemical tests ( Schleifer, 1986 ). Many of these species have been found in milk. Hogan et al ( 1987a ) isolated 11 species of coagulase negative staphylococci from milk samples.

During the present trial, no attempt was made to identify these isolates to the species level, the group being referred to simply as " coagulase negative staphylococci ". For the purpose of comparison, this group may be considered equivalent to " Staphylococcus epidermidis ", " nonhaemolytic staphylococci ", or to " micrococci ", terms which have been used in previous studies ( Stabenfeldt and Spencer, 1966; Schmidt Madsen et al, 1974; Bramley, 1978; Rainard and Poutrel, 1982 ).

## (3) Major pathogens

This term refers to a number of bacterial species which are capable of causing both clinical and subclinical mastitis, often involving severe damage to the mammary gland. This group includes:

- (a) Coagulase positive staphylococci
- (b) Streptococcus agalactiae
- (c) Streptococcus dysgalactiae
- (d) Streptococcus uberis
- (e) Escherichia coli

Other species which have from time to time been included in the term " major pathogens " were not isolated during the present trial.

## (4) Minor pathogens

This term refers to the group of bacterial species of low pathogenicity, which may infect the mammary gland, but usually cause little damage. This group includes:

- (a) Coagulase negative staphylococci
- (b) Corynebacterium bovis

## RESULTS

Tables 5.2a, 5.2b and 5.2c give the incidence, the average duration of infection, and the average level of infection for the various infection categories, in each of the herds A, B and C respectively.

### (1) INCIDENCE OF INFECTION

The incidence of uninfected quarters, ie the percentage of quarters which at some stage of the dairy season were bacteriologically negative, was 90 % for herd A ( table 5.2a ). Accordingly, 10 % of quarters in this herd were infected throughout the sampling period.

In herd B, 29 % of quarters were infected throughout the eight month sampling period ( table 5.2b ), while for herd C, the corresponding figure was 17 % ( table 5.2c ). The difference between the herds in incidence of infection, overall, was highly significant (  $p < 0.01$  ).

While the overall incidence of infection differed between herds, so too did that of individual bacterial species. The incidence of Corynebacterium bovis was highest in herd B, with 72 % of quarters yielding this organism at least once during the season. Herd C also had a high incidence of Corynebacterium bovis, but in herd A only 17 % of quarters harboured this organism at least once during the season. The difference between herds with respect to the incidence of Corynebacterium bovis infection was highly significant (  $p < 0.01$  ).

Coagulase negative staphylococci were isolated from 22 % of quarters from the most severely affected herd ( herd C ), but from only 7 % of quarters in herd A, this difference being significant (  $p < 0.1$  ).

Coagulase positive staphylococci were isolated from between 8 % to 28 % of quarters at least once during the season, herd C having the highest incidence, and herd A the lowest. This difference was significant (  $p < 0.1$  ).

Because of the low numbers of streptococci which were isolated, the species Streptococcus uberis and Streptococcus dysgalactiae were combined, the group being termed " nonagalactiae streptococci ". Within this group, Streptococcus uberis was the more commonly isolated of the two species, this being true for each of the three herds. The highest incidence of these organisms was again in herd C, in which they were isolated on at least one occasion from 14 % of quarters.

Streptococcus agalactiae was isolated from herd B only, and from one quarter of one cow within this herd. Similarly, coliforms were isolated only within herd C.

### THE EFFECT OF AGE OR LACTATION NUMBER ON THE INCIDENCE OF INFECTION

A trend toward an increasing incidence of infection with increasing lactation number was observed during the present trial. This increase was significant for Corynebacterium bovis (  $p < 0.01$  ), coagulase positive staphylococci (  $p < 0.01$  ) and for the nonagalactiae streptococcus group (  $p < 0.01$  ), when the data from the three herds were combined.

#### (2) AVERAGE DURATION OF INFECTION

The milk samples were taken at monthly ( four-weekly ) intervals. If an organism was isolated at an arbitrary sampling but had not been present at the preceeding sampling, and was not present at the next sampling, then this infection is deemed to have been present for one month only. It is recognized that this figure is arbitrary and that the infection may have been present for less than or for more than one month. For the purpose of comparison, this figure was used as a compromise. Similarly, if the same organism was isolated at two consecutive samplings, then the infection is deemed to have lasted for two months. A more accurate picture of the duration of infection would have been provided by the more frequent sampling of each quarter. This however, was not practical during the present trial, given the limited resources available.

While the incidence of Corynebacterium bovis infection varied markedly between the herds, the average duration of infection for this organism, when present, showed less variability ( tables 5.2a, 5.2b, 5.2c ). Infection of a quarter with Corynebacterium bovis tended to be stable, the duration ranging from 44 % to 55 % of the sampling period.

By contrast, the coagulase negative staphylococci caused infections of relatively short duration, particularly within herd B, in which the average duration was only one month ( table 5.2b ).

The coagulase positive staphylococci were most stable within herd A, in which the average duration of infection was approximately two thirds of the

sampling period ( table 5.2a ), while the figures for the other two herds were close to one third of the sampling period ( tables 5.2b and 5.2c ).

Another group of major pathogens, the nonagalactiae streptococci caused infection of a longer duration within herd A than within the other herds.

The only isolate of Streptococcus agalactiae was present for a period of three months, which was 38 % of the sampling period. A similar duration was recorded for the coliform isolate.

In general, the udder infections were of longest duration within herd A, and of shortest duration in herd B.

### (3) BACTERIOLOGICAL FINDINGS AT A SUBSEQUENT SAMPLING

Table 5.3 gives a breakdown of the bacteriological status at a second sampling, given a specific finding at an arbitrary first sampling. The data therefore provides an indication of the stability of the bacteriological status within quarters, between consecutive samplings. The table includes data from each of the three herds.

Within herd A, 96 % of quarters which were bacteriologically negative at an arbitrary first sampling were still bacteriologically negative one month later. For herds B and C, the figures were 80 % and 76 % respectively.

In each of the three herds, Corynebacterium bovis showed the highest new infection rate, with as many as 16 % of the quarters acquiring an infection with this organism per month in quarters which were previously free of infection, while 19 % of the pre-existing infections were replaced by Corynebacterium bovis infection ( figures for herd C ).

Once a Corynebacterium bovis infection had occurred, a total of 21 % were eliminated by the next sampling within herd A, with the majority of quarters reverting to a bacteriologically negative status, the remainder having the infection replaced by infection with a different pathogen.

The stability of infection with the coagulase negative staphylococci was relatively low, particularly within herd B ( table 5.3 ). In the majority of such cases, the infection was spontaneously eliminated, leaving an uninfected gland, although in a number of quarters the coagulase negative staphylococci were replaced by other minor or major pathogens.

Coagulase positive staphylococci caused relatively stable infections. Within herd A, the majority of quarters which eliminated this group of organisms were bacteriologically negative at the next sampling. Within herd B, in which the coagulase positive staphylococci caused infections of shorter duration, the numbers of quarters which were bacteriologically negative, and the number of quarters which yielded Corynebacterium bovis following elimination of coagulase positive staphylococci were similar.

Herd B showed a high proportion of mixed infections following the isolation of coagulase positive staphylococci at the preceeding sampling. Herd C showed the majority of eliminated coagulase positive staphylococcal infections to be replaced by a bacteriologically negative quarter, although 10 % of established infections were followed by infection with a different pathogen, and a further 5 % were followed by a mixed infection.

The nonagalactiae streptococcal group caused relatively stable infections within herds A and C, but within herd B, tended to be eliminated or else replaced by other organisms, although the figures for this group of organisms must be interpreted with caution, since the number of isolations was low. Streptococcus agalactiae was isolated on only one occasion, hence firm conclusions cannot be drawn regarding the duration of infection or of its displacement by other organisms.

Mixed infections were unstable, tending to resolve into infection with a single organism. This single organism was almost invariably one of the two organisms which formed the original mixture. The makeup of these mixed infections varied, but for herds B and C were usually composed of Corynebacterium bovis and one other organism, often a coagulase positive staphylococcus or a member of the nonagalactiae streptococcal group.

#### (4) THE NUMBER OF DIFFERENT BACTERIAL SPECIES ISOLATED PER QUARTER, DURING THE SAMPLING PERIOD

Tables 5.4a, 5.4b and 5.4c show the number of different species isolated from each quarter within the lactation period, for the herds A, B and C respectively.

Within herd A, the vast majority of quarters were either uninfected throughout the lactation period, or yielded one type of bacterial species only ( table 5.4a ). Overall, only 4 % of the quarters contained more than one species of bacteria, although not necessarily concurrently. The average number

of different organisms isolated from each quarter increased with lactation number.

Within herd B, > 30 % of the quarters yielded two or more different pathogens during the season ( table 5.4b ). The figure for herd C was higher still, with over 35 % of quarters being infected by two or more different pathogens during the season ( table 5.4c ).

In both herds B and C, the average number of organisms isolated increased with increasing lactation number, up to the sixth lactation in herd C. It must be remembered that the makeup of herds A and B in terms of ages of the cows was similar, while herd C included some older animals ( see chapter three for details ).

#### (5) PREVALENCE OF INFECTION ACROSS THE LACTATION IN INDIVIDUAL QUARTERS

Graphs 5.1a, 5.1b and 5.1c show the prevalence of infection in individual quarters, across the lactation.

The three herds showed major changes in infection status during the season. Within herd A, there was a small but significant decrease in the number of quarters which were uninfected, as the season progressed ( graph 5.1a ). This was caused by a significant (  $p < 0.1$  ) increase in the infected quarters when all pathogens were combined, but the low levels of infection precluded significance from being reached for individual species or pathogen groups within herd A.

Within herd B, uninfected quarters constituted over 60 % of the total number of quarters at the first sampling, by drying off, this figure had decreased to less than 30 % of quarters ( graph 5.1b ). Minor pathogens showed a significant increase (  $p < 0.01$  ) from approximately 30 % to 65 % of quarters during this period, with Corynebacterium bovis being largely responsible for this increase. Quarters infected by major pathogens increased significantly in number (  $p < 0.01$  ) early in the season, but by the fourth sampling, had returned to the level which had been present at the beginning of the trial, with little further change prior to drying off.

Herd C showed similar trends to those observed for herd B. There was a rapid decline in the percentage of quarters which were uninfected, from over 60 % during the first two samplings, to less than 30 % of quarters at the final sampling ( graph 5.1c ). As was the case for herd B, minor pathogens,

particularly Corynebacterium bovis accounted for the significant (  $p < 0.01$  ) increase in the " all pathogens " group. The major pathogens accounted for approximately 20 % of quarters within herd C, this figure remaining relatively stable during the course of the season.

#### (6) PREVALENCE OF INFECTION ACROSS THE LACTATION IN COWS

Up to this point, the results relate to infection at the level of the individual quarter. Graphs 5.2a, 5.2b and 5.2c show the prevalence of infection on a whole udder basis, across the lactation, for herds A, B and C respectively.

Within herd A, the number of cows which were uninfected in all quarters decreased slightly during the season, but increased during the final month ( graph 5.2a ). The number of cows infected by minor pathogens fluctuated, but showed no real trend, while those infected by major pathogens decreased initially then increased during mid-lactation.

Herd B showed a more marked decrease in the number of cows which were uninfected in all quarters ( graph 5.2b ), this decrease being due to the increase in the number of cows which yielded minor pathogens only. The number of animals which were infected by major pathogens showed little overall change during the course of the season.

Within herd C, there was a decrease in the number of cows which were uninfected, as the season progressed ( graph 5.2c ). This was associated with a doubling of the number of cows which were infected by minor pathogens only, while there was an overall decrease in the number of cows which harboured major pathogens.

A greater percentage of cows were infected with a major pathogen in herd C than within herds A or B. Herd B showed the lowest percentage of cows infected with a major pathogen, but the highest percentage of cows infected with a minor pathogen of the three herds studied.

### DISCUSSION

In trials involving repeated sampling, the average level of infection is a function of both the total number of infected cows, and the average duration of infection.

Thus we have:

$$A = B \times C$$

where  $A$  = the average level of infection.

$B$  = the total number of cows infected ( during the period ).

$C$  = the average duration of infection.

( Dodd, 1971 ).

If this formula is applied to quarters, we have:

$$L = I \times D$$

where  $L$  = the average level of infection in quarters at any one time during the sampling period.

$I$  = the incidence of infection, ie the total number of quarters which were infected at least once during the period, expressed as a percentage of all quarters.

$D$  = the average duration of infection, given that the quarter was infected at least once, expressed as a percentage of the period.

By dividing the samples according to infection status, the formula may be used to give an indication of the dynamics of udder infection due to various species of bacteria.

### (1) INCIDENCE OF INFECTION

Tables 5.2a, 5.2b and 5.2c show the incidence, the average duration of infection, and the average level of infection for cows from the three herds A, B and C respectively.

The herds varied with respect to the incidence of infection with the different pathogens. Corynebacterium bovis, coagulase positive staphylococci, coagulase negative staphylococci and the nonagalactiae streptococci were isolated from the quarters of all three herds.

In the present study, Corynebacterium bovis showed the highest incidence of the bacterial groups in each of the three herds. Corynebacterium bovis is reported to be the organism most commonly isolated from aseptically taken foremilk samples in herds not using teat dips ( Honkanen-Buzalski *et al*, 1984 ). Dodd ( 1971 ) claimed that over 90 % of infections are caused by the major pathogens Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae and Streptococcus uberis.

These two claims serve to highlight the uncertain status of the minor pathogens in general, and of Corynebacterium bovis in particular as mammary pathogens, these organisms often being ignored.

Coagulase negative staphylococci were isolated on at least one occasion from between 7 % of quarters in herd A and 28 % of quarters in herd C during the present trial. The incidence of these organisms was markedly lower than that found by Brolund ( 1985 ), who isolated these organisms on at least one occasion from 83 % of quarter samples from Swedish Friesian cows.

The same was true of the incidence of coagulase positive staphylococci. Brolund ( 1985 ) reported a higher overall incidence of coagulase positive staphylococci than was seen even in herd C, the most severely affected herd within the present study.

During the present trial, the incidence of infection with the nonagalactiae streptococci was low. These organisms show certain similarities in terms of natural habitat and of pathogenesis, and for the purpose of analysis, are grouped together. This serves to distinguish them from the other major pathogen Streptococcus agalactiae, which is an obligate udder parasite, an important consideration in terms of management and control.

During the present study, there was a trend towards increased incidence of infection with lactation number, or age of cow. A similar finding has been reported by Schmidt Madsen et al. ( 1974 ), who reported that the percentage of quarters with positive microbiological findings increases through the first six lactations and suggested that the decrease seen beyond the sixth lactation was due to the increase in rate of culling for infected cows over those with a healthy udder.

## (2) DURATION OF INFECTION

The average duration of infection in the present study was shorter for the coagulase negative staphylococci than for the other organisms in each of the three herds. Similar findings have been reported by Rainard and Poutrel ( 1982 ) who found that the coagulase negative staphylococci are eliminated significantly more readily than are other types of bacteria. The high elimination rate distinguishes the coagulase negative staphylococci from the other minor pathogen Corynebacterium bovis which persisted for 44 % to 55 % of the lactation during the present study. Infection of a quarter with Corynebacterium bovis is persistent unless the quarter is treated, or becomes infected with a major pathogen ( Honkanen-Buzalski et al., 1984 ).

Infections with the major pathogens were generally of longer duration within herd A, than within herds B or C. Overall, the duration of infections during the present trial were shorter than were those reported by Rainard and Poutrel ( 1982 ), the latter authors reported that approximately 80 % of infections persist until drying off for both major and for minor pathogens.

At least some of the difference between the results of the present trial and those of Rainard and Poutrel ( 1982 ) may lie in the definition of infection. Rainard and Poutrel ( 1982 ) regarded a quarter as being infected when the same organism was isolated from two consecutive samples taken three weeks apart. In the present trial, a quarter was deemed to be infected if a pathogen was isolated on a single occasion.

## (3) AVERAGE LEVEL OF INFECTION

Table 5.5 gives the results of a number of field trials which have been undertaken to describe the infection status of udder quarters or of cows. These studies relate to the collection of samples on one occasion only, from a large number of animals. Some difficulties arise in the drawing of comparisons between the results of such surveys, and those of the present trial ( which

involved the repeated sampling of the same cows ). Nevertheless, the average levels of infection given in tables 5.2a, 5.2b and 5.2c represent a similar point estimate to that provided by the prevalence figures used in single sampling trials.

The average level of infection with Corynebacterium bovis was 8 % of quarters in herd A, this low figure being due mainly to the low incidence of infection. Within herd B, the average level of infection for Corynebacterium bovis was 41 % of quarters, whilst in herd C the corresponding figure was 27 % of quarters.

In both herds B and C, the greater average level of infection was due to the greater incidence of infection; a higher proportion of quarters yielded this organism at least once during the season than was the case in herd A. The average duration of infection with Corynebacterium bovis was similar in the three herds studied.

The prevalence of Corynebacterium bovis infection has been reported in a small number of studies. Hogan *et al* ( 1987a ) isolated Corynebacterium bovis from 37 % of the quarters in herds which were not using postmilking teat dipping, whilst in those herds which did employ teat dipping, the isolation rate varied from 8 % to 28 % of quarters, depending upon the type of dip. During the present trial, teat dips were used in each of the three herds, although only intermittantly within herd B. In both herds B and C, the percentage of quarters infected with Corynebacterium bovis increased as the season progressed.

In herds using dry cow therapy without effective teat dipping, the high infectivity of Corynebacterium bovis results in a rapid increase in the level of infection during the lactation ( Honkanen-Buzalski *et al*, 1984 ).

While Kitchen *et al* ( 1984a ) isolated Corynebacterium bovis from 24 % of quarter samples during a repeated sampling trial, in many surveys this organism has been either disregarded completely, or combined with the coagulase negative staphylococci to form a " minor pathogen " group, ( table 5.5 ).

The average level of infection with Corynebacterium bovis during the present trial agrees with the levels found in the limited number of references which have included this organism.

The average level of infection with the coagulase negative staphylococci ranged from 2 % to 5 % of quarters within the three herds. Both the low

incidence and the short duration of infection contributed to the low average level of infection. Higher figures have been reported by other workers. Elliot *et al* ( 1976 ) found coagulase negative staphylococci in 31 % of cows. Figures for the prevalence in individual quarters are not given. The average level of infection with coagulase negative staphylococci was 28 % of quarters in a Swedish trial ( Brolund, 1985 ) while Andrews *et al* ( 1983 ) isolated this group of organisms from 41.6 % of quarter foremilk samples. The figures observed in the present trial are closer to those of Schmidt Madsen *et al* ( 1974 ) who isolated coagulase negative staphylococci from 5.3 % of quarter samples from Danish dairy herds.

The coagulase negative staphylococci are a heterogeneous group of related organisms. A number of individual species have been isolated from infected quarters. Hodges *et al* ( 1984 ) isolated six species of coagulase negative staphylococci from clinically affected or from subclinically affected quarters.

The prevalence of coagulase negative staphylococci may be related to the use of teat dips. Hogan *et al* ( 1987a ) isolated coagulase negative staphylococci from 11 % of quarters in herds in which no teat dips were used, compared with 7.8 % to 11.8 % of quarters when teat dips were used. Eleven species were identified, the distribution of these species varying between herds according to their use of different teat dips. Teat dipping appears to suppress Staphylococcus epidermidis, but the isolation of Staphylococcus hyicus increases when teat dips are used. The above results show that a number of organisms make up this group of minor pathogens, and that the particular makeup within a herd will depend upon management factors. This variation may be responsible for the vast differences between the prevalence figures for coagulase negative staphylococci which have been reported in the literature.

The average levels of infection with coagulase positive staphylococci in the three herds are similar to those reported in the literature ( table 5.5 ). Although strain differences may occur, the coagulase positive staphylococci which are associated with cows would appear to be a more homogeneous group of organisms than are their coagulase negative counterparts.

The average levels of infection for the nonagalactiae streptococci in the three herds, fall within the range of values shown in table 5.5. In a repeated sampling study Kitchen *et al* ( 1984a ) found this group of organisms in 3.2 % of quarter samples.

In studies undertaken in New Zealand, Brookbanks ( 1966 ) and Elliot *et al* ( 1976 ) isolated Streptococcus agalactiae from 15.9 % and 14.5 % of cows respectively. Figures for individual quarters are not given, but had each cow

been infected in one quarter only, then the total percentage of quarters which were infected with this organism must have been between 3 % and 4 %. During the present trial, only one quarter yielded Streptococcus agalactiae. This represents less than 1 % of the total number of cows and less than 0.25 % of the total number of quarters.

The marked reduction in the average level of infection by Streptococcus agalactiae is probably due to the implementation of mastitis control measures over the past 20 years. This organism is an obligate udder parasite and may be eliminated from a herd by the application of appropriate control techniques ( McDonald, 1977 ).

The figures for the average level of infection with Streptococcus agalactiae are lower than the prevalence figures in a number of reports ( table 5.5 ), but are similar to those of Schmidt Madsen et al ( 1974 ).

Coliform organisms showed a low average level of infection during the present trial. This is in general agreement with a number of the surveys cited in table 5.5, but it is in marked contrast to the figures of Elliot et al ( 1976 ), which although relating to cows rather than to individual quarter samples, show much higher levels of infection with coliforms. A reason for this difference may be the fact that milk samples taken within one month of calving were not included in the analysis. Coliform mastitis frequently occurs shortly after parturition ( Eberhart, 1977 ).

A number of studies have shown that under close confinement conditions, bedding materials, particularly sawdust may harbour large numbers of coliform organisms, and serve as a source of infection for the cows ( Newman and Kowalski, 1973; Rendos et al, 1975 ). Under New Zealand dairying conditions, cows are grazed on pasture throughout the year and are not confined under crowded conditions on artificial bedding materials. Nevertheless, Jasper ( 1976 ) showed that in the case of some New Zealand dairy herds, high populations of coliform bacteria do exist within the environment, and that coliform mastitis may become a problem. One suggested source of contamination of teats was the spraying of cowshed effluent onto the pasture. It is possible that stripgrazing may result in an increase in the exposure of teats to environmental organisms including coliforms.

#### (4) THE PREVALENCE OF INFECTION DURING THE SEASON IN INDIVIDUAL QUARTERS

During the present trial, the percentage of quarters which were free of infection decreased within all three of the herds as the lactation progressed. This was due almost entirely to the increase in one pathogen, Corynebacterium bovis.

There was little change in the percentage of quarters which were infected with other pathogens, although it must be remembered that any samples taken within one month of parturition were not included in the analysis. Schmidt Madsen *et al* ( 1974 ) reported very little variation in the microbiological findings during the first eight months of lactation, while differences were noted during the dry period. These authors did not, apparently, culture for Corynebacterium bovis. It would appear that with the exception of Corynebacterium bovis, the prevalence of infection with specific pathogens shows little variation from the start of the second month of lactation until the cows are dried off at the end of the season.

Corynebacterium bovis is sensitive to dry cow therapy. Crist *et al* ( 1982 ) have reported that three different dry cow therapy preparations resulted in the elimination of between 93.8 % and 100 % of infections, while 47.6 % of Corynebacterium bovis udder infections were spontaneously eliminated over the dry period. The combination of this sensitivity to therapy, and the ease with which infection is established when teats are exposed to this minor pathogen ( Honkanen-Buzalski and Bramley, 1984 ) explains the rapid increase in prevalence of Corynebacterium bovis during the dairy season.

#### (5) THE PREVALENCE OF INFECTION DURING THE SEASON IN COWS

The changes in prevalence of infection across the season in cows, tended to mirror those of the prevalence within individual quarters, although exceptions did occur between stages 2 to 3 for herd B, and between stages 3 to 4 for herd C ( graphs 5.1a & 5.2a, 5.1b & 5.2b, 5.1c & 5.2c ).

An increase in the prevalence of infection in cows, relative to that in quarters suggests that previously uninfected animals are becoming infected at a greater rate than are uninfected quarters within animals which are already infected. Conversely, an increase in the prevalence of infection in quarters, relative to that in cows, suggests that previously infected cows are becoming infected in a greater number of quarters.

Because of the limited extent to which the bacteriological identification of the isolates was carried out, it is not possible to speculate whether the infections with major pathogens were spreading from one quarter to another within the udder, spreading from one udder to another, or were resulting from contact with pathogens in the environment.

As was the case for individual quarters, infection of cows with major pathogens was most prevalent within herd C, and least prevalent within herd B. The prevalence of minor pathogens was greatest within herd B, and least within herd A. The results for minor pathogens given in graphs 5.2a, 5.2b and 5.2c refer to those cows which harboured minor pathogens only. If a cow was infected by minor and by major pathogens, then these animals were included in the " major pathogen " group.

Other workers have shown little change in the prevalence of infection in cows during the lactation even when Corynebacterium bovis was included ( Brooks et al, 1982 ), although the herds were not " seasonal " ( in contrast to those of the present study ).

#### (6) THE EFFECT OF PRE-EXISTING INFECTION OF THE MAMMARY GLAND BY MINOR PATHOGENS ON INFECTION OF THE GLAND BY MAJOR PATHOGENS

The possibility of a protective effect due to infection of a gland with a minor pathogen, against superinfection by a second pathogen has been raised by other workers. Schalm et al ( 1966 ) showed that quarters which had a pre-existing leukocytosis of the order of 200,000 to 500,000 cells per ml of milk were effectively protected against experimental exposure to Streptococcus agalactiae. Similar results were obtained for another major pathogen, Escherichia coli, when 12 out of 17 quarters were experimentally infected with this organism. The five quarters which did not become infected each had a cell count of over 300,000 cells per ml prior to exposure to the pathogen, while those 12 quarters which did become infected by the Escherichia coli all had cell counts below 300,000 cells per ml ( Bramley 1976 ).

In a later trial, 62 % of the quarters which were infected by the minor pathogen Staphylococcus epidermidis became infected following challenge by Escherichia coli, while 93 % of uninfected quarters became infected. It was suggested that the increased resistance to infection was due to the increased polymorph neutrophil count ( somatic cell count ) rather than to any direct inhibitory effects produced by Staphylococcus epidermidis ( Bramley, 1978 ).

While regarded as being less pathogenic than haemolytic coagulase positive staphylococci, nonhaemolytic coagulase negative staphylococci can produce lesions in the alveolar epithelium and induce the migration of leukocytes into the mammary tissue ( Stabenfeldt and Spencer, 1966 ).

Corynebacterium bovis stimulates a leukocytosis, with a modest increase in the somatic cell count of the milk ( Griffin et al, 1974; Honkanen-Buzalski et al, 1984; Honkanen-Buzalski and Bramley, 1984 ).

Corynebacterium bovis is generally associated with the keratinaceous tissue of the streak canal, with only 25 % of samples which were positive to milk drawn through the teat canal yielding this organism when re-examined by teat puncture ( Black et al, 1972 ), although Pankey et al ( 1985 ) isolated Corynebacterium bovis from 75 % of infected quarters when samples were taken through the teat wall. Honkanen-Buzalski et al ( 1984 ) reported that 44 % of infections by Corynebacterium bovis were confined to the teat canal.

Brooks and Barnum ( 1984 ) reported that quarters which were shedding Corynebacterium bovis were more resistant to an experimental infection when Staphylococcus aureus was infused by cisternal puncture into the teat cistern. However, no resistance to infection with Streptococcus agalactiae was associated with quarters which had a pre-existing Corynebacterium bovis infection ( Brooks and Barnum, 1984 ). Similarly, Pankey et al ( 1985 ) reported that the rate of intramammary infection by Staphylococcus aureus in quarters infected with Corynebacterium bovis was reduced by 50 % in comparison with infection in bacteriologically negative quarters. In contrast, quarters infected with Corynebacterium bovis were more than eight times more susceptible to experimental infection by Streptococcus agalactiae ( Pankey et al, 1985 ).

The difference between Streptococcus agalactiae and Staphylococcus aureus with respect to superinfection of quarters with pre-existing Corynebacterium bovis infection probably lies in the effects of competition for growth limiting substances in the teat canal, rather than to the inhibitory effects of Corynebacterium bovis metabolites on the growth of the major pathogens ( Hogan et al, 1987b ).

Other workers have shown that while normal milk may be regarded as bacteriostatic, mastitis pathogens generally grow more rapidly in the whey prepared from mastitic milk than from that of normal milk, this being ascribed, in part, to the breakdown of proteins by proteinase enzymes such as plasmin, which are present in the mastitic milk ( Mattila et al, 1986c ). This suggests that a naturally occurring infection would tend to make the gland

more susceptible to either re-infection or to superinfection with a second organism, rather than exerting any protective effect.

During the present trial, the findings at a subsequent sampling, given a specific finding at an arbitrary first sampling, are given in table 5.3. Should infection caused by minor pathogens have protected the quarters against infection with a major pathogen, then the percentage of bacteriologically negative quarters which became infected by major pathogens should be greater than the percentage of Corynebacterium bovis or coagulase negative staphylococci infected quarters which showed an infection by a major pathogen at the subsequent sampling. Herd A showed a very low incidence of minor pathogens, making a comparison difficult, while within herds B and C, 2 % and 3 % respectively of bacteriologically negative quarters were infected by coagulase positive staphylococci at the subsequent sampling. Only 1 % of the quarters which were infected with Corynebacterium bovis, were infected by coagulase positive staphylococci at the subsequent sampling. Coagulase negative staphylococci did not show any protective effect, since a greater percentage of quarters infected by these organisms were subsequently infected by coagulase positive staphylococci than was the case for quarters which were bacteriologically negative at the first sampling. It is not clear whether the relatively small decrease in the infection rate which was associated with Corynebacterium bovis infection, was real, or merely the result of sampling variation.

The percentage of quarters which were infected with Corynebacterium bovis increased markedly during the season both in herd B and in herd C. Had there been a true protective effect, then the percentage of quarters which were infected with major pathogens should have decreased during the season. A small decrease in the percentage of quarters which were infected by major pathogens did occur during the season within herd B, while the level in herd C remained remarkably stable during the season.

It is difficult to draw any firm conclusions regarding the presence or absence of a protective effect on the basis of the results of the present trial, this being partly due to the comparatively small number of samples taken, and to the low overall infection rate which was observed. If protection was present, the effect was small, and was confined to Corynebacterium bovis.

The suggestion has been made that use of the protective effects of minor pathogens should be encouraged, particularly within herds with a low prevalence of subclinical mastitis ( Brooks and Barnum, 1984 ), since the effects of the minor pathogens on the quality and on the quantity of the milk produced are not great. However, minor pathogens have been shown to be

capable of causing outbreaks of clinical mastitis, ( Cobb and Walley, 1962; Counter, 1981 ). Furthermore, by causing changes to the composition of the milk, the presence of minor pathogens would make detection of udders infected by major pathogens less reliable.

The effects of the minor pathogens on the composition of milk will be dealt with in chapters six and eight of this thesis.

### SUMMARY AND CONCLUSIONS

- (1) The three herds which were studied during the present trial differed in the incidence of infection, both overall, and for individual major or minor pathogens.
- (2) Of the three herds, Herd A showed the lowest overall incidence of infection. The incidence of minor pathogens was highest in herd B, while that of major pathogens was highest in herd C. The incidence of infection tended to increase with lactation number.
- (3) The average duration of infection with certain pathogens differed between herds. In general, the duration of infection was longer within herd A, than within herds B or C.
- (4) The prevalence of infection showed some change during the course of the lactation, particularly for the organism Corynebacterium bovis within herds B and C. The percentage of quarters and of cows which were uninfected tended to decrease as the dairy season progressed.
- (5) The changes in prevalence of infection in cows, during the sampling period generally paralleled those of the individual quarters.

TABLE 5.1THE NUMBER OF COWS AND OF QUARTERS INCLUDED IN THE ANALYSIS

| HERD                                              |                                                                            |                                                                                 |                                                                                     |
|---------------------------------------------------|----------------------------------------------------------------------------|---------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
|                                                   | A                                                                          | B                                                                               | C                                                                                   |
| NUMBER OF COWS<br>ORIGINALLY<br>SELECTED:         | 32                                                                         | 33                                                                              | 64                                                                                  |
| NUMBER OF COWS<br>INCLUDED IN<br>ANALYSIS:        | 32                                                                         | 33                                                                              | 56                                                                                  |
| NUMBER OF<br>QUARTERS<br>INCLUDED IN<br>ANALYSIS: | 127                                                                        | 130                                                                             | 223                                                                                 |
| REASON FOR<br>EXCLUSION OF<br>COW(S) :            |                                                                            |                                                                                 | A NUMBER OF<br>COWS WERE<br>CULLED FOR<br>REASONS NOT<br>CONNECTED WITH<br>MASTITIS |
| REASON FOR<br>EXCLUSION OF<br>QUARTER(S) :        | A LACERATED TEAT,<br>EARLY IN THE<br>SEASON MADE<br>SAMPLING<br>IMPOSSIBLE | TWO QUARTERS WERE<br>VIRTUALLY DRY, DUE<br>TO SEVERE MASTITIS<br>PRIOR TO TRIAL | ONE QUARTER WAS<br>DRY DUE TO AN<br>INFECTION IN<br>THE PRECEEDING<br>SEASON        |

TABLE 5.2a

THE INCIDENCE, AVERAGE DURATION AND AVERAGE LEVEL  
OF INFECTION IN INDIVIDUAL UDDER QUARTERS - HERD A

| BACTERIOLOGICAL STATUS ( EXPRESSED AS A % ) |   |              |                 |                 |                 |                   |                   |                |                          |
|---------------------------------------------|---|--------------|-----------------|-----------------|-----------------|-------------------|-------------------|----------------|--------------------------|
|                                             |   | NO<br>GROWTH | CORYNE<br>BOVIS | COAG -<br>STAPH | COAG +<br>STAPH | STREPT<br>NONAGAL | STREPT<br>AGALACT | COLI-<br>FORMS | NUMBER<br>OF<br>QUARTERS |
| P<br>A<br>R<br>T<br>I<br>T<br>Y             |   |              |                 |                 |                 |                   |                   |                |                          |
| 1                                           | I | 91           | 3               | 3               | 9               | 0                 | 0                 | 0              |                          |
|                                             | D | 99           | 12              | 100             | 71              | 0                 | 0                 | 0              | 32                       |
|                                             | L | 90           | <1              | 3               | 6               | 0                 | 0                 | 0              |                          |
| 2                                           | I | 91           | 13              | 9               | 9               | 0                 | 0                 | 0              |                          |
|                                             | D | 93           | 56              | 13              | 71              | 0                 | 0                 | 0              | 32                       |
|                                             | L | 85           | 7               | 1               | 7               | 0                 | 0                 | 0              |                          |
| 3                                           | I | 94           | 16              | 3               | 3               | 13                | 0                 | 0              |                          |
|                                             | D | 89           | 33              | 13              | 38              | 75                | 0                 | 0              | 31                       |
|                                             | L | 84           | 5               | <1              | 1               | 10                | 0                 | 0              |                          |
| 4                                           | I | 84           | 38              | 13              | 9               | 13                | 0                 | 0              |                          |
|                                             | D | 76           | 46              | 38              | 58              | 50                | 0                 | 0              | 32                       |
|                                             | L | 64           | 17              | 5               | 5               | 7                 | 0                 | 0              |                          |
| O<br>V<br>E<br>R<br>A<br>L<br>L             | I | 90           | 17              | 7               | 8               | 6                 | 0                 | 0              |                          |
|                                             | D | 90           | 45              | 33              | 66              | 71                | 0                 | 0              | 127                      |
|                                             | L | 81           | 8               | 2               | 5               | 4                 | 0                 | 0              |                          |

I = Incidence of infection ( expressed as a percentage of the quarters ).

D = Average duration of infection ( expressed as a percentage of the period ).

L = Level of Infection ( = I x D, expressed as a percentage ).

TABLE 5.2b

THE INCIDENCE, AVERAGE DURATION AND AVERAGE LEVEL  
OF INFECTION IN INDIVIDUAL UDDER QUARTERS - HERD B

| BACTERIOLOGICAL STATUS ( EXPRESSED AS A % ) |     |              |                 |                 |                 |                   |                   |                |                          |
|---------------------------------------------|-----|--------------|-----------------|-----------------|-----------------|-------------------|-------------------|----------------|--------------------------|
|                                             |     | NO<br>GROWTH | CORYNE<br>BOVIS | COAG -<br>STAPH | COAG +<br>STAPH | STREPT<br>NONAGAL | STREPT<br>AGALACT | COLI-<br>FORMS | NUMBER<br>OF<br>QUARTERS |
| P<br>A<br>R<br>I<br>T<br>Y                  | 1 I | 89           | 50              | 22              | 11              | 6                 | 0                 | 0              | 36                       |
|                                             | D   | 79           | 42              | 17              | 24              | 20                | 0                 | 0              |                          |
|                                             | L   | 70           | 21              | 4               | 3               | 1                 | 0                 | 0              |                          |
| 2                                           | I   | 71           | 71              | 10              | 19              | 6                 | 3                 | 0              | 31                       |
|                                             | D   | 62           | 65              | 20              | 34              | 38                | 38                | 0              |                          |
|                                             | L   | 44           | 46              | 2               | 6               | 2                 | 1                 | 0              |                          |
| 3                                           | I   | 65           | 81              | 23              | 29              | 6                 | 0                 | 0              | 31                       |
|                                             | D   | 56           | 51              | 14              | 40              | 40                | 0                 | 0              |                          |
|                                             | L   | 36           | 41              | 3               | 12              | 2                 | 0                 | 0              |                          |
| 4                                           | I   | 56           | 84              | 25              | 28              | 6                 | 0                 | 0              | 32                       |
|                                             | D   | 52           | 60              | 16              | 34              | 29                | 0                 | 0              |                          |
|                                             | L   | 29           | 50              | 4               | 10              | 2                 | 0                 | 0              |                          |
| O<br>V<br>E<br>R<br>A<br>L<br>L             | I   | 71           | 72              | 19              | 22              | 6                 | 1                 | 0              | 130                      |
|                                             | D   | 65           | 55              | 16              | 35              | 31                | 38                | 0              |                          |
|                                             | L   | 45           | 41              | 3               | 8               | 2                 | <1                | 0              |                          |

I = Incidence of infection ( expressed as a percentage of the quarters ).  
D = Average duration of infection ( expressed as a percentage of the period ).  
L = Level of infection ( = I x D, expressed as a percentage ).

TABLE 5.2c

THE INCIDENCE, AVERAGE DURATION AND AVERAGE LEVEL  
OF INFECTION IN INDIVIDUAL UDDER QUARTERS - HERD C

| BACTERIOLOGICAL STATUS ( EXPRESSED AS A % ) |   |              |                 |                 |                 |                   |                   |                |                          |
|---------------------------------------------|---|--------------|-----------------|-----------------|-----------------|-------------------|-------------------|----------------|--------------------------|
| P<br>A<br>R<br>I<br>T<br>Y                  |   | NO<br>GROWTH | CORYNE<br>BOVIS | COAG -<br>STAPH | COAG +<br>STAPH | STREPT<br>NONAGAL | STREPT<br>AGALACT | COLI-<br>FORMS | NUMBER<br>OF<br>QUARTERS |
| 2                                           | I | 85           | 55              | 28              | 10              | 3                 | 0                 | 0              | 40                       |
|                                             | D | 68           | 51              | 19              | 36              | 100               | 0                 | 0              |                          |
|                                             | L | 58           | 28              | 5               | 4               | 3                 | 0                 | 0              |                          |
| 3                                           | I | 100          | 50              | 33              | 33              | 6                 | 0                 | 0              | 16                       |
|                                             | D | 68           | 40              | 23              | 32              | 14                | 0                 | 0              |                          |
|                                             | L | 68           | 20              | 8               | 11              | 8                 | 0                 | 0              |                          |
| 4                                           | I | 85           | 70              | 8               | 25              | 13                | 0                 | 0              | 40                       |
|                                             | D | 65           | 46              | 17              | 26              | 26                | 0                 | 0              |                          |
|                                             | L | 55           | 32              | 1               | 7               | 3                 | 0                 | 0              |                          |
| 5                                           | I | 81           | 67              | 21              | 27              | 8                 | 0                 | 0              | 52                       |
|                                             | D | 53           | 46              | 21              | 39              | 72                | 0                 | 0              |                          |
|                                             | L | 43           | 31              | 4               | 11              | 6                 | 0                 | 0              |                          |
| 6                                           | I | 90           | 68              | 58              | 32              | 11                | 0                 | 0              | 19                       |
|                                             | D | 57           | 35              | 33              | 21              | 24                | 0                 | 0              |                          |
|                                             | L | 51           | 24              | 19              | 7               | 3                 | 0                 | 0              |                          |
| 8                                           | I | 80           | 60              | 10              | 60              | 20                | 0                 | 0              | 20                       |
|                                             | D | 50           | 34              | 13              | 35              | 39                | 0                 | 0              |                          |
|                                             | L | 40           | 20              | 1               | 21              | 8                 | 0                 | 0              |                          |
| 9                                           | I | 75           | 50              | 22              | 39              | 42                | 0                 | 6              | 36                       |
|                                             | D | 43           | 41              | 24              | 45              | 43                | 0                 | 36             |                          |
|                                             | L | 32           | 20              | 5               | 18              | 18                | 0                 | 2              |                          |
| O<br>V<br>E<br>R<br>A<br>L<br>L             | I | 83           | 61              | 22              | 28              | 14                | 0                 | 1              | 223                      |
|                                             | D | 58           | 44              | 23              | 35              | 52                | 0                 | 36             |                          |
|                                             | L | 48           | 27              | 5               | 10              | 7                 | 0                 | <1             |                          |

I = Incidence of infection ( expressed as a percentage of the quarters ).

D = Average duration of infection ( expressed as a percentage of the period ).

L = Level of Infection ( = I x D, expressed as a percentage ).

**TABLE 5.3**

**BACTERIOLOGICAL FINDINGS AT A SUBSEQUENT SAMPLING,**  
**GIVEN A SPECIFIC FINDING AT AN ARBITRARY FIRST SAMPLING**

| FINDINGS AT SUBSEQUENT SAMPLING            |      |              |                 |                 |                 |                   |                  |                 |     |
|--------------------------------------------|------|--------------|-----------------|-----------------|-----------------|-------------------|------------------|-----------------|-----|
|                                            | HERD | NO<br>GROWTH | CORYNE<br>BOVIS | COAG -<br>STAPH | COAG +<br>STAPH | STREPT<br>NONAGAL | STREPT<br>AGALAC | MIXED<br>GROWTH | #   |
| FINDINGS<br>AT AN<br>ARBITRARY<br>SAMPLING |      |              |                 |                 |                 |                   |                  |                 |     |
| NO<br>GROWTH                               | A    | 96           | 2               | 1               | <1              | <1                | 0                | <1              | 714 |
|                                            | B    | 80           | 13              | 4               | 2               | <1                | 0                | <1              | 378 |
|                                            | C    | 76           | 16              | 4               | 3               | <1                | 0                | <1              | 732 |
| CORYNE<br>BOVIS                            | A    | 18           | 79              | 0               | 0               | 3                 | 0                | 0               | 67  |
|                                            | B    | 4            | 90              | <1              | 1               | 0                 | 0                | 4               | 313 |
|                                            | C    | 10           | 86              | <1              | 1               | 1                 | 0                | 1               | 350 |
| COAG -<br>STAPH                            | A    | 30           | 0               | 65              | 5               | 0                 | 0                | 0               | 20  |
|                                            | B    | 76           | 6               | 6               | 6               | 0                 | 0                | 6               | 17  |
|                                            | C    | 34           | 13              | 43              | 3               | 3                 | 0                | 4               | 68  |
| COAG +<br>STAPH                            | A    | 9            | 0               | 2               | 89              | 0                 | 0                | 0               | 45  |
|                                            | B    | 15           | 13              | 0               | 59              | 0                 | 0                | 13              | 47  |
|                                            | C    | 17           | 5               | 2               | 68              | 3                 | 0                | 5               | 143 |
| STREPT<br>NONAGL                           | A    | 6            | 0               | 0               | 0               | 94                | 0                | 0               | 36  |
|                                            | B    | 15           | 15              | 0               | 8               | 31                | 31               | 0               | 13  |
|                                            | C    | 8            | 1               | 0               | 0               | 80                | 0                | 11              | 83  |
| STREPT<br>AGALAC                           | A    | 0            | 0               | 0               | 0               | 0                 | 0                | 0               | 0   |
|                                            | B    | 0            | 0               | 0               | 0               | 33                | 67               | 0               | 3   |
|                                            | C    | 0            | 0               | 0               | 0               | 0                 | 0                | 0               | 0   |
| MIXED<br>GROWTH                            | A    | 0            | 0               | 0               | 0               | 100               | 0                | 0               | 1   |
|                                            | B    | 3            | 52              | 3               | 13              | 13                | 0                | 16              | 31  |
|                                            | C    | 15           | 28              | 0               | 7               | 13                | 0                | 37              | 46  |

All figures are expressed as a percentage of quarters.

TABLE 5.4a

THE NUMBER OF DIFFERENT SPECIES OF BACTERIA  
ISOLATED PER QUARTER, DURING THE LACTATION - HERD A

| PARITY  | NUMBER OF<br>DIFFERENT<br>SPECIES<br>ISOLATED | 0  | 1  | 2 | 3 | 4 | Average |
|---------|-----------------------------------------------|----|----|---|---|---|---------|
|         | PERCENTAGE OF QUARTERS                        |    |    |   |   |   |         |
| 1       | 81                                            | 19 | 0  | 0 | 0 | 0 | 0.19    |
| 2       | 72                                            | 25 | 3  | 0 | 0 | 0 | 0.31    |
| 3       | 65                                            | 35 | 0  | 0 | 0 | 0 | 0.35    |
| 4       | 41                                            | 47 | 12 | 0 | 0 | 0 | 0.72    |
| Overall | 65                                            | 31 | 4  | 0 | 0 | 0 | 0.39    |

TABLE 5.4b

THE NUMBER OF DIFFERENT SPECIES OF BACTERIA  
ISOLATED PER QUARTER, DURING THE LACTATION - HERD B

|         | NUMBER OF<br>DIFFERENT<br>SPECIES<br>ISOLATED | 0  | 1  | 2  | 3  | 4  | Average |
|---------|-----------------------------------------------|----|----|----|----|----|---------|
| PARITY  | PERCENTAGE OF QUARTERS                        |    |    |    |    |    |         |
| 1       |                                               | 36 | 47 | 14 | 0  | 3  | 0.86    |
| 2       |                                               | 16 | 61 | 20 | 3  | 0  | 1.10    |
| 3       |                                               | 10 | 48 | 36 | 6  | 0  | 1.39    |
| 4       |                                               | 9  | 50 | 28 | 13 | 0  | 1.44    |
| Overall |                                               | 18 | 51 | 24 | 6  | <1 | 1.18    |

TABLE 5.4c

THE NUMBER OF DIFFERENT SPECIES OF BACTERIA  
ISOLATED PER QUARTER, DURING THE LACTATION - HERD C

| NUMBER OF<br>DIFFERENT<br>SPECIES<br>ISOLATED | PERCENTAGE OF QUARTERS |    |    |    |    | Average |
|-----------------------------------------------|------------------------|----|----|----|----|---------|
|                                               | 0                      | 1  | 2  | 3  | 4  |         |
| PARITY                                        |                        |    |    |    |    |         |
| 1                                             | ND <sup>1</sup>        | ND | ND | ND | ND | ND      |
| 2                                             | 30                     | 50 | 15 | 5  | 0  | 0.95    |
| 3                                             | 38                     | 31 | 31 | 0  | 0  | 0.94    |
| 4                                             | 23                     | 55 | 13 | 8  | 1  | 1.18    |
| 5                                             | 15                     | 48 | 35 | 2  | 0  | 1.23    |
| 6                                             | 16                     | 21 | 47 | 16 | 0  | 1.63    |
| 8                                             | 10                     | 45 | 30 | 15 | 0  | 1.50    |
| 9                                             | 0                      | 53 | 33 | 14 | 0  | 1.61    |
| OVERALL                                       | 18                     | 47 | 27 | 8  | <1 | 1.27    |

1 ND = No data available

TABLE 5.5

THE PREVALENCE OF SPECIFIC INFECTIONS IN A NUMBER  
OF SURVEYS CARRIED OUT BY OTHER RESEARCHERS

|                                            | STUDY NUMBER ( SEE KEY - OVERLEAF ) |      |                 |      |      |      |      |      |
|--------------------------------------------|-------------------------------------|------|-----------------|------|------|------|------|------|
|                                            | 1                                   | 2    | 3a              | 3b   | 4a   | 4b   | 5    | 6    |
| Coag +<br>Staph                            | 26.6                                | 40.7 | 12.2            | 8.0  | 20.7 | 8.1  | 6.3  | 8.5  |
| Strept.<br>agalact                         | 15.9                                | 14.5 | 6.6             | 4.1  | 7.2  | 3.4  | 0.1  | 16.5 |
| Strept.<br>dysgalact                       | 3.1                                 | 4.0  | ND <sup>1</sup> | ND   | 3.5  | 1.1  | 1.3  | ND   |
| Strept.<br>uberis                          | 0.8                                 | <7.2 | ND              | ND   | 4.6  | 1.5  | 2.5  | ND   |
| Strept.<br>nonagal                         | ND                                  | ND   | 8.8             | 4.5  | ND   | ND   | ND   | 2.7  |
| Coliform                                   | ND                                  | 20.7 | 0.9             | 0.7  | ND   | 0.1  | 0.1  | 0.7  |
| Coag -<br>Staph                            | ND                                  | 31.1 | ND              | ND   | ND   | ND   | 5.3  | 11.0 |
| Coryne.<br>bovis                           | ND                                  | ND   | ND              | ND   | ND   | ND   | ND   | 37.4 |
| Coag -<br>Staph<br>and<br>Coryne.<br>bovis | ND                                  | ND   | 53.1            | 60.1 | 70.0 | 43.5 | ND   | ND   |
| Total<br>Infected                          | 40.6                                | ND   | 92.9            | 85.9 | ND   | 54.5 | 19.1 | 76.9 |

1 ND = NO DATA AVAILABLE

KEY TO TABLE 5.5

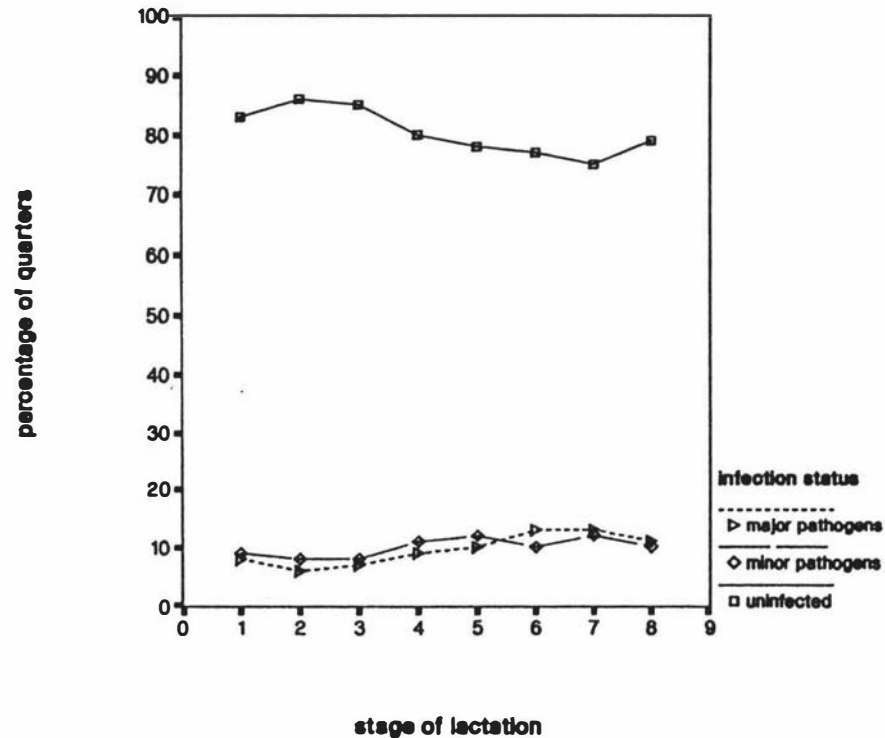
- 
- |                                     |   |                                                                                                                                                        |
|-------------------------------------|---|--------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1 Brookbanks 1966                   | - | figures given represent the percentage of cows infected in a survey of 42 herds from New Zealand. Total of 2795 cows randomly selected.                |
| 2 Elliot <u>et al</u> 1976          | - | figures given represent the percentage of cows infected in 399 randomly selected herds from throughout New Zealand. Total of 3956 samples.             |
| 3a Brooks <u>et al</u> 1982         | - | figures given represent the percentage of cows infected. Seventy four herds randomly selected in Ontario. Total of 2423 samples.                       |
| 3b Brooks <u>et al</u> 1982         | - | figures given represent the percentage of quarters infected. A total of 9602 samples from 74 Ontario dairy herds ( see 3a ).                           |
| 4a Wilson and Richards 1980.        | - | figures given represent the percentage of cows infected in 501 dairy herds throughout Britain, containing > 10 cows. Total of 27,526 cows.             |
| 4b Wilson and Richards 1980.        | - | figures given represent the percentage of quarters infected. A total of approximately 110,000 quarter samples ( see 4a )                               |
| 5 Schmidt Madsen <u>et al</u> 1974. | - | figures given represent the percentage of quarters infected from 24622 quarter samples, approximately 400 herds from four Danish dairying districts.   |
| 6 Hogan <u>et al</u> 1987           | - | figures given represent the percentage of quarters infected in four herds not using a teat dip. Approximately 800 quarter samples from Vermont, U.S.A. |
-

# PREVALENCE OF INFECTION DURING LACTATION - HERD A

**graph 5.1a**

prevalence of infection within quarters during the lactation

herd A

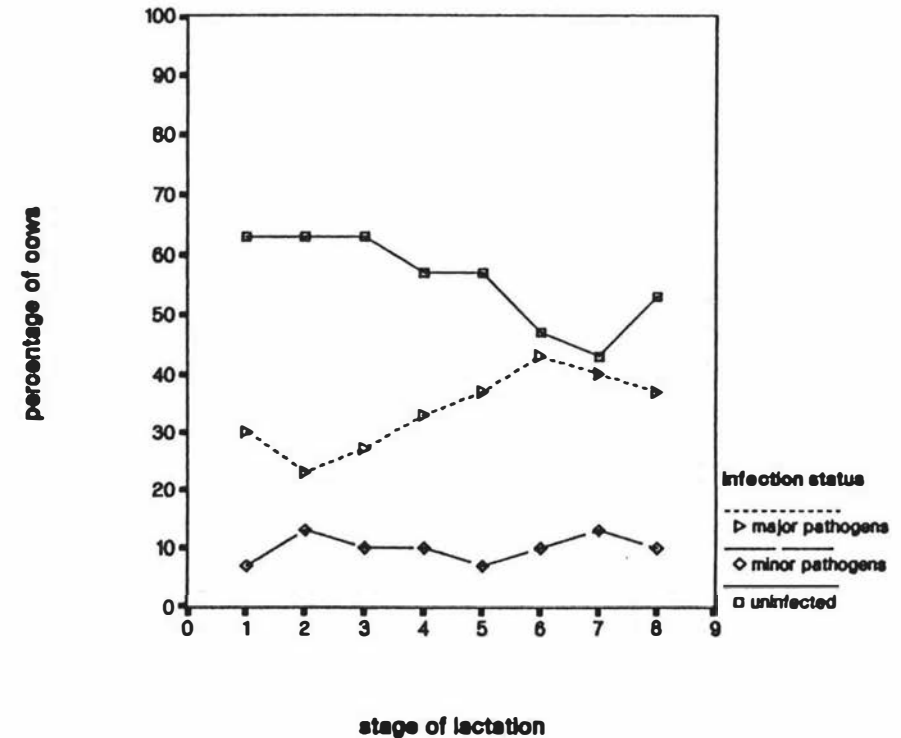


minor pathogens = quarters infected by minor pathogens only  
major pathogens = quarters may also have harboured minor pathogens

**graph 5.2a**

prevalence of infection within cows during the lactation

herd A



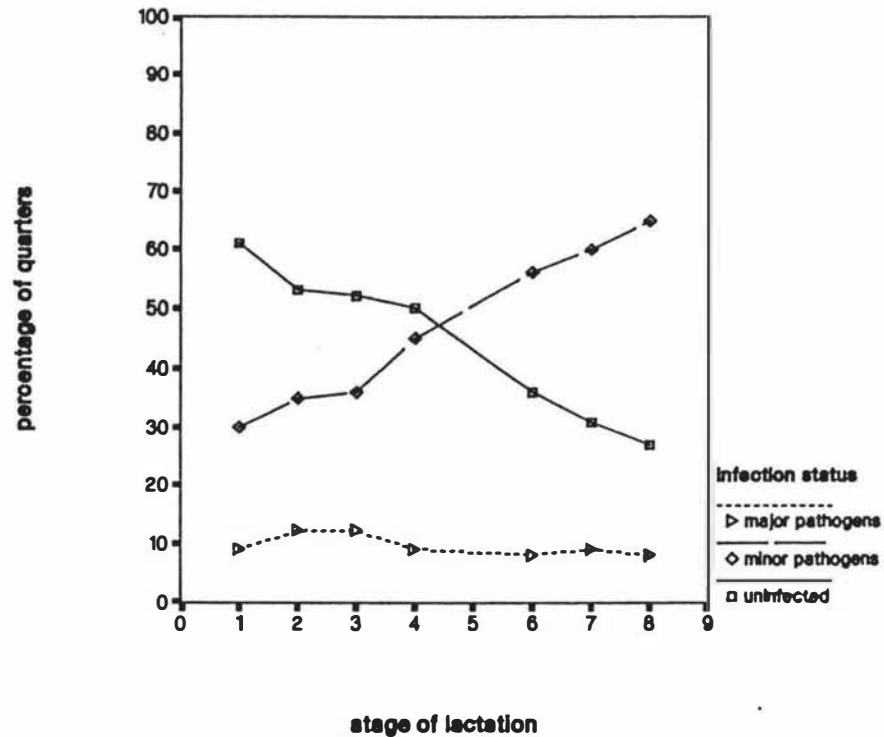
minor pathogens = cows infected by minor pathogens only  
major pathogens = cows may also have harboured minor pathogens

# PREVALENCE OF INFECTION DURING LACTATION - HERD B

**graph 5.1b**

prevalence of infection within quarters during the lactation

herd B

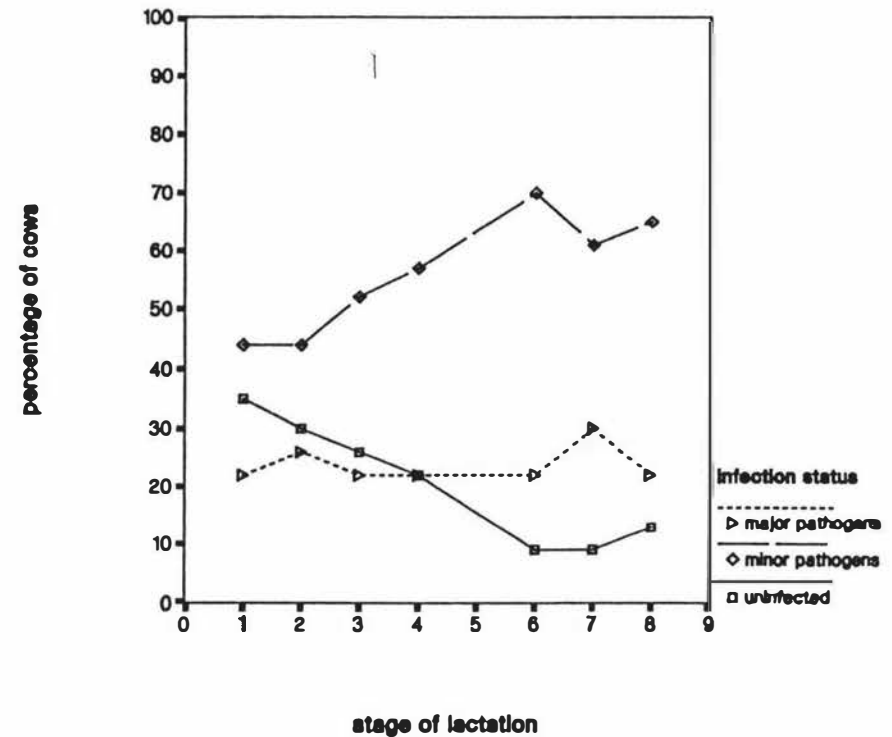


minor pathogens = quarters infected by minor pathogens only  
major pathogens = quarters may also have harboured minor pathogens

**graph 5.2b**

prevalence of infection within cows during the lactation

herd B



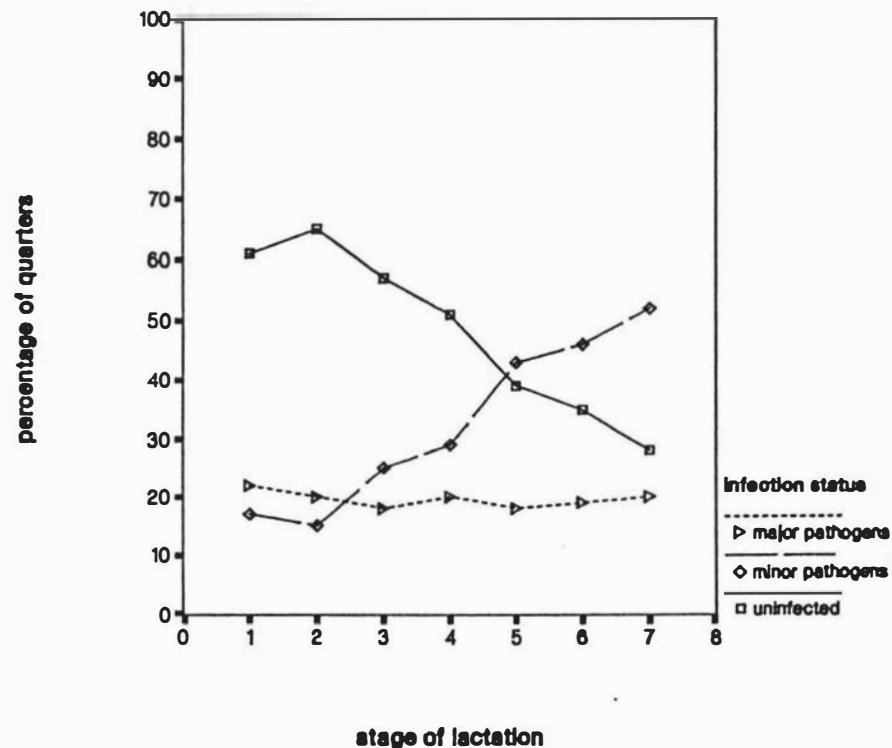
minor pathogens = cows infected by minor pathogens only  
major pathogens = cows may also have harboured minor pathogens

# PREVALENCE OF INFECTION DURING LACTATION - HERD C

**graph 5.1c**

prevalence of infection within quarters during the lactation

herd C

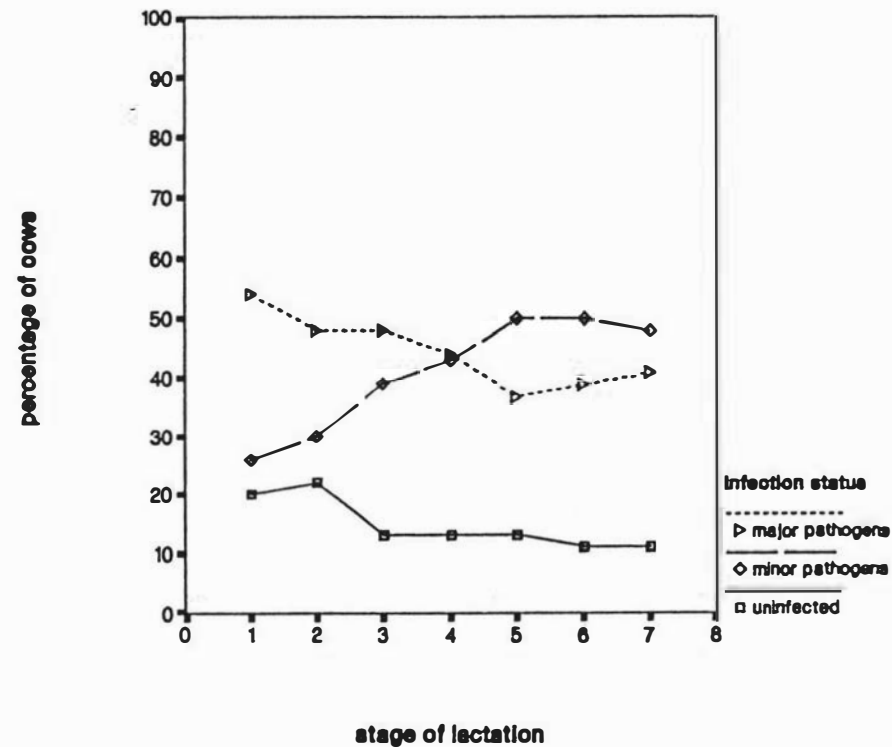


minor pathogens = quarters infected by minor pathogens only  
major pathogens = quarters may also have harboured minor pathogens

**graph 5.2c**

prevalence of infection within cows during the lactation

herd C



minor pathogens = cows infected by minor pathogens only  
major pathogens = cows may also have harboured minor pathogens

## CHAPTER SIX

### THE EFFECT OF BACTERIOLOGICAL STATUS, STAGE OF LACTATION AND AGE OF COW ON LEVELS OF PARAMETERS IN MILK FROM INDIVIDUAL QUARTERS

#### INTRODUCTION

An ideal indicator of bovine subclinical mastitis should be completely unaffected by factors other than the current health status of the quarter or udder. Should other factors exert an influence on a mastitis indicator, these factors and their degree of influence must be identified, if reliable interpretation of the health status is to be achieved.

A number of trials have been carried out during which the influence of environmental or physiological factors on mastitis indicators have been studied ( Natzke *et al*, 1972a; Reichmuth, 1975; Schultz, 1977a; Gill and Holmes, 1978; Lindström *et al*, 1981; Dohoo and Meek, 1982; Kennedy *et al*, 1982; Mijnen *et al*, 1982; Ng-Kwai-Hang *et al*, 1982; Sheldrake *et al*, 1983b; Jaartveld *et al*, 1983; Mattila and Sandholm, 1985; Mattila *et al*, 1986b; Berning *et al*, 1987a; Berning *et al*, 1987b; Emanuelson *et al*, 1988 ).

The results of these trials are not always in total agreement on the degree of influence of a factor on a particular mastitis indicator, perhaps reflecting the differing managemental and environmental conditions between the countries in which the trials have been performed.

In New Zealand, cows are grazed on pasture throughout the lactation with supplementary feed given if available, and as required, when pasture growth is reduced. In contrast, in many northern hemisphere countries, the animals are housed indoors during the colder winter months with consequent differences in the diet, bedding materials and degree of contact between animals.

Other factors may also differ between countries, such as the average size of the dairy herd and the predominant breeds of cow. These factors mean that results pertinent to cows in one region, may be less relevant for those in another region. For these reasons, it is important to study the effects of the physiological factors on potential indicators of mastitis under New Zealand conditions of management.

Differences are known to exist between the somatic cell counts of Friesian and of Swedish Red breed cows ( Brolund, 1985 ). The majority of cows in the present study were Friesian or Friesian-Jersey cross, with relatively few purebred Jersey cows. It was therefore not possible to test the effect of breed of cow on the mastitis indicators.

The season can influence the somatic cell count of the milk ( Nelson et al, 1967; Bodoh et al, 1976; Kennedy et al, 1982 ). Within New Zealand seasonal supply dairy herds, calving occurs over a relatively short period, so that at any given stage of the year, the effects of season will be confounded with those of stage of lactation.

The milk fraction used for analysis may affect the somatic cell count ( Paape and Tucker, 1966; Fernando et al, 1985; Berning et al, 1987a ) as well as other potential mastitis indicators including the concentrations of sodium ions, potassium ions, lactose, the electrical conductivity of milk, and the NAGase activity ( Fernando et al, 1985; Berning et al, 1987a ).

The effect of milk fraction on indicators of mastitis was addressed in chapter four, and will not be dealt with in the present chapter. All analyses in the present chapter were carried out on individual quarter foremilk samples, taken after the first 10 to 15 ml of milk had been discarded.

While it is desirable for an indicator of mastitis to be unaffected by these physiological factors, there should be a strong response on the part of the indicator to the presence of and/or damage caused by infecting microbes. The effect of infection on each of the selected indicators of mastitis will be dealt with in this chapter, in order to determine which of these indicators are likely to be of use in the diagnosis of subclinical mastitis.

Two factors which have been shown to affect indicators of mastitis are the age or parity of the cow, and the stage of lactation at which the milk sample is taken. The effect of these two factors on the somatic cell count, the pH of milk, the electrical conductivity, the NAGase activity, and the concentrations of sodium ions, potassium ions, lactose and antitrypsin will be studied in this chapter.

## MATERIALS AND METHODS

### (1) STATISTICAL ANALYSIS

Data from each of the three herds were analysed separately using SAS ( Statistical Analytical Systems, 1985 ). Repeated measures analysis was used to study the effects of age of cow, stage of lactation, bacteriological status and the interactions of these factors on each parameter, using the GLM ( General Linear Models ) package. The results of the analyses are given in table 6.1. All tables and graphs are located at the end of the chapter.

When an effect was found to be significant, the individual differences were determined using contrast analysis as the multiple comparison technique. Low numbers of quarters, particularly uninfected quarters of the older animals, precluded the analysis of the effect of age within bacteriological status in the present study.

### (2) BACTERIOLOGICAL STATUS

Quarters were divided into three bacteriological statuses on the basis of bacterial isolations during the season:

- (a) Status 0 quarters: these were quarters from which no pathogens, major or minor, were isolated at any stage during the season.
- (b) Status 1 quarters: these were quarters from which no major pathogens were isolated at any stage during the season.
- (c) Status 2 quarters: these were quarters from which major pathogens were isolated on three or fewer occasions during the season.
- (d) Status 3 quarters: these were quarters from which major pathogens were isolated on four or more occasions during the season.

The results of the contrast analyses between bacteriological statuses are given in table 6.2.

The status 0 quarters were not included in the original analysis, but were analysed separately in order to determine the effect of the stage of lactation on each parameter in quarters which were uninfected throughout the lactation. The results of these analyses are given in tables 6.5a, 6.5b and 6.5c for herds A, B, and C respectively.

### (3) AGE GROUPS

Cows within herds A and B ranged in age from 2 to 5 years, ie first to fourth parity, with approximately equal numbers in each category at the start of the trial. In herd C, the ages ranged from 3 to 10 years, ie second to ninth parity and for the analysis were divided into five age groups as follows:

- (a) Age group 3, comprising the three year old cows
- (b) Age group 4, comprising the four year old cows
- (c) Age group 5, comprising the five year old cows
- (d) Age group 6, comprising the 6 to 8 year old cows
- (e) Age group 7, comprising the 9 to 10 year old cows

Numbers of cows in each age group differed, and no two year old cows were included from herd C

The results of the contrast analyses between age groups are given in tables 6.3a, 6.3b and 6.3c for herds A, B and C respectively.

### (4) STAGES OF LACTATION

Milk samples which were taken during the first month after calving were not included in the analysis, but each cow was sampled monthly thereafter until each herd was dried off, giving a maximum of eight monthly samples which represent the eight stages of lactation. Part of the data for herd B during the fifth month was unavailable and the remaining data for that month was excluded from the data set to avoid effects of missing data.

The results of the contrast analyses between the stages of lactation are given in tables 6.4a, 6.4b and 6.4c for herds A, B and C respectively. Mean levels of each parameter at each month are given in graphical form, for bacteriological status and for age of cow.

Graph 6.9 shows the mean monthly milk production for each of the three herds.

## RESULTS AND DISCUSSION

### (1) SOMATIC CELL COUNT

#### (a) EFFECT OF BACTERIOLOGICAL STATUS

Graphs 6.1ai, 6.1bi and 6.1ci show the mean values of log (10) somatic cell count for the different statuses at each stage of lactation, for the three herds A, B and C respectively.

The effect of the bacteriological status on the somatic cell count was highly significant within each of the three herds (  $p < 0.01$  ). Within each herd, status 0 quarters showed lower somatic cell counts than did those of the other statuses, although the significance of the difference was not tested. Within each herd, status 3 quarters showed significantly higher somatic cell counts than did quarters of either status 1 or status 2. There was no significant difference between status 1 and 2 quarters within herd A, but status 2 quarters showed significantly higher somatic cell counts than did status 1 quarters in herds B and C (  $p < 0.05$  and  $p < 0.01$  respectively ).

While status 1 and 2 quarters did not differ significantly with respect to the somatic cell count within herd A, there was a highly significant stage by status interaction effect for this herd, with status 2 quarters showing significantly higher somatic cell counts than did status 1 quarters during stages 6 to 8 of lactation. From graph 5.1a it may be seen that the number of quarters which were infected with a major pathogen increased during this period, indicating that most of the status 2 quarters probably became infected at this stage, rather than earlier in the season.

The increase in somatic cell count with bacterial infection has been well recognized ( Giesecke and Van den Heever, 1974 ) and the somatic cell count has become the standard method for the routine diagnosis of subclinical mastitis in New Zealand, and in other dairying countries.

A number of authors have shown differences in the mean cell counts of udder quarters which are infected with different pathogens ( McEwen and Cooper, 1947; Blackburn, 1968; Ward and Schultz, 1972 ). However, it does not appear possible to differentiate between species of pathogen on the basis of the somatic cell counts alone ( Dohoo and Meek, 1982 ).

### (b) EFFECT OF STAGE OF LACTATION

The effect of stage of lactation on the somatic cell count was highly significant within each of the three herds (  $p < 0.01$  ), with an increase in the somatic cell count of the milk occurring during the later stages of the lactation. In each of the three herds, the status 0 quarters showed similar changes to those observed in the other statuses.

Within herd A, a slight decrease in the cell count occurred prior to stage 4, while there was a sharp increase between stages 6 to 7. No significant change in the somatic cell count occurred during the final month of lactation. The decrease in cell count between stages 3 to 4 may have been partly due to the greater percentage of quarters which were infected by a major pathogen during the first three months ( graph 5.1a ), although a similar decrease was seen both in status 0 quarters, which were not infected, and in status 1 quarters, which were infected by minor pathogens only. Data for individual quarter milk yield are not available, but composite milk samples, comprised of the milk from all four quarters, were taken at each sampling.

While the mean somatic cell count decreased over the first four months, so too did the composite milk yields ( see graph 6.9 ). Although the use of composite yield data may not necessarily reflect the situation within individual quarters, due to the possibility of yield compensation ( Woolford *et al.*, 1983 ), it would appear that the decrease in the somatic cell count of quarter samples cannot be fully explained by a simple dilution effect due to increased milk production.

The increase in the somatic cell count between months 6 to 7 may be partly attributed to the preceeding increase in the percentage of infected quarters ( graph 5.1a ). However, a corresponding increase in cell count (  $p < 0.05$  ) was seen in status 0 quarters. This was associated with an increase in the composite milk yield due to the increased pasture availability caused by more favourable climatic conditions ( graph 6.9 ), therefore simple concentration effects cannot fully explain the increase in cell count.

An increase in the somatic cell count may occur during the later stages of lactation, in quarters which are uninfected ( Sheldrake *et al.*, 1983b; Brolund, 1985; Emanuelson *et al.*, 1988 ). In the cow, involutionary changes do not proceed uniformly throughout the year, with involuting lobules and active lobules often being found side by side ( Lascelles and Lee, 1978 ). Involution is associated with an increase in the somatic cells ( Lee *et al.*, 1969 ), thus the increase in the cell count may be attributed to involutionary changes in some lobules during advancing lactation.

Within herd B, the nonsignificant decrease in cell count between stages 1 to 2, was followed by a significant decrease in cell count between stages 2 to 3 (  $p < 0.01$  ). From

stages 3 to 6 there were significant monthly increases in cell count (  $p < 0.05$  or less ), with no significant change during the final month. As with herd A, the decrease in cell count early in the lactation was not associated with an increase in the composite milk yield ( graph 6.9 ). The increase in somatic cell count from stages 3 to 6 was associated with a decrease in the composite milk yield, although the increase in cell count between stages 6 to 7 was highly significant (  $p < 0.01$  ) despite an increase in the milk yield during that month. While the milk yield decreased further during the final month, the mean somatic cell count did not change significantly.

Within herd C, the somatic cell count increased significantly (  $p < 0.01$  ) from stages 1 to 5. There was no significant change in the somatic cell count during the final two months of sampling. Composite milk yields for herd C decreased during stages 1 to 5 and this may have contributed to the observed increase in the somatic cell counts.

Because of the shortage of feed due to climatic conditions over the summer, the cows in herd C were dried off prior to the sampling being completed for stage 8.

#### (c) EFFECT OF AGE OF COW

Graphs 6.1aii, 6.1bii and 6.1cii show the mean values for log (10) somatic cell counts for the different age groups, at each month, for the three herds A, B and C respectively.

The effect of age on the somatic cell count was significant only within herd C (  $p < 0.05$  ), in which an increase in somatic cell count with age was observed. The age group 7 animals, ie those cows aged nine years or older, showed significantly higher cell counts than did any other age group except for the age group 5 animals. There were no significant differences in somatic cell count between the age groups 3 to 4, or 5 to 6, with all other age group pairs being significantly different (  $p < 0.05$  or less ).

An increase in somatic cell counts with age of cow has been shown by a number of authors ( Waite and Blackburn, 1957; Lindström *et al.*, 1981; Brolund, 1985; Emanuelson *et al.*, 1988 ). Much of this increase may be attributed to the increase in the prevalence or the severity of infection with advancing age ( Duitschaeffer and Ashton, 1972; Reichmuth, 1975 ) rather than to any physiological process, although Natzke *et al.* ( 1972a ) have reported small, statistically nonsignificant increases with advancing age in composite milk samples from uninfected cows. During the present trial the incidence of infection increased with advancing age of the cow ( chapter five ).

While the effects of age on the somatic cell count were not significant for herds A or B, in both herds, the stage by age interaction was highly significant (  $p < 0.01$  ). Within herd A, five year old cows showed significantly higher somatic cell counts than did all

other age groups during the final month of lactation, and significantly higher cell counts than those of the three year old animals during the final three months.

Herd A had a very low incidence of infection, and a history of low bulk milk somatic cell counts ( see chapters three and five ). Thus a large increase in somatic cell count with age was not expected.

Within herd B, the two year old cows showed significantly lower (  $p < 0.01$  ) cell counts than did the other age groups during the second half of lactation, while only the 2 and 5 year old cows differed prior to this. Herd B had a history of medium to high bulk milk somatic cell counts, and a high incidence of clinical mastitis, therefore an increase in the cell count with age might have been predicted. While the main effect of age on the somatic cell count within this herd was nonsignificant, there were significant differences between the age groups towards the end of the season.

The cows within herds A and B were aged from 2 to 5 years, in contrast to those of herd C, which covered a wider range of ages. This, plus the smaller number of quarters available for analysis in herd B may account for the greater effect of age on the somatic cell count within herd C than was the case within herds A or B.

## (2) SODIUM CONCENTRATION

### (a) EFFECT OF BACTERIOLOGICAL STATUS

Graphs 6.2ai, 6.2bi and 6.2ci show the mean values for the log (10) sodium concentration, expressed as millimoles per litre, for the different bacteriological statuses, at each stage of lactation, in herds A, B and C respectively.

The effect of the bacteriological status on the sodium concentration was highly significant within each of the three herds. In each herd, status 0 quarters showed a lower sodium concentration than did the those of other statuses, although the statistical significance of this difference was not tested.

In each of the three herds, the status 3 quarters showed a significantly higher sodium concentration than did those of either status 1 or status 2 (  $p < 0.01$  ). There was no significant difference between status 1 or 2 quarters with respect to sodium concentration in herds A or B, but within herd C status 2 quarters showed a significantly higher sodium concentration than did status 1 quarters (  $p < 0.05$  ).

Within herds A and C, the sodium concentration of quarters within each bacteriological status behaved similarly across the lactation; in herd B there was a significant stage by

status interaction effect on the sodium concentration of the milk. The status 2 quarters showed a high sodium concentration at stage 1, and consequently there was no significant difference between the sodium concentration in status 2 and 3 quarters at stage 1 of lactation. While status 1 and 2 quarters did not differ overall with respect to sodium concentration, they did differ significantly at the first sampling.

Bacterial infection is associated with an increase in the sodium concentration of the milk ( Wheelock *et al*, 1966; Wegner and Stull, 1978; Daniel and Pavithran, 1985 ).

The sodium concentration is closely correlated with the somatic cell count of the milk ( Corbellini, 1984; Wegner and Stull, 1978 ).

Bacterial infection of the udder is associated with a much greater degree of involution than is normally observed at a given stage of lactation ( Waite and Blackburn, 1963 ). The changes in the sodium concentration which occur during involution may be explained at least in part by the operation of the " paracellular pathway " ( Peaker, 1977 ) in which ions may be exchanged between the milk and the extracellular fluid by passage between, as opposed to through, the secretory cells.

#### (b) EFFECT OF STAGE OF LACTATION

The effect of the stage of lactation on the sodium concentration was highly significant in each of the three herds (  $p < 0.01$  ), with an initial decrease in the sodium concentration being followed by increases as the season progressed. These changes were seen both for the infected quarters, and for those of status 0.

Within herd A, the slight decrease in the sodium concentration which occurred between stages 2 to 3 (  $p < 0.05$  ), was followed by an increase between stages 3 to 4. There were further significant increases in sodium concentration from stage 5 onwards (  $p < 0.01$  ). The composite milk yield decreased from stages 1 to 6 and this may have contributed to the observed increases in sodium concentration. However, the increase in sodium concentration between stages 6 to 7 occurred in conjunction with an increase in the milk yield, so concentration effects alone cannot explain the changes, which may be related to the unequal rate of involution of individual lobules ( see discussion of somatic cell count results ).

Within herd B there was an initial decrease (  $p < 0.05$  ) in the sodium concentration between stages 1 to 2, with no further significant change during the next two months. The sodium concentration increased sharply between stages 4 to 6 (  $p < 0.01$  ) and increased again during the final stage of lactation.

The initial decrease in sodium concentration in the overall figures was due largely to that of the status 2 quarters. During this early period there was an increase in the number of quarters which harboured major pathogens ( graph 5.1b ), and the sharp decrease in sodium concentration may have been due to a decreasing severity of infection. From stage 2 onwards, the sodium concentration showed an inverse relationship with the composite milk yield, this being true both for the infected and for the uninfected quarters. The percentage of quarters which were infected with a major pathogen decreased during the later stages of lactation, yet the sodium concentration continued to increase for all quarters.

Within herd C there was an initial decrease in the sodium concentration (  $p < 0.05$  ) between stages 1 to 2. From stage 3 onwards there were significant monthly increases (  $p < 0.01$  ) at each stage. Each status showed a similar pattern over the season, although statistical significance was not reached in every instance. As was the case for the other herds, the composite milk yield decreased as the sodium concentration increased. This suggests that concentration effects may have contributed to the results. However, there was an increase in milk yield between stages 5 to 6, at which time the sodium concentration also increased. The number of quarters which were infected with a major pathogen decreased slightly between stages 1 to 2, but remained relatively stable throughout the remainder of the season ( graph 5.1c ).

Little published data is available on the effects of the stage of lactation on the concentration of sodium ions in quarter samples, but a number of authors have shown changes in the sodium concentration in composite ( whole udder ) samples during the lactation. Rook and Campling ( 1965 ) observed comparatively high sodium concentrations in colostrum, with the concentration steady during early to mid lactation, but increasing towards the end of the lactation. Similar findings in relation to late lactation have been reported by other authors ( Sbodio *et al.* 1985; Daniel and Pavithran, 1985 ). In contrast, Safwate *et al.* ( 1981 ) found a significant decrease in the sodium concentration during the first month postcalving (  $p < 0.01$  ), followed by an increase until day 50, after which the concentration remained steady until lactation ceased.

### (c) EFFECT OF AGE OF COW

Graphs 6.2a<sub>ii</sub>, 6.2b<sub>ii</sub> and 6.2c<sub>ii</sub> show the mean values of log (10) sodium concentration expressed as millimoles per litre for the different age groups, at each stage of lactation, in herds A, B and C respectively.

The effect of age on the sodium concentration was highly significant in all three herds (  $p < 0.01$  ), with an increase in the sodium concentration of the milk being associated with an increase in the age of the cow.

Within herd A, there was an overall increase in the sodium concentration with age, although not all of the age groups differed significantly. The five year old cows showed a higher concentration of sodium than did those animals aged 2 or 3 years (  $p < 0.05$  ), but the difference between the 4 to 5 year old cows did not reach significance. The two year old cows did not show significantly lower levels of sodium than did the 3 or 4 year old animals.

There was a significant age by stage interaction effect on the sodium concentration in herd A. A large increase in the sodium concentration at stage 7 in the four year old cows caused significant differences between ages 2 to 4 and ages 3 to 4 at this stage, while overall, these age groups did not differ significantly with respect to sodium concentration.

Within herd B, the five year old cows showed a higher sodium concentration than did any of the other age groups (  $p < 0.01$  ). Although there were no significant differences between cows in the other age groups overall, there was a significant age by stage interaction effect on the sodium concentration within herd B, the four year old cows showing a higher sodium concentration than did the 2 or 3 year old cows at stage 1 of lactation (  $p < 0.05$  ). The difference in sodium concentration between the age groups tended to be greatest towards the end of the lactation.

Within herd C, there was a higher sodium concentration in the oldest age group, ie those animals nine years or older, than in any other age group (  $p < 0.01$  ). None of the other age groups differed significantly, but there was a significant age by stage interaction effect on sodium concentration in herd C, with significant differences between some of the other age groups occurring at individual samplings, particularly towards the end of the season. Thus at stages 6 and 7, the three year old cows showed a significantly lower sodium concentration than was seen in cows aged five years or older (  $p < 0.05$  or less ). Thus while only the oldest age group differed significantly overall, there was a trend towards increasing sodium concentration with age, particularly towards the end of the lactation. To what extent the increases in sodium with age were due to infection, or to an increase in the baseline levels is unclear. Numbers of observations were too small to enable an effect of age within status to be assessed.

### (3) POTASSIUM CONCENTRATION

#### (a) EFFECT OF BACTERIOLOGICAL STATUS

Graphs 6.3ai, 6.3bi and 6.3ci show the mean values for log (10) potassium concentration expressed as millimoles per litre, at each month for the different bacteriological statuses in herds A, B, and C respectively.

The effect of bacteriological status on the potassium concentration of the milk was highly significant in herd B (  $p < 0.01$  ), but was not significant in either herd A or herd C.

The potassium concentration in the status 0 quarters was similar to that of status 1 quarters in herds A and C. The potassium concentration of status 0 quarters was slightly lower than that from status 1 quarters within herd B, although the difference was not tested for significance. Within herd B, the status 3 quarters showed a significantly lower mean potassium concentration than did either statuses 1 or 2 (  $p < 0.01$  ). The difference between status 1 and 2 quarters did not reach significance at the 5 % level.

While the overall effects of status on the potassium concentration were not significant within herd A, there was a significant stage by status effect in this herd (  $p < 0.05$  ). Status 1 quarters showed a higher potassium concentration in stages 7 and 8 (  $p < 0.05$  ) than did status 3 quarters, while statuses 1 and 2 differed only at stage 7. Status 2 and 3 quarters did not differ significantly at any stage of lactation with respect to milk potassium concentration.

There was a significant stage by status interaction effect on the potassium concentration within herd B, with the status 3 quarters showing a lower concentration of potassium than did status 1 quarters in all but stage 2, and a lower concentration than status 2 quarters from stage 4 onwards (  $p < 0.05$  or less ). Status 2 quarters showed a significantly lower potassium concentration than did status 1 quarters at stage 1 of the lactation only.

The stage by status interaction effect on the potassium concentration was also significant for herd C. While the potassium concentration in quarters of statuses 1 and 3 did not differ overall, the potassium concentration in status 3 quarters was significantly lower than that of status 1 quarters from stages 3 to 7 (  $p < 0.05$  or less ). The mean potassium concentration in quarters of statuses 2 and 3 also differed significantly towards the end of the season (  $p < 0.05$  ).

Other researchers have found the potassium concentration to decrease as a result of infection. During experimental mastitis, the concentration of potassium in the milk is

invariably reduced ( Wheelock *et al.*, 1966 ), while similar findings have been reported in cows with naturally acquired infection ( Wegner and Stull, 1978 ). A lower concentration of potassium occurs in the milk from infected quarters than that from healthy quarters, the difference being greater when strippings rather than foremilk samples are analysed ( Fernando *et al.*, 1985 ).

The potassium concentration of the milk decreases during involution of the mammary gland ( Thompson, 1988 ). As is the case for the sodium concentration, changes in the milk potassium in the presence of infection are probably due to the operation of the paracellular pathway in areas of the gland which have undergone damage and involution, this pathway allowing potassium ions to diffuse out of the milk and into the extracellular fluid ( Holt, 1985 ).

#### (b) EFFECT OF STAGE OF LACTATION

The effect of stage of lactation on the potassium concentration of milk was highly significant in each of the three herds, with lower values being observed in the later stages of lactation. The pattern seen in status 0 quarters was similar to that observed for those of the other statuses.

Within herd A, the initial increase in the potassium concentration was followed by a decrease during midlactation. The potassium concentration did not change significantly between stages 6 to 7, but decreased again during the final month of lactation.

The potassium concentration of milk from herd B quarters fluctuated between stages 1 to 4, decreasing significantly from stage 4 until the cows were dried off (  $p < 0.05$  or less ). Status 3 quarters showed a decrease in potassium concentration from stages 2 to 7 (  $p < 0.05$  or less ). During this period the percentage of quarters infected with major pathogens halved. This may indicate an increasing severity of infection in those quarters which remained infected.

Within herd C, there were no significant changes in the potassium concentration during the first four stages. Between stages 4 and 5 there was a significant decrease (  $p < 0.01$  ), with a second decrease in the concentration of potassium during the final month of lactation (  $p < 0.01$  ). Similar changes in potassium concentration were observed within status 0 quarters as within those quarters which contained pathogens.

The status 3 quarters showed a greater decrease in milk potassium concentration than did those of status 1 or 2, suggesting an increasing severity of infection. The number of quarters infected with major pathogens changed little during the season in herd C ( graph 5.1c ). There was a decrease in the composite milk yield during the season, although a temporary increase in yield occurred between stages 5 to 6. This decreased

yield tends to mask the decrease in the potassium concentration to some extent, while accentuating increases in parameters such as the somatic cell count, or the sodium concentration.

Other researchers have found similar results, with little change in the potassium concentration early in the season, but a decrease in concentration towards the end of the lactation ( White and Davies, 1958; Rook and Campling, 1965 ). Sbodio *et al.* ( 1985 ) reported a gradual decrease in potassium concentration as lactation advanced, while Daniel and Pavithran ( 1985 ) reported the potassium concentration to be at a maximum at midlactation in composite milk samples.

While significant changes in potassium concentration did occur during the course of the lactation in the present study, the scale of these changes were generally smaller than were those seen with the sodium concentration of milk.

#### (c) EFFECT OF AGE OF COW

Graphs 6.3aii, 6.3bii and 6.3cii show the mean values for log (10) potassium concentration expressed as millimoles per litre, for each age group, at each stage of lactation for the herds A, B and C respectively.

The effect of age on the potassium concentration was highly significant in herds A and B (  $p < 0.01$  ) and significant in herd C (  $p < 0.05$  ), with the older animals tending to show a lower potassium concentration than did their younger herdmates.

Within herd A, the four year old cows had a significantly lower potassium concentration than did any other age group, while that of age groups 2, 3 and 5 did not differ significantly overall.

The effect of the age by stage interaction on the potassium concentration was highly significant in herd A. During the first four stages, the two year old cows had a significantly lower mean potassium concentration than did the three year old cows (  $p < 0.05$  ), while during the first two stages the two year old cows showed a lower potassium concentration than did the five year old cows (  $p < 0.05$  ). Thus there was no clear pattern of decrease in potassium concentration with age, despite the increasing incidence of infection which occurred with age ( table 5.2a ).

By contrast, herd B did show evidence of a decreasing milk potassium concentration with age, in that there existed significant differences (  $p < 0.05$  or less ) between the mean potassium concentration of milk from the five year old cows, and that for each of the other age groups. The mean potassium concentrations for the 2, 3 and 4 year old cows did not differ overall. The age by stage interaction effect on the potassium

concentration was highly significant (  $p < 0.01$  ), with the greatest differences in potassium concentration occurring between age groups, towards the end of lactation.

Within herd C the four year old cows showed a significantly higher (  $p < 0.05$  or less ) potassium concentration than did any other age group except the three year old cows. The age group 7 cows, ie those cows nine years and older, showed a significantly lower potassium concentration than did the age group 3, 4 or 6 cows (  $p < 0.05$  or less ), although significance between age groups 5 and 7 was not reached.

As was the case with the other herds, the age by stage interaction effect on the potassium concentration was highly significant within herd C (  $p < 0.01$  ). Differences between the mean potassium levels for the age groups varied during the lactation, but showed no clear pattern, other than an increase in these differences towards the end of the lactation.

Little recently published data is available regarding the effect of age on the potassium concentration of milk.

In the present study, only the eldest age groups in herds B and C showed a significantly lower potassium concentration than did their younger herdsmates. In herd A, although the effect of age was significant, there was no obvious trend towards a decrease in potassium concentration as the age of the cow increased.

#### (4) ELECTRICAL CONDUCTIVITY

##### (a) EFFECT OF BACTERIOLOGICAL STATUS

Graphs 6.4ai and 6.4bi show the mean values for log (10) electrical conductivity at each month, for the various bacteriological statuses in herds A and B respectively. Electrical conductivity measurements were not carried out on samples from herd C.

Bacteriological status exerted a significant effect (  $p < 0.05$  ) on electrical conductivity in both herds A and B.

Within herd A, the status 0 quarters showed similar conductivity readings to those of status 1 quarters, while in herd B the conductivity of status 0 quarters was lower than that seen in status 1 quarters, suggesting that in herd B, the minor pathogens exerted some effect on milk conductivity, although the significance of these differences was not tested in either herd.

In both herds A and B, the status 3 quarters showed significantly higher electrical conductivity values than did either status 1 or status 2 quarters (  $p < 0.05$  ), while the conductivity of status 1 and 2 quarters did not differ significantly.

The three components which are largely responsible for the electrical conductivity of milk are sodium, potassium and chloride ions. Milk has a similar osmolarity to that of the extracellular fluid. During mastitis, the concentration of sodium and of chloride ions in milk increase, while that of the potassium decreases. The lactose concentration also decreases, but as lactose is un-ionized, the increase in the sodium and chloride ions more than compensate for the decrease in potassium ions, with the result that the electrical conductivity of the milk increases ( Linzell, 1975 ).

Infection of the quarter with either a major or a minor pathogen is associated with an increase in the electrical conductivity of the milk ( Fernando *et al*, 1982; Mijnen *et al*, 1982; Sheldrake *et al*, 1983b; Fernando *et al* 1985 ). Thus the findings of the present study support those of other workers, with respect to the effect of infection on the electrical conductivity of milk.

#### (b) EFFECT OF STAGE OF LACTATION

The effect of the stage of lactation on the electrical conductivity was highly significant within both herds A and B (  $p < 0.01$  ), with an increase in conductivity occurring towards the end of the lactation. These effects were seen both in infected and in status 0 quarters.

Within herd A, there was a significant increase in the milk conductivity between stages 1 to 2 (  $p < 0.01$  ) followed by decreases between stages 2 to 4. From stages 4 to 7 there were significant increases at each sampling (  $p < 0.05$  or less ) although there was a decrease in the conductivity during the final month of lactation. Although the composite milk yield increased between stages 6 to 7, there was no corresponding decrease in the electrical conductivity within herd A.

Within herd B, there was a nonsignificant increase in the conductivity between stages 1 to 2, with a further increase between stages 2 to 3. A significant decrease in electrical conductivity was recorded between stages 3 to 4 although an increase occurred between stages 4 to 6. The decrease in conductivity between stages 6 to 7, and the increase between stages 7 to 8 were significant (  $p < 0.01$  ).

While a similar pattern of change was observed across the lactation, between statuses within herd A, this was not the case within herd B, where there was a highly significant stage by status interaction effect on the electrical conductivity of the milk (  $p < 0.01$  ).

Within herd B the status 1 quarters showed an increase in conductivity between stages 1 and 2, while the conductivity of the status 2 quarters decreased sharply. A similar decrease was seen with the sodium concentration in status 2 quarters during this period while there was a smaller increase in potassium concentration.

Between stages 1 to 2, there was an increase in the number of quarters which were infected with a major pathogen ( graph 5.1b ), and the decrease in the conductivity in status 2 quarters did not follow the increases which occurred in statuses 0,1 and 3. It is possible that some of the status 2 quarters were infected around the time of calving, with the infections being eliminated between stages 1 to 2, ie months 2 to 3 of lactation, with a subsequent improvement in indicators of udder health such as electrical conductivity.

The increase in conductivity during lactation within herd B was greatest for the status 3 quarters, indicating an increase in the severity of established infections. The decrease in conductivity within each status between stages 6 to 7 was probably due to the increase in milk yield ( graph 6.9 ).

Other researchers have shown the electrical conductivity of milk to vary during the season. Linzell and Peaker ( 1975 ) reported that the conductivity reaches the normal lactation levels between 3 to 5 days postpartum, and that late lactation is not necessarily associated with an increase in the conductivity, although their conclusions were based on a small number of cows. Sheldrake *et al* ( 1983b ) found the effect of stage of lactation on the electrical conductivity of milk to be highly significant (  $p < 0.01$  ) with the conductivity being high following calving, but decreasing to a low point at 35 days postpartum in uninfected cows. The conductivity increased from day 120 until the end of the lactation.

The electrical conductivity of milk from quarters infected with major pathogens tends to increase during the course of the lactation ( Sheldrake *et al*, 1983b ). This feature was observed in both herds A and B during the present study but was more marked within herd B.

### (c) EFFECT OF AGE OF COW

Graphs 6.4a<sub>ii</sub> and 6.4b<sub>ii</sub> show the mean log (10) conductivity values at each month, for each age group in herds A and B respectively.

Age had a significant effect (  $p < 0.05$  ) on conductivity within both herds A and B, with some evidence of increasing conductivity with age being recorded.

Within herd A, the five year old cows showed significantly higher conductivity readings than did the 2 or 4 year old cows (  $p < 0.01$  ), although the three year old cows showed significantly higher readings than did the four year old cows. Thus while the oldest age group showed high conductivity values, there was not a linear increase in conductivity with age. From table 5.2a it may be seen that the average level of infection with coagulase positive staphylococci was low for the parity 3 cows, ie the four year old cows. This may partly explain the low conductivity of milk from the four year old cows, although the potassium concentration was also low in these samples.

The effect of the age by stage interaction on electrical conductivity was highly significant (  $p < 0.01$  ) for herd A, with the five year old cows showing greater conductivity readings than did the three year old cows only towards the end of the season. The three year old cows showed significantly higher conductivity readings than did the two year old cows during stages 1 to 5 of the lactation.

Within herd B the two year old cows showed lower conductivity readings than did either the 3 or the 5 year old cows. There were no significant differences between any other age group pairs.

The effect of the age by stage interaction on electrical conductivity was highly significant within herd B (  $p < 0.01$  ). While the 2 and 4 year old cows did not differ overall with respect to the electrical conductivity, the differences were significant at stages 1 to 3, with the four year olds showing the higher conductivity readings. The five year old cows showed higher readings than did the four year old cows at stages 6 and 8. Thus at certain stages of the season, a definite trend towards increasing electrical conductivity with age could be seen.

Age, or parity, of the cow has been shown by other workers to have a significant effect on the electrical conductivity of milk ( Sheldrake *et al*, 1983b ). These authors found little effect in quarters which were free of infection, while quarters which were infected with Staphylococcus aureus showed a marked increase in conductivity with age of the cow.

The low numbers of quarters, particularly uninfected quarters in older cows, precluded an analysis of the effect of the age within each bacteriological status during the present study.

## (5) pH OF MILK

### (a) EFFECT OF BACTERIOLOGICAL STATUS

Graphs 6.5ai and 6.5bi show the mean log (10) values for pH at each month, for the different bacteriological statuses, within herds A and B respectively.

The effect of the bacteriological status on the milk pH was highly significant within herd B (  $p < 0.01$  ), but was not statistically significant within herd A. pH readings were not recorded for herd C samples.

Within herd B, the status 3 quarters showed significantly higher pH values than did either status 1 or status 2 quarters (  $p < 0.01$  ).

Other workers have shown bacterial infection to cause an increase in the pH of the milk ( Feagan *et al.*, 1966b ). The mean pH of quarter foremilk samples and the corresponding bromothymol blue test scores increase with the somatic cell count of milk ( Marschke and Kitchen, 1981; Marschke and Kitchen, 1985 ).

While the pH of milk does increase in response to bacterial infection, the pH is of little use as an indicator of mastitis ( Mijnen *et al.*, 1982 ).

The acidity of normal milk is due to free acidic groups of casein, citrate and phosphate as well as to the presence of dissolved carbon dioxide ( Schalm *et al.*, 1971 ). Damage caused by bacterial toxins, and by the ensuing inflammatory response causes leakage between the extracellular fluid and milk compartments, allowing diffusion of these compounds, and of the bicarbonate ions, down their respective concentration gradients, with the nett result that the pH of the milk increases.

### (b) EFFECT OF STAGE OF LACTATION

The effect of the stage of lactation on the pH of the milk was highly significant in both herds A and B (  $p < 0.01$  ) with decreases in the milk pH occurring early in lactation, followed by increases during mid to late lactation, these changes being observed both within infected and in status 0 quarters.

Within herd A, an initial decrease in the pH (  $p < 0.01$  ) occurred between stages 1 to 2, this being followed by increases between stages 2 to 5 of lactation (  $p < 0.05$  or less ). There was a significant decrease in the pH between stages 5 to 6 (  $p < 0.01$  ). No samples were analysed for pH during month 7, and there was no significant change in the pH between stages 6 to 8.

The stage by status interaction effect on pH was highly significant for herd A (  $p < 0.01$  ), due largely to changes towards the end of the lactation, at which time the status 3 quarters showed significantly higher pH values than did status 1 quarters (  $p < 0.01$  ).

Within herd B there was a decrease in the pH between stages 1 to 2 (  $p < 0.01$  ), followed by increases between stages 2 to 4 (  $p < 0.01$  ). There was a significant decrease in the pH between stages 4 to 6 (  $p < 0.01$  ), with increases between stages 6 to 7 (  $p < 0.01$  ) and between stages 7 to 8 (  $p < 0.01$  ).

As was the case within herd A, the effect of the stage by status interaction was highly significant for herd B. Much larger increases in pH occurred in status 3 quarters towards the end of the season than occurred within the other statuses in herd B. Thus differences in the pH of milk from quarters which were infected for the majority of the season, and those which were uninfected or infected for only a portion of the season were greater towards the end of the season. This sharper increase in status 3 quarters was seen with other parameters within herd B, and points to an increasing severity of infection with advancing lactation.

The pH of milk is low at the beginning of the lactation, but increases rapidly to the midlactation value, thereafter increasing gradually until the end of lactation, at which time the increase becomes more rapid ( White and Davies, 1958 ). A similar increase in the pH of milk towards the end of lactation has been reported by Kossila ( 1967 ).

During the present trial, status 0 quarters showed similar changes to those described for the infected quarters, thus the changes which occurred during the season were not due merely to a change in the infection status.

### (c) EFFECT OF AGE OF COW

Graphs 6.5a<sub>ii</sub> and 6.5b<sub>ii</sub> give the mean log (10) pH values at each month, for the different age groups within herds A and B respectively.

The effect of age on milk pH was not significant in herd A, but was highly significant within herd B (  $p < 0.01$  ), although there was no clear pattern of increasing pH with

age of the cow. Within herd B, milk from the quarters of four year old cows showed significantly lower pH readings than did those from either the two year old or five year old animals (  $p < 0.05$  ).

While age per se was not a significant source of variation in pH within herd A, the stage by age interaction was highly significant (  $p < 0.01$  ). The two year old cows showed higher pH readings than did the other age groups at various stages of the season (  $p < 0.05$  or less ). The four year old cows showed significantly higher pH values than did their five year old herdmates at stages 1 and 2 (  $p < 0.01$  ) but lower values than the five year old cows at stage 6 (  $p < 0.05$  ).

Within herd B the stage by age interaction was highly significant  $p < 0.01$  ), but as was the case with herd A, there was no clear pattern of increasing pH with increasing age of cow at any stage of the lactation.

## (6) LACTOSE CONCENTRATION

### (a) EFFECT OF BACTERIOLOGICAL STATUS

Graphs 6.6ai, 6.6bi and 6.6ci give the mean log (10) lactose concentration at each month, for the different bacteriological statuses within herds A, B and C respectively.

The effect of bacteriological status on the lactose concentration was significant in herd A (  $p < 0.05$  ) and highly significant in herds B and C (  $p < 0.01$  ).

Within herds B and C, the status 0 quarters showed a higher lactose concentration than did the status 1 quarters, suggesting that the minor pathogens may cause a small decrease in the lactose concentration of milk, although the significance of the difference was not tested. Within herd A, the status 3 quarters showed a significantly lower mean lactose concentration than that recorded for status 1 quarters (  $p < 0.05$  ). Quarters of statuses 1 and 2, and 2 and 3 did not differ significantly with respect to lactose concentration. The stage by status interaction did not exert a significant effect on the lactose concentration of the milk within herd A.

In herd B, the status 3 quarters showed a lower lactose concentration than did either status 1 or 2 quarters (  $p < 0.01$  ), while status 1 and 2 quarters did not differ significantly.

The stage by status interaction was highly significant for the lactose concentration within herd B (  $p < 0.01$  ) with the lactose concentration of status 3 quarters declining

more rapidly than did those of the other statuses, giving a better separation between bacteriological statuses towards the end of the season.

Within herd C, status 3 quarters showed a significantly lower mean lactose concentration than did those of status 1 (  $p < 0.01$  ). Status 2 quarters also showed a lower lactose concentration than did status 1 quarters (  $p < 0.05$  ), but the difference between status 2 and 3 quarters did not quite reach significance at the 5 % level. The effect of the stage by status interaction on lactose concentration was not significant within herd C, suggesting that the quarters within each bacteriological status behaved in a similar manner with respect to lactose concentration across the season.

Other workers have shown bacterial infection to affect the lactose concentration of cow's milk, with a lower lactose concentration occurring in the milk from infected animals than that from healthy animals ( Mijnen *et al*, 1982; Daniel and Pavithran, 1985; Wiesner, 1985 ). The effect of infection on lactose concentration is greater for major than for minor pathogens ( Fernando *et al*, 1985 ).

The decrease in the lactose concentration which has been observed during infection may be due to either the reduced synthesis of lactose ( Wheelock *et al*, 1966 ), the breakdown of lactose by bacteria in the milk ( Wiesner, 1985 ) or to increased permeability of the secretory tissue, allowing the passage of lactose down the concentration gradient between milk and extracellular fluid. The three common streptococcal udder pathogens: Streptococcus agalactiae, Streptococcus dysgalactiae and Streptococcus uberis are all capable of fermenting lactose, as are staphylococci and coliforms ( Merchant and Packer, 1971 ). Evidence for the leakage of lactose has been shown in that the concentration of lactose in the urine increases during mastitis ( Wiesner, 1985 ).

#### (b) EFFECT OF STAGE OF LACTATION

The effect of stage of lactation on the concentration of lactose was highly significant in all three herds (  $p < 0.01$  ) with an initial increase, followed by a decrease in the lactose concentration later in the lactation. Status 0 quarters showed similar changes to those of the other statuses within each herd.

Within herd A, an initial increase in lactose concentration was seen between stages 1 and 2 (  $p < 0.01$  ) with decreases occurring between stages 3 to 6 (  $p < 0.05$  or less ). There was a significant increase in the lactose concentration between stages 6 to 7 (  $p < 0.01$  ) with no further change during the final month of testing.

An increase in lactose concentration occurred early in the season within herd B (  $p < 0.01$  ) followed by significant decreases between stages 2 to 6 (  $p < 0.01$  or less ). The lactose concentration did not change significantly during the final two months of testing.

Within herd C there was no significant change in lactose concentration during the first three stages of lactation. Between stages 3 to 5 there were significant decreases in lactose concentration (  $p < 0.01$  ), while the increase between stages 5 to 6 did not reach significance at the 5 % level. There was no significant change in the lactose concentration during the final month of testing.

Previous studies have shown the lactose concentration of milk to change during the course of the lactation. The concentration of lactose in the milk increases early in the lactation, and then decreases during mid to late lactation ( White and Davies, 1958; Rook and Campling, 1965 ).

The reason for the changes in lactose concentration during the lactation are likely to be complex. Lactose is a major osmotically active component of milk and as such will exert some control over the final volume of milk produced. The decrease in lactose concentration which has been observed during mid to late lactation may reflect an increasing area of the mammary gland undergoing involutionary changes. This would allow the leakage of lactose out of the milk and into the extracellular fluid, while sodium and chloride ions would flow in the opposite direction, maintaining osmolarity, but resulting in a lower lactose concentration of the milk as the lactation progressed.

#### (c) EFFECT OF AGE OF COW

Graphs 6.6aii, 6.6bii and 6.6cii give the mean log (10) lactose concentration at each month for the different age groups in each of the three herds A, B and C respectively.

The effect of age on the concentration of lactose in the milk was highly significant in each of the three herds (  $p < 0.01$  or less ), with a lower lactose concentration being recorded in milk samples from older cows than that from their younger herdmates.

Within herd A, the quarters from five year old cows showed a significantly lower lactose concentration than did those of any other age group (  $p < 0.01$  or less ). The three year old cows showed a significantly lower lactose concentration than did the two year old cows (  $p < 0.05$  ).

The effect of the stage by age interaction on the lactose concentration was highly significant within herd A (  $p < 0.01$  ), with differences between the lactose

concentration in milk from five year old animals and that from the other age groups being greater during the early and late stages of lactation, than during mid-lactation.

Within herd B, the milk from the five year old cows showed a significantly lower concentration of lactose than did that from the two year old cows (  $p < 0.01$  ) and from the four year old cows (  $p < 0.05$  ). No other age group pairs differed significantly with respect to the lactose concentration. The effect of the stage by age interaction on the lactose concentration was highly significant for herd B (  $p < 0.01$  ), with the oldest age group, ie five year old cows, showing greater differences from the 3 or 4 year old cows towards the end of the lactation, at which stage there were no significant differences between the age group 2, 3 or 4 animals with respect to the lactose concentration of quarter foremilk.

Within herd C, the oldest age group, ie those cows aged nine years or older, showed a significantly lower lactose concentration than did any other age group (  $p < 0.01$  or less ). Furthermore, the three year old cows, the youngest age group, showed a significantly higher mean lactose concentration than did the five year old cows (  $p < 0.05$  ) or the six year old cows (  $p < 0.01$  ). The effect of the stage by age interaction on lactose concentration was highly significant within herd C, the four year old cows showing a marked increase in the lactose concentration between stages 2 to 3, and the age group 6 and 7 animals showing a greater decline in lactose concentration during mid lactation than did their younger herdmates.

With respect to the lactose concentration, although not all of the age groups differed overall, differences did exist at various stages of the lactation which suggest a decrease in lactose concentration with increasing age of the cow, this finding applying to all three herds. Other researchers have shown a decrease in the lactose concentration with age during the first three lactations ( Rook and Campling, 1965 ). Mijnen *et al* ( 1982 ) reported parity to be an important source of variation in the milk lactose concentration of composite milk samples, the concentration decreasing with increasing age of the cow.

## (7) N-ACETYL- $\beta$ -D-GLUCOSAMINIDASE ( NAGase ) ACTIVITY

### (a) EFFECT OF BACTERIOLOGICAL STATUS

Graphs 6.7ai, 6.7bi and 6.7ci show the mean log (10) NAGase activity at each month, for the different bacteriological statuses within herds A, B and C respectively.

The effect of bacteriological status on the NAGase activity was highly significant (  $p < 0.01$  ) in each of the three herds.

The NAGase activity appeared to be lower for the status 0 quarters than for the status 1 quarters, particularly within herds B and C, although the significance of these differences was not tested.

In each herd, the NAGase activity of status 3 quarters was significantly greater than were those of status 1 or status 2 quarters (  $p < 0.01$  ). While status 1 and 2 quarters did not differ significantly in herds A or B, status 2 quarters showed a greater mean NAGase activity than did the status 1 quarters within herd C (  $p < 0.01$  ).

A number of researchers have reported increases in NAGase activity in infected quarters ( Mattila and Sandholm, 1985; Mattila et al, 1986b; Emanuelson et al, 1987; Nagahata et al, 1987; Miller and Paape, 1988 ), while Kitchen ( 1976 ) found an increased NAGase activity to be associated with a high somatic cell count of milk. Corbellini et al ( 1987 ) have reported an increased NAGase activity to be associated with streptococcal infections, but differences between quarters infected with other pathogens and those quarters which were uninfected were not statistically significant.

The source of NAGase in milk is still unclear. Although regarded as an indicator of damage to secretory tissue, a number of other potential sources of this enzyme have been suggested. These sources include: the milk neutrophil leukocytes, milk macrophages, and blood plasma, as well as the mammary secretory cells ( Mellors, 1968; Kitchen, 1976; Kitchen et al, 1978b; Kitchen et al, 1980; Dulin et al, 1984; Capuco et al, 1986; Nagahata et al, 1987 ) .

The relative contributions of the enzyme in healthy and in diseased glands remains to be fully elucidated. The investigation of isoenzyme patterns might clarify the situation.

#### (b) EFFECT OF STAGE OF LACTATION

The stage of lactation exerted a highly significant effect on the NAGase activity of the milk in each of the three herds (  $p < 0.01$  ), decreases in activity being recorded early in the lactation, followed by increasing NAGase activity during the second half of the lactation period. Status 0 quarters showed similar changes to those seen in the other statuses during the season.

Within herd A there was a decrease in the NAGase activity between stages 1 to 2 (  $p < 0.01$  ), followed by an increase during the following month (  $p < 0.01$  ). There was a further decrease in NAGase activity between stages 3 to 4 (  $p < 0.01$  ) but no significant change occurred between stages 4 to 6. The NAGase activity increased between stages 6 to 7 (  $p < 0.01$  ), but decreased significantly during the final month.

The effect of the stage by status interaction on the NAGase activity was not significant for herd A. The reason for the peak in NAGase activity which was observed at stage 3 is unclear. A similar peak was not observed for the somatic cell count.

Within herd B, there were highly significant decreases in NAGase activity between stages 1 to 4 (  $p < 0.01$  ). These were followed by a significant increase in the NAGase activity between stages 4 to 6 (  $p < 0.01$  ). There was a small but significant decrease in activity between stages 6 to 7 (  $p < 0.05$  ), but no change occurred during the final month of testing.

The effect of the stage by status interaction on the NAGase activity was highly significant within herd B (  $p < 0.01$  ). While the status 1 quarters showed similar changes to those observed for the overall data, the status 3 quarters showed only a slight and nonsignificant decrease in milk NAGase activity during the first half of the season, followed by a significant increase in activity between stages 4 to 6. During the final two months of testing there were nonsignificant increases in activity. The lack of significance may be due in part to the relatively small number of quarters which were involved. A decrease in NAGase activity between stages 6 to 7 was not seen in status 3 quarters.

While the somatic cell count increased between stages 6 to 7 and then decreased during the following month in status 1 or status 2 quarters within herd B, ( see section 6.1 ), the opposite was true of the NAGase activity, thus changes in the cell count did not necessarily mirror those of the NAGase activity.

Within herd C there was no significant change in the mean NAGase activity between stages 1 to 2, although significant decreases in activity were observed between stages 2 to 4 (  $p < 0.01$  ). From stage 4 until the end of the season there were significant monthly increases in NAGase activity (  $p < 0.01$  ).

The effect of the status by stage interaction on the NAGase activity was not significant within herd C, although there was a peak in the NAGase activity at stage 3 for status 3 quarters which was not seen within quarters of the other statuses. This peak was not associated with an increase in the number of quarters which were infected with a major pathogen ( see graph 5.1c ), nor with an increase in the somatic cell count. The results suggest that the infections which were present at stage 3 had a particularly severe effect on the mammary epithelium with the release of the cytoplasmic contents into the milk.

Similar stage of lactation effects on NAGase activity have been found during previous studies ( Mattila *et al.*, 1986b; Miller and Paape, 1988 ). The effects are inversely related to milk production and may be largely ascribed to the dilution of the NAGase with varying amounts of milk ( Mattila *et al.*, 1986 ).

### (c) EFFECT OF AGE OF COW

Graphs 6.7a<sub>ii</sub>, 6.7b<sub>ii</sub> and 6.7c<sub>ii</sub> give the mean log (10) NAGase activity at each month, for the different age groups within herds A, B and C respectively.

The effect of age on the milk NAGase activity was highly significant within each herd ( $p < 0.01$ ), with a trend towards an increase in NAGase activity in older cows.

Within herd A, quarters from the five year old cows showed a significantly higher NAGase activity than did those from 3 or 4 year old cows. However, the youngest age group, the two year old animals, also showed a significantly higher NAGase activity than did the 3 or 4 year old cows.

The effect of the stage by age interaction was highly significant within herd A ( $p < 0.01$ ) with the four year old cows showing a higher NAGase activity than did the three year old cows at stage 7.

Within herd B, quarters from the five year old cows showed a higher mean NAGase activity than did those of any other age group ( $p < 0.05$  or less), while none of the other age groups differed significantly.

The stage by age interaction effect was highly significant within herd B ( $p < 0.01$ ) with the five year old cows showing an increased NAGase activity from stage 3 onwards, the activity being similar between age groups prior to this point.

Within herd C, the three year old cows showed a lower NAGase activity than did any other age group with the exception of the four year old cows ( $p < 0.05$  or less), whilst the oldest age group showed a significantly higher NAGase activity than did any other age group ( $p < 0.01$  or less). The effect of the stage by age interaction on the NAGase activity was highly significant within herd C ( $p < 0.01$ ), with differences between the age groups generally being greater towards the end of the lactation.

Conflicting results concerning the effect of age on NAGase activity have been published. Mattila *et al* (1986b) found lactation number to be a significant source of variation in NAGase activity, with the activity increasing during the first four lactations, while Miller and Paape (1988) found the main effect of parity to be statistically nonsignificant.

While there was evidence of increasing NAGase activity with age during the present trial, the increase was not linear. Heifers within herd A showed a comparatively high NAGase activity, while the other mastitis indicators within this age group were unremarkable. Infection in these young cows may have caused a greater degree of

tissue damage, with greater enzyme release than occurred in older cows within herd A. The prevalence of infection in the heifers within herd A was low in comparison with the prevalence of infection within the older cows.

## (8) ANTITRYPSIN CONCENTRATION

### (a) EFFECT OF BACTERIOLOGICAL STATUS

Graphs 6.8ai, 6.8bi and 6.8ci give the mean log (10) antitrypsin concentration at each month for the different bacteriological statuses within herds A, B and C respectively.

The effect of the bacteriological status on the antitrypsin concentration was highly significant in each of the three herds (  $p < 0.01$  ).

The status 0 quarters showed a similar mean antitrypsin concentration to that of status 1 quarters, except within herd B, where milk from status 0 quarters appeared to have a higher antitrypsin concentration than that of status 1 quarters, although the significance of this difference was not tested.

Within herd A, the status 3 quarters showed a significantly higher antitrypsin concentration than did either status 1 (  $p < 0.05$  ) or status 2 (  $p < 0.01$  ) quarters. Moreover, status 1 quarters showed a significantly higher antitrypsin concentration than did status 2 quarters (  $p < 0.05$  ).

Within herds B and C there were no significant differences between status 1 and 2 quarters with respect to antitrypsin concentration, but in each herd, status 3 quarters showed a significantly higher antitrypsin concentration than did either status 1 or 2 quarters (  $p < 0.01$  ).

Several researchers have shown that infection causes an increase in the milk antitrypsin concentration. Mattila *et al* ( 1985 ), Mattila *et al* ( 1986b ) and Emanuelson *et al* ( 1987 ) have reported a positive bacteriological culture to be associated with a 1.4 to 10 fold increase in the antitrypsin concentration, depending upon the bacteriological species concerned, with major pathogens generally causing a greater increase than do minor pathogens.

Antitrypsin has been found in high concentration in serum of cows ( Honkanen-Buzalski and Sandholm, 1981 ). Mastitis is associated with increased permeability between the blood and milk compartments, with leakage of plasma proteins into the milk. Theoretically, any plasma protein which is not actively transported across the blood-milk barrier may be used to indicate this increased permeability, but the low

molecular weight proteins appear in the milk in greater proportion than do high molecular weight proteins, and have therefore been used as markers ( Honkanen-Buzalski and Sandholm, 1981 ). Bovine serum albumin has been advocated as a marker of increased permeability ( Giesecke and Viljoen, 1974 ), although the use of antitrypsin has several advantages over bovine serum albumin ( see literature review, chapter two ).

#### (b) EFFECT OF STAGE OF LACTATION

The effect of stage of lactation on milk antitrypsin was significant in each of the three herds, with an increase in the antitrypsin concentration being observed during the second half of the lactation. The pattern of change was seen both within infected, and within status 0 quarters.

Within herd A, the decrease in the antitrypsin concentration between stages 1 to 2 (  $p < 0.01$  ), was followed by increases between stages 2 to 4 (  $p < 0.01$  ). There was a sharp decrease in antitrypsin concentration between stages 4 to 5 (  $p < 0.01$  ). No significant change occurred during the following month, but between stages 6 to 7 there was an increase in concentration (  $p < 0.01$  ) with a decrease in antitrypsin concentration during the final month of testing. The pattern of change for the antitrypsin within herd A, resembles that observed for the milk NAGase activity ( graph 6.7ai ), but with the midlactation peak occurring one month later than was the case with the NAGase activity.

The decrease in antitrypsin concentration which occurred towards the end of the lactation is unlikely to have been due to the break in the drought, as the increase in milk production occurred during the preceeding month, at which stage the antitrypsin concentration was increasing. Similar changes in antitrypsin concentration were seen in status 0 quarters, suggesting that changes in the level or severity of infection were not involved.

The effect of the stage by status interaction on the antitrypsin concentration was not significant for herd A.

Within herd B there was a significant decrease in the antitrypsin concentration between stages 1 to 2 (  $p < 0.01$  ), but the concentration increased during the following month (  $p < 0.01$  ). Between stages 3 to 4 the antitrypsin concentration decreased significantly (  $p < 0.05$  ), before increasing again between stages 4 to 6 (  $p < 0.01$  ). The increase in the antitrypsin concentration between stages 6 to 7 did not reach significance and there was no change in antitrypsin concentration during the final month of testing.

The status 0 quarters, which were never infected during the season, showed similar changes across the season, but the absolute antitrypsin concentration in the milk for these uninfected quarters within herd B was surprisingly high in comparison with that of uninfected quarters within herds A or C.

The effect of the stage by status interaction on the mean antitrypsin concentration was highly significant within herd B ( $p < 0.01$ ), due to the greater increase in the antitrypsin concentration in status 3 quarters than within those of other statuses between stages 4 to 7, giving a better separation of infected and uninfected quarters during the second half of lactation, than during the first half.

Within herd C, a similar pattern of change occurred to that seen in herd B, with an initial decrease in antitrypsin concentration ( $p < 0.01$ ) followed by a temporary increase ( $p < 0.05$ ) before the concentration dipped to a low point at stage 4. There was a sharp increase in the antitrypsin concentration between stages 4 to 5 ( $p < 0.01$ ) with no change between stages 5 to 6. The increase in the antitrypsin concentration during the final month did not reach significance at the 5 % level.

The stage by status effect on antitrypsin was significant for herd C ( $p < 0.05$ ). There was no initial decrease in the antitrypsin concentration in status 3 quarters and the difference between the antitrypsin concentration of status 3 quarters and that of other statuses was smaller at the first sampling than was the case later in the lactation.

The antitrypsin concentration is very high early in the lactation, decreases to a low point at approximately 100 days postpartum, before increasing during the remainder of the lactation (Sandholm *et al*, 1984). Mattila *et al* (1986b) reported the effect of the stage of lactation on the antitrypsin concentration to be highly significant.

The very high concentrations of antitrypsin which have been observed early in the dairy season are due to colostral antitrypsin, a low molecular weight protein which is produced within the mammary gland (Sandholm *et al*, 1984). The presence of this colostral antitrypsin may mask the blood derived protein if this test is performed early in the lactation.

During the present trial, samples were not taken during the first month of lactation, thus colostral effects were not seen, but the increase in the antitrypsin concentration which was observed during the second half of the season is in agreement with that reported in the literature, and is probably due to a combination of decreasing milk yield, and an increase in the amount of the mammary tissue which is undergoing involutionary change.

### (c) EFFECT OF AGE OF COW

Graphs 6.8a<sub>ii</sub>, 6.8b<sub>ii</sub> and 6.8c<sub>ii</sub> give the mean log (10) antitrypsin concentration at each month for the different age groups, within herds A, B and C respectively.

The effect of age on antitrypsin concentration was significant in herd B only ( $p < 0.05$ ) in which there was a decrease in antitrypsin concentration with increasing age of the cow. Quarters from the two year old cows showed a significantly higher antitrypsin concentration than did those of each other age group ( $p < 0.01$  or less), while the three year old cows showed a higher concentration than did the five year old cows ( $p < 0.05$ ).

The effect of the stage by age interaction on the antitrypsin concentration was significant for herd B ( $p < 0.05$ ), with greater differences between age groups being observed both early and late in the lactation, than was the case during mid-lactation.

The main effect of age on the antitrypsin concentration was not significant within herd A, but there was a highly significant stage by age interaction effect ( $p < 0.01$ ) on the mean milk antitrypsin concentration, with greater differences between age groups at certain stages of the season. The two year old cows showed a higher mean antitrypsin concentration than did 3, 4 or 5 year old cows, although significance was not reached within each month. The three year old cows showed a significantly higher antitrypsin concentration than did the four year old cows at stages 1 to 3 and a higher concentration than did the five year old cows at stages 1 to 6 ( $p < 0.05$  or less). The four year old cows showed a significantly higher antitrypsin concentration than did the five year old cows at stages 4 to 6 ( $p < 0.05$ ).

Neither the age of the cow, nor the stage by age interaction exerted a statistically significant effect on the antitrypsin concentration within herd C.

The antitrypsin concentration of milk has been shown to increase between the first and second parities, but beyond the second parity, the concentration shows little further change (Sandholm *et al*, 1984). Similarly, Mattila *et al* (1986b) reported little increase in the antitrypsin concentration with increasing lactation number, with no overall statistically significant effect being detected. During the present investigation, both herds A and B showed evidence of a decrease in the antitrypsin concentration with age of cow. This does not agree with the few published reports which are available. In both herds A and B the percentage of quarters which were infected with a major pathogen increased with age of cow.

In the absence of age group within bacteriological status analysis, which could not be supported with the relatively small data set, it must be concluded that the infections which were present in the younger animals had a more severe effect in terms of the

increased permeability of small blood vessels than was the case in their older herdmates. It is also possible that the concentration of antitrypsin in the blood increased more rapidly in response to infection than was the case for older animals although no data in support of this proposal is currently available.

### SUMMARY AND CONCLUSIONS

- (1) The effect of the bacteriological status, which takes into account both the presence and the duration of the udder infection, on the somatic cell count, the electrical conductivity, the NAGase activity and the concentrations of sodium, antitrypsin and lactose in individual quarter foremilk samples was significant for each parameter, within each of the three herds. A strong effect of the bacteriological status on a given parameter suggests that the parameter is likely to be of use in the discrimination between infected and uninfected quarters. The effect of bacteriological status on the potassium concentration and on the pH differed between herds.
- (2) There was some variation in degree of response of parameters to infection between herds. This variation may reflect differences in the bacterial flora, with certain types of infection being more severe in one herd than in another.
- (3) The effect of stage of lactation was significant for all parameters in each of the three herds. The cows were sampled at four-weekly intervals throughout the lactation, the mean time lag between calving and first sampling being 30 days. There was some variation about this mean, so that some cows were slightly closer to their calving date than were others. This fact may explain some of the changes which were observed early in the season.
- (4) In general, the stage of lactation effects observed during the present trial were in reasonable agreement with those found in the literature. These effects are probably due to the changes in milk yield, as well as to involutionary change within the gland, rather than to the changes in the prevalence of infection which occurred during the lactation. It must be remembered however, that the stage of lactation effects are confounded with the seasonal effects in New Zealand seasonal supply dairy herds, and this may partly account for departures from the predicted stage of lactation effects. The results show that the effect of stage of lactation should be taken into account when discriminating between infected and uninfected udder quarters.

- (5) In many cases, the effect of the interaction between the bacteriological status and the stage of lactation was significant, with a tendency towards greater differences between bacteriological statuses at different stages of the lactation period, particularly towards the end of the lactation. This has implications for the timing of sampling for mastitis diagnosis, since the greater the difference between bacteriological statuses, the more successful will be the test in discriminating between infected and uninfected quarters.
- (6) The age of the cow was shown to have significant effects on a number of parameters, the effect being most noticeable within herd B. There was only a weak association between pH or potassium and the age of the cow. The effect of age on a parameter, may well be due to the increasing rate of infection in older cows which was shown in chapter five. The number of observations included in the data set did not permit an analysis of the effect of age within each of the bacteriological statuses.

TABLE 6.1RESULTS OF ANALYSIS OF VARIANCE - INDIVIDUAL QUARTERS

| PARAMETER           | HERD | STATUS          | AGE  | STAGE | STATUS<br>BY AGE | STATUS<br>BY STAGE | AGE BY<br>STAGE |
|---------------------|------|-----------------|------|-------|------------------|--------------------|-----------------|
| Pr > F <sup>1</sup> |      |                 |      |       |                  |                    |                 |
| S.C.C.              | A    | 0.01            | 0.91 | 0.01  | 0.93             | 0.01               | 0.01            |
|                     | B    | 0.01            | 0.07 | 0.01  | 0.63             | 0.11               | 0.01            |
|                     | C    | 0.01            | 0.02 | 0.01  | 0.44             | 0.49               | 0.11            |
| SODIUM              | A    | 0.01            | 0.01 | 0.01  | 0.41             | 0.06               | 0.01            |
|                     | B    | 0.01            | 0.01 | 0.01  | 0.01             | 0.01               | 0.01            |
|                     | C    | 0.01            | 0.01 | 0.01  | 0.76             | 0.08               | 0.01            |
| POTASSIUM           | A    | 0.29            | 0.01 | 0.01  | 0.54             | 0.02               | 0.01            |
|                     | B    | 0.01            | 0.01 | 0.01  | 0.01             | 0.01               | 0.01            |
|                     | C    | 0.83            | 0.02 | 0.01  | 0.36             | 0.01               | 0.01            |
| CONDUCTIVITY        | A    | 0.03            | 0.04 | 0.01  | 0.99             | 0.08               | 0.01            |
|                     | B    | 0.02            | 0.05 | 0.01  | 0.07             | 0.01               | 0.01            |
|                     | C    | ND <sup>2</sup> | ND   | ND    | ND               | ND                 | ND              |
| pH                  | A    | 0.30            | 0.85 | 0.01  | 0.55             | 0.01               | 0.01            |
|                     | B    | 0.01            | 0.01 | 0.01  | 0.01             | 0.01               | 0.01            |
|                     | C    | ND              | ND   | ND    | ND               | ND                 | ND              |
| LACTOSE             | A    | 0.02            | 0.01 | 0.01  | 0.31             | 0.09               | 0.01            |
|                     | B    | 0.01            | 0.01 | 0.01  | 0.05             | 0.01               | 0.01            |
|                     | C    | 0.01            | 0.01 | 0.01  | 0.88             | 0.17               | 0.01            |
| NAGase              | A    | 0.01            | 0.01 | 0.01  | 0.28             | 0.22               | 0.01            |
|                     | B    | 0.01            | 0.01 | 0.01  | 0.08             | 0.01               | 0.01            |
|                     | C    | 0.01            | 0.01 | 0.01  | 0.48             | 0.09               | 0.01            |
| ANTITRYPSIN         | A    | 0.01            | 0.12 | 0.01  | 0.93             | 0.33               | 0.01            |
|                     | B    | 0.01            | 0.03 | 0.01  | 0.79             | 0.01               | 0.02            |
|                     | C    | 0.01            | 0.35 | 0.01  | 0.40             | 0.03               | 0.06            |

1 Pr > F indicates the probability that sampling variation alone was responsible for the difference between means.

2 ND = No data available.

TABLE 6.2RESULTS OF CONTRAST ANALYSIS BETWEEN EACH BACTERIOLOGICAL STATUS

| PARAMETER    | HERD | STATUS<br>1 VS 2 | STATUS<br>1 VS 3    | STATUS<br>2 VS 3 |
|--------------|------|------------------|---------------------|------------------|
| <hr/>        |      |                  |                     |                  |
|              |      |                  | Pr > F <sup>1</sup> |                  |
| S.C.C.       | A    | 0.56             | 0.01                | 0.01             |
|              | B    | 0.04             | 0.01                | 0.01             |
|              | C    | 0.01             | 0.01                | 0.01             |
| SODIUM       | A    | 0.63             | 0.01                | 0.01             |
|              | B    | 0.42             | 0.01                | 0.01             |
|              | C    | 0.04             | 0.01                | 0.01             |
| POTASSIUM    | A    | NT <sup>2</sup>  | NT                  | NT               |
|              | B    | 0.58             | 0.01                | 0.01             |
|              | C    | NT               | NT                  | NT               |
| CONDUCTIVITY | A    | 0.87             | 0.01                | 0.05             |
|              | B    | 0.60             | 0.01                | 0.02             |
|              | C    | ND <sup>3</sup>  | ND                  | ND               |
| pH           | A    | NT               | NT                  | NT               |
|              | B    | 0.45             | 0.01                | 0.01             |
|              | C    | ND               | ND                  | ND               |
| LACTOSE      | A    | 0.85             | 0.01                | 0.06             |
|              | B    | 0.62             | 0.01                | 0.01             |
|              | C    | 0.05             | 0.01                | 0.07             |
| NAGase       | A    | 0.41             | 0.01                | 0.01             |
|              | B    | 0.52             | 0.01                | 0.01             |
|              | C    | 0.01             | 0.01                | 0.01             |
| ANTITRYPSIN  | A    | 0.05             | 0.02                | 0.01             |
|              | B    | 0.53             | 0.01                | 0.01             |
|              | C    | 0.55             | 0.01                | 0.01             |

1 Pr > F indicates the probability that sampling variation alone was responsible for the differences between the means.

2 NT = Not tested.

3 ND = No data.

TABLE 6.3aRESULTS OF CONTRAST ANALYSIS BETWEEN EACH AGE GROUP - HERD A

| PARAMETER    | AGE GROUP           |        |        |        |        |        |
|--------------|---------------------|--------|--------|--------|--------|--------|
|              | 2 vs 3              | 2 vs 4 | 2 vs 5 | 3 vs 4 | 3 vs 5 | 4 vs 5 |
|              | Pr > F <sup>1</sup> |        |        |        |        |        |
| S.C.C.       | NT <sup>2</sup>     | NT     | NT     | NT     | NT     | NT     |
| SODIUM       | 0.55                | 0.06   | 0.01   | 0.02   | 0.01   | 0.10   |
| POTASSIUM    | 0.23                | 0.02   | 0.79   | 0.01   | 0.36   | 0.01   |
| CONDUCTIVITY | 0.11                | 0.63   | 0.01   | 0.05   | 0.10   | 0.01   |
| pH           | NT                  | NT     | NT     | NT     | NT     | NT     |
| LACTOSE      | 0.03                | 0.37   | 0.01   | 0.20   | 0.01   | 0.01   |
| NAGase       | 0.01                | 0.01   | 0.40   | 0.06   | 0.01   | 0.01   |
| ANTITRYPSIN  | NT                  | NT     | NT     | NT     | NT     | NT     |

1 Pr > F indicates the probability that sampling variation alone was responsible for the differences between the means.

2 NT = Not tested.

TABLE 6.3b

RESULTS OF CONTRAST ANALYSIS BETWEEN EACH AGE GROUP - HERD B

| PARAMETER    | AGE GROUP           |        |        |        |        |        |
|--------------|---------------------|--------|--------|--------|--------|--------|
|              | 2 vs 3              | 2 vs 4 | 2 vs 5 | 3 vs 4 | 3 vs 5 | 4 vs 5 |
|              | Pr > F <sup>1</sup> |        |        |        |        |        |
| S.C.C.       | NT <sup>2</sup>     | NT     | NT     | NT     | NT     | NT     |
| SODIUM       | 0.16                | 0.69   | 0.01   | 0.30   | 0.01   | 0.01   |
| POTASSIUM    | 0.30                | 0.19   | 0.05   | 0.81   | 0.01   | 0.01   |
| CONDUCTIVITY | 0.04                | 0.18   | 0.01   | 0.40   | 0.40   | 0.09   |
| pH           | 0.10                | 0.03   | 0.89   | 0.59   | 0.08   | 0.02   |
| LACTOSE      | 0.25                | 0.69   | 0.01   | 0.43   | 0.09   | 0.02   |
| NAGase       | 0.45                | 0.29   | 0.03   | 0.79   | 0.01   | 0.01   |
| ANTITRYPSIN  | 0.01                | 0.01   | 0.01   | 0.33   | 0.02   | 0.17   |

1 Pr > F indicates the probability that sampling variation alone was responsible for the differences between the means.

2 NT = Not tested.

TABLE 6.3cRESULTS OF CONTRAST ANALYSIS BETWEEN EACH AGE GROUP - HERD C

| PARAMETER    | AGE GROUP           |      |      |      |      |      |      |      |      |      |
|--------------|---------------------|------|------|------|------|------|------|------|------|------|
|              | 3vs4                | 3vs5 | 3vs6 | 3vs7 | 4vs5 | 4vs6 | 4vs7 | 5vs6 | 5vs7 | 6vs7 |
|              | Pr > F <sup>1</sup> |      |      |      |      |      |      |      |      |      |
| S.C.C.       | 0.60                | 0.01 | 0.01 | 0.01 | 0.02 | 0.05 | 0.01 | 0.35 | 0.32 | 0.03 |
| SODIUM       | 0.77                | 0.13 | 0.07 | 0.01 | 0.14 | 0.10 | 0.01 | 0.99 | 0.01 | 0.01 |
| POTASSIUM    | 0.11                | 0.20 | 0.56 | 0.02 | 0.01 | 0.03 | 0.01 | 0.37 | 0.30 | 0.03 |
| CONDUCTIVITY | ND <sup>2</sup>     | ND   | ND   | ND   | ND   | ND   | ND   | ND   | ND   | ND   |
| pH           | ND                  | ND   | ND   | ND   | ND   | ND   | ND   | ND   | ND   | ND   |
| LACTOSE      | 0.32                | 0.05 | 0.01 | 0.01 | 0.58 | 0.24 | 0.01 | 0.46 | 0.01 | 0.01 |
| NAGase       | 0.66                | 0.04 | 0.02 | 0.01 | 0.25 | 0.19 | 0.01 | 0.94 | 0.01 | 0.01 |
| ANTITRYPSIN  | NT <sup>3</sup>     | NT   | NT   | NT   | NT   | NT   | NT   | NT   | NT   | NT   |

1 Pr > F indicates the probability that sampling variation alone was responsible for the differences between the means.

2 ND = No data.

3 NT = Not tested.

TABLE 6.4aRESULTS OF CONTRAST ANALYSIS BETWEEN STAGES OF LACTATION - HERD A

| PARAMETER    | STAGE OF LACTATION  |        |        |        |        |                 |        |
|--------------|---------------------|--------|--------|--------|--------|-----------------|--------|
|              | 1 vs 2              | 2 vs 3 | 3 vs 4 | 4 vs 5 | 5 vs 6 | 6 vs 7          | 7 vs 8 |
|              | Pr > F <sup>1</sup> |        |        |        |        |                 |        |
| S.C.C.       | 0.87                | 0.26   | 0.08   | 0.36   | 0.73   | 0.01            | 0.47   |
| SODIUM       | 0.63                | 0.03   | 0.04   | 0.89   | 0.01   | 0.01            | 0.01   |
| POTASSIUM    | 0.01                | 0.69   | 0.01   | 0.01   | 0.06   | 0.60            | 0.01   |
| CONDUCTIVITY | 0.01                | 0.11   | 0.01   | 0.01   | 0.46   | 0.01            | 0.01   |
| pH           | 0.01                | 0.01   | 0.01   | 0.01   | 0.22   | ND <sup>2</sup> | 0.03   |
| LACTOSE      | 0.01                | 0.38   | 0.01   | 0.01   | 0.01   | 0.01            | 0.32   |
| NAGase       | 0.01                | 0.01   | 0.01   | 0.73   | 0.06   | 0.01            | 0.01   |
| ANTITRYPSIN  | 0.01                | 0.01   | 0.01   | 0.01   | 0.42   | 0.01            | 0.01   |

1 Pr > F indicates the probability that sampling variation alone was responsible for the differences between the means.

2 ND = No data.

TABLE 6.4bRESULTS OF CONTRAST ANALYSIS BETWEEN STAGES OF LACTATION - HERD B

| PARAMETER    | STAGE OF LACTATION  |        |        |        |        |        |
|--------------|---------------------|--------|--------|--------|--------|--------|
|              | 1 vs 2              | 2 vs 3 | 3 vs 4 | 4 vs 6 | 6 vs 7 | 7 vs 8 |
|              | Pr > F <sup>1</sup> |        |        |        |        |        |
| S.C.C.       | 0.29                | 0.01   | 0.04   | 0.01   | 0.01   | 0.22   |
| SODIUM       | 0.04                | 0.36   | 0.51   | 0.01   | 0.28   | 0.01   |
| POTASSIUM    | 0.01                | 0.01   | 0.05   | 0.01   | 0.02   | 0.01   |
| CONDUCTIVITY | 0.10                | 0.02   | 0.02   | 0.01   | 0.01   | 0.01   |
| pH           | 0.01                | 0.01   | 0.01   | 0.01   | 0.01   | 0.01   |
| LACTOSE      | 0.01                | 0.01   | 0.01   | 0.01   | 0.95   | 0.06   |
| NAGase       | 0.01                | 0.01   | 0.01   | 0.01   | 0.02   | 0.16   |
| ANTITRYPSIN  | 0.01                | 0.01   | 0.02   | 0.01   | 0.11   | 0.75   |

\* Pr > F indicates the probability that sampling variation alone was responsible for the differences between the means.

TABLE 6.4c

RESULTS OF CONTRAST ANALYSIS BETWEEN STAGES OF LACTATION - HERD C

| PARAMETER    | STAGE OF LACTATION  |        |        |        |        |        |
|--------------|---------------------|--------|--------|--------|--------|--------|
|              | 1 vs 2              | 2 vs 3 | 3 vs 4 | 4 vs 5 | 5 vs 6 | 6 vs 7 |
|              | Pr > F <sup>1</sup> |        |        |        |        |        |
| S.C.C.       | 0.01                | 0.01   | 0.01   | 0.01   | 0.22   | 0.31   |
| SODIUM       | 0.05                | 0.15   | 0.01   | 0.01   | 0.01   | 0.01   |
| POTASSIUM    | 0.10                | 0.17   | 0.50   | 0.01   | 0.70   | 0.01   |
| CONDUCTIVITY | ND <sup>2</sup>     | ND     | ND     | ND     | ND     | ND     |
| pH           | ND                  | ND     | ND     | ND     | ND     | ND     |
| LACTOSE      | 0.84                | 0.52   | 0.01   | 0.01   | 0.13   | 0.96   |
| NAGase       | 0.05                | 0.01   | 0.01   | 0.01   | 0.01   | 0.01   |
| ANTITRYPSIN  | 0.01                | 0.02   | 0.01   | 0.01   | 0.71   | 0.07   |

1 Pr > F indicates the probability that sampling variation alone was responsible for the differences between the means.

2 ND = No data.

TABLE 6.5aRESULTS OF CONTRAST ANALYSIS BETWEEN STAGES OF LACTATION - HERD ASTATUS 0 QUARTERS ONLY

| PARAMETER    | STAGE OF LACTATION |        |        |                     |        |                 |        |
|--------------|--------------------|--------|--------|---------------------|--------|-----------------|--------|
|              | 1 vs 2             | 2 vs 3 | 3 vs 4 | 4 vs 5              | 5 vs 6 | 6 vs 7          | 7 vs 8 |
|              |                    |        |        | Pr > F <sup>1</sup> |        |                 |        |
| S.C.C.       | 0.95               | 0.16   | 0.01   | 0.18                | 0.37   | 0.01            | 0.17   |
| SODIUM       | 0.87               | 0.02   | 0.52   | 0.67                | 0.01   | 0.01            | 0.03   |
| POTASSIUM    | 0.01               | 0.83   | 0.01   | 0.01                | 0.02   | 0.17            | 0.01   |
| CONDUCTIVITY | 0.01               | 0.11   | 0.01   | 0.01                | 0.46   | 0.01            | 0.01   |
| pH           | 0.01               | 0.01   | 0.01   | 0.01                | 0.22   | ND <sup>2</sup> | 0.03   |
| LACTOSE      | 0.01               | 0.03   | 0.01   | 0.01                | 0.01   | 0.01            | 0.02   |
| NAGase       | 0.01               | 0.01   | 0.01   | 0.26                | 0.05   | 0.04            | 0.02   |
| ANTITRYPSIN  | 0.01               | 0.02   | 0.01   | 0.01                | 0.80   | 0.01            | 0.01   |

1 Pr > F indicates the probability that sampling variation alone was responsible for the differences between the means.

2 ND = No data.

TABLE 6.5bRESULTS OF CONTRAST ANALYSIS BETWEEN STAGES OF LACTATION - HERD BSTATUS 0 QUARTERS ONLY

| PARAMETER    | STAGE OF LACTATION  |        |        |        |        |        |
|--------------|---------------------|--------|--------|--------|--------|--------|
|              | 1 vs 2              | 2 vs 3 | 3 vs 4 | 4 vs 6 | 6 vs 7 | 7 vs 8 |
|              | Pr > F <sup>1</sup> |        |        |        |        |        |
| S.C.C.       | 0.32                | 0.01   | 0.07   | 0.11   | 0.01   | 0.12   |
| SODIUM       | 0.50                | 0.71   | 0.04   | 0.01   | 0.87   | 0.13   |
| POTASSIUM    | 0.40                | 0.21   | 0.12   | 0.01   | 0.09   | 0.01   |
| CONDUCTIVITY | 0.82                | 0.03   | 0.17   | 0.01   | 0.01   | 0.01   |
| pH           | 0.01                | 0.01   | 0.02   | 0.01   | 0.01   | 0.68   |
| LACTOSE      | 0.01                | 0.43   | 0.01   | 0.01   | 0.20   | 0.02   |
| NAGase       | 0.04                | 0.01   | 0.01   | 0.01   | 0.78   | 0.74   |
| ANTITRYPSIN  | 0.99                | 0.55   | 0.12   | 0.10   | 0.31   | 0.96   |

1 Pr > F indicates the probability that sampling variation alone was responsible for the differences between the means.

TABLE 6.5cRESULTS OF CONTRAST ANALYSIS BETWEEN STAGES OF LACTATION - HERD CSTATUS 0 QUARTERS ONLY

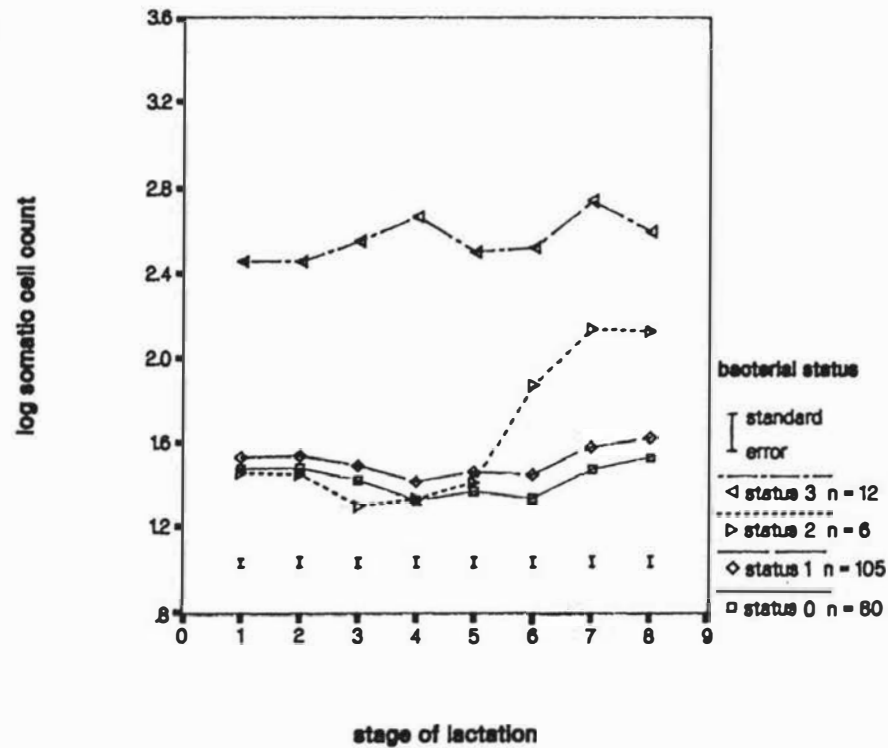
| PARAMETER    | STAGE OF LACTATION  |        |        |        |        |        |
|--------------|---------------------|--------|--------|--------|--------|--------|
|              | 1 vs 2              | 2 vs 3 | 3 vs 4 | 4 vs 5 | 5 vs 6 | 6 vs 7 |
|              | Pr > F <sup>1</sup> |        |        |        |        |        |
| S.C.C.       | 0.14                | 0.06   | 0.81   | 0.05   | 0.36   | 0.08   |
| SODIUM       | 0.07                | 0.73   | 0.01   | 0.01   | 0.01   | 0.01   |
| POTASSIUM    | 0.25                | 0.25   | 0.15   | 0.01   | 0.79   | 0.01   |
| CONDUCTIVITY | ND <sup>2</sup>     | ND     | ND     | ND     | ND     | ND     |
| pH           | ND                  | ND     | ND     | ND     | ND     | ND     |
| LACTOSE      | 0.57                | 0.01   | 0.01   | 0.01   | 0.92   | 0.04   |
| NAGase       | 0.99                | 0.01   | 0.01   | 0.25   | 0.01   | 0.21   |
| ANTITRYPSIN  | 0.01                | 0.23   | 0.05   | 0.01   | 0.24   | 0.82   |

1 Pr > F indicates the probability that sampling variation alone was responsible for the differences between the means.

2 ND = No data.

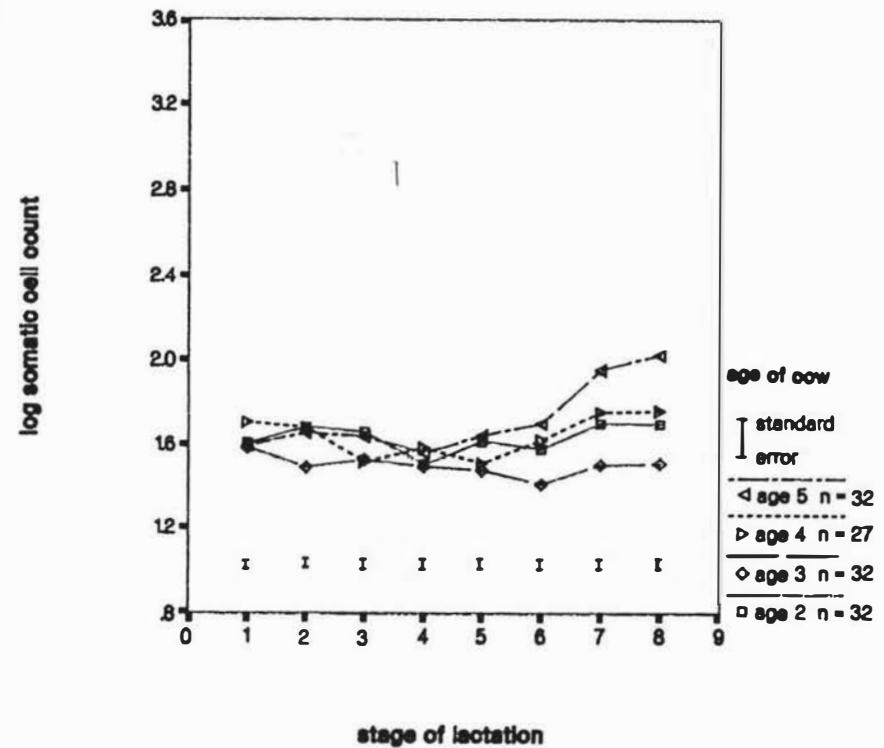
# SOMATIC CELL COUNT - HERD A

**graph 6.1ai**  
**logscs vs stage of lactation**  
by bacteriological status



logscs =  $\log_{10}$  somatic cell count  $\times 1000$   
standard error = pooled monthly standard error

**graph 6.1aii**  
**logscs vs stage of lactation**  
by age of cow

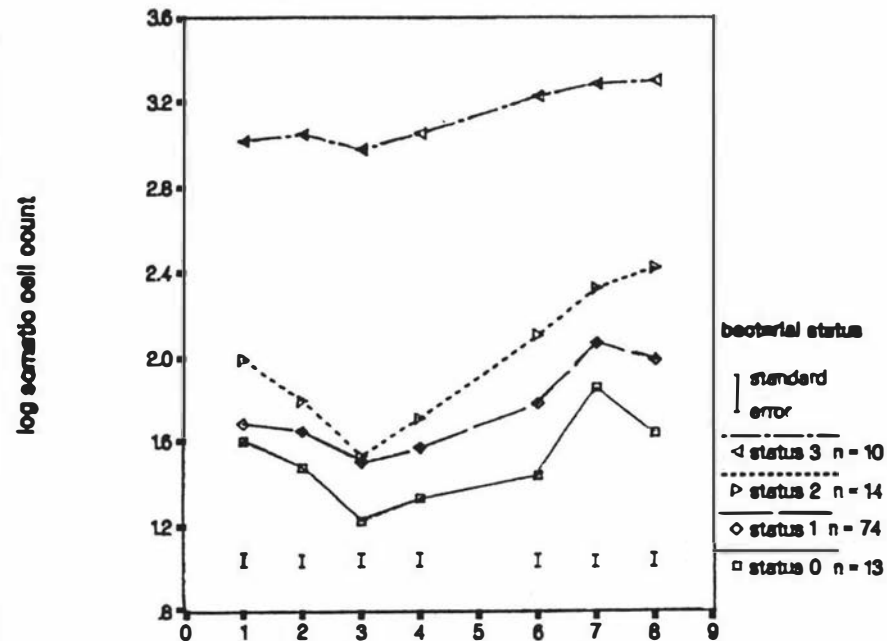


logscs =  $\log_{10}$  somatic cell count  $\times 1000$   
standard error = pooled monthly standard error

# SOMATIC CELL COUNT - HERD B

**graph 6.1bi**  
**logscs vs stage of lactation**

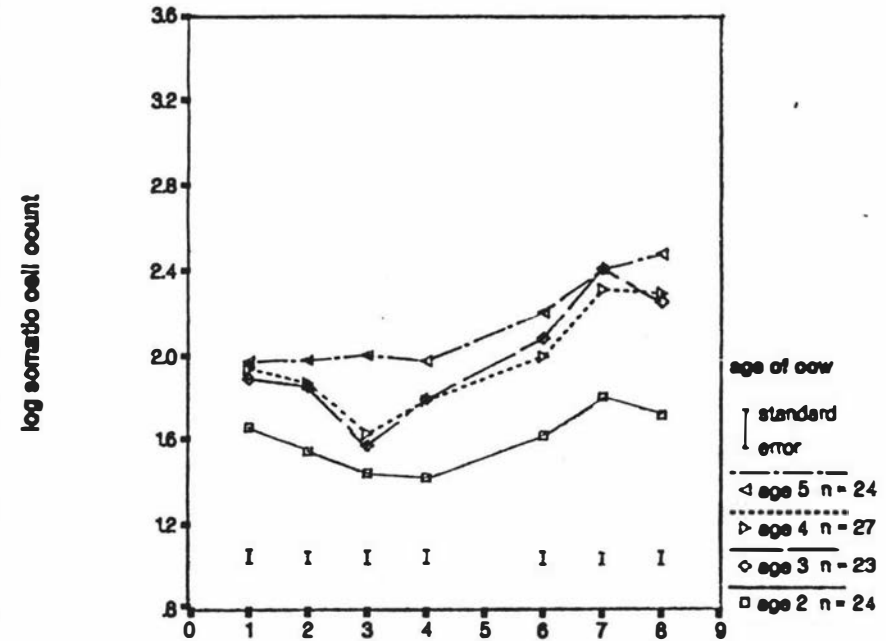
by bacteriological status



logscs =  $\log_{10}$  somatic cell count  $\times 1000$   
standard error = pooled monthly standard error

**graph 6.1bii**  
**logscs vs stage of lactation**

by age of cow

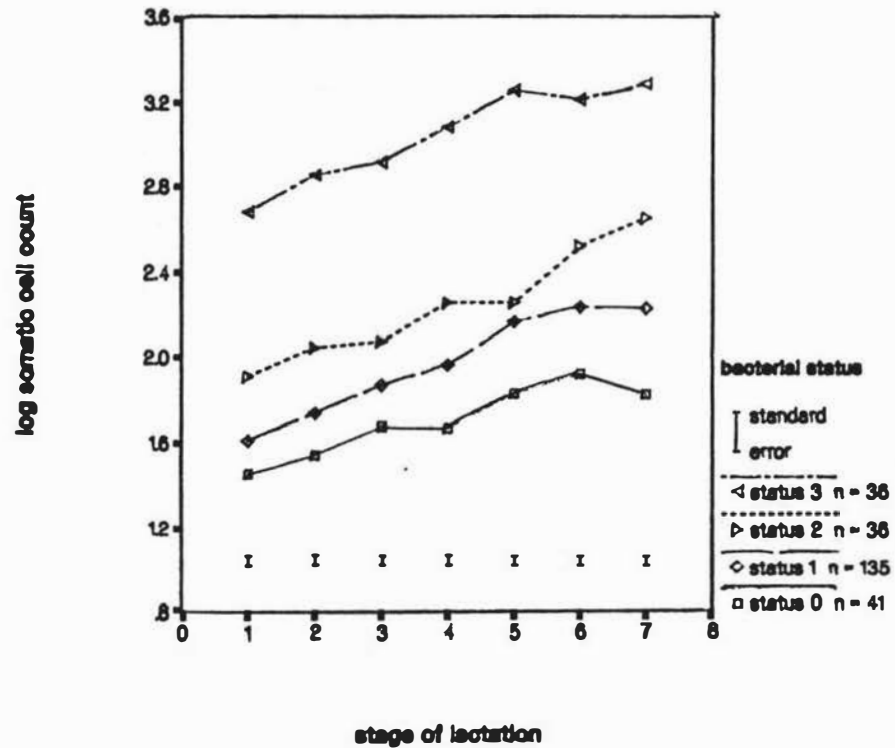


logscs =  $\log_{10}$  somatic cell count  $\times 1000$   
standard error = pooled monthly standard error

# SOMATIC CELL COUNT - HERD C

**graph 6.1ci**  
**logscv vs stage of lactation**

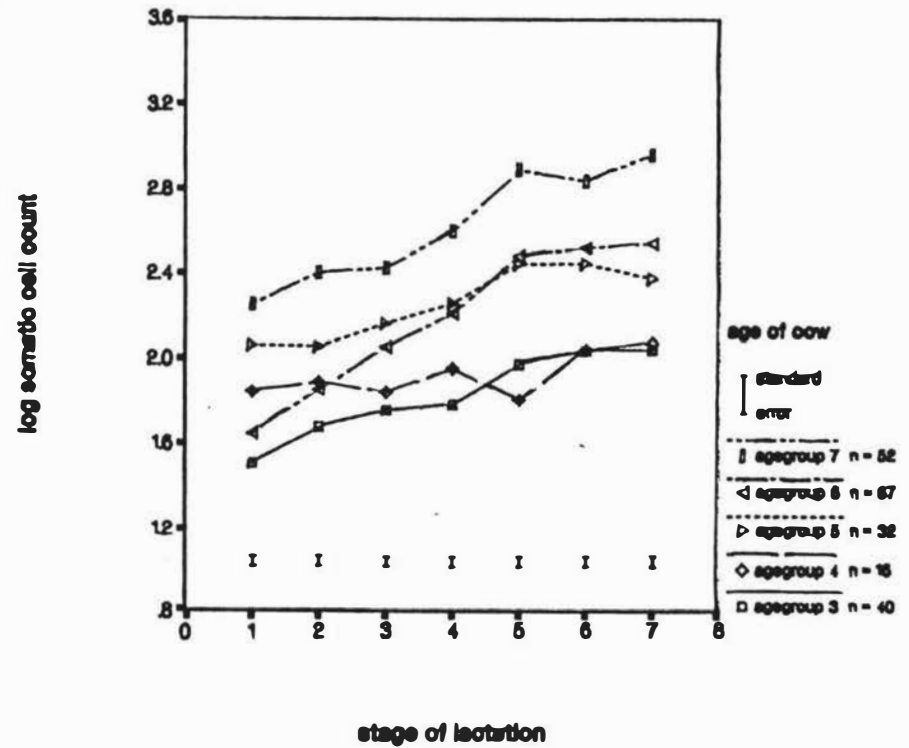
by bacteriological status



logscv = log (10) somatic cell count x 1000  
standard error = pooled monthly standard error

**graph 6.1cii**  
**logscv vs stage of lactation**

by age of cow

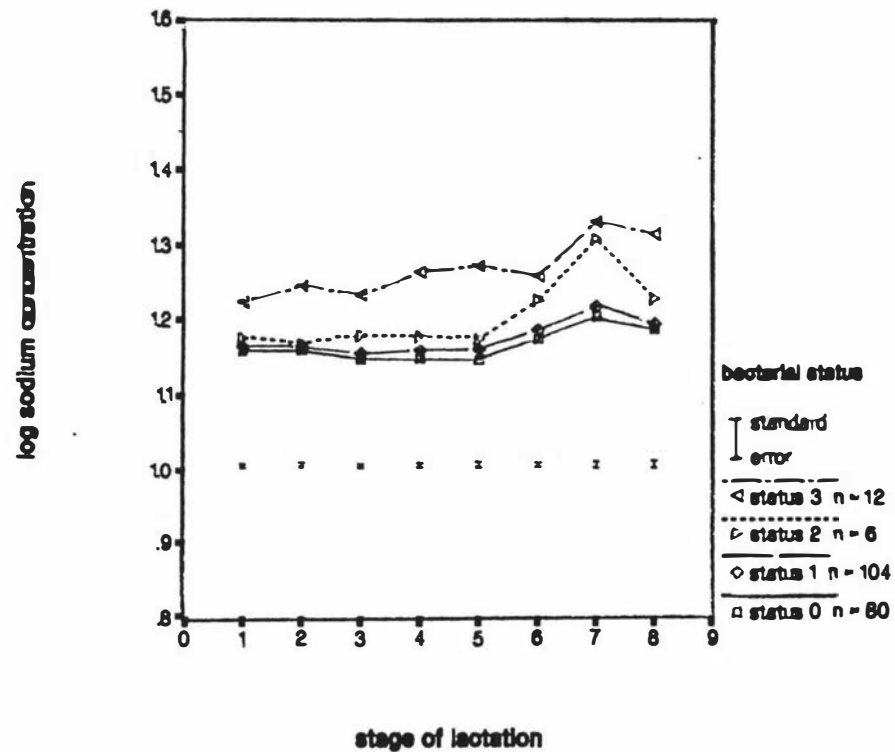


logscv = log (10) somatic cell count x 1000  
standard error = pooled monthly standard error

# SODIUM CONCENTRATION - HERD A

**graph 6.2ai**  
**logsod vs stage of lactation**

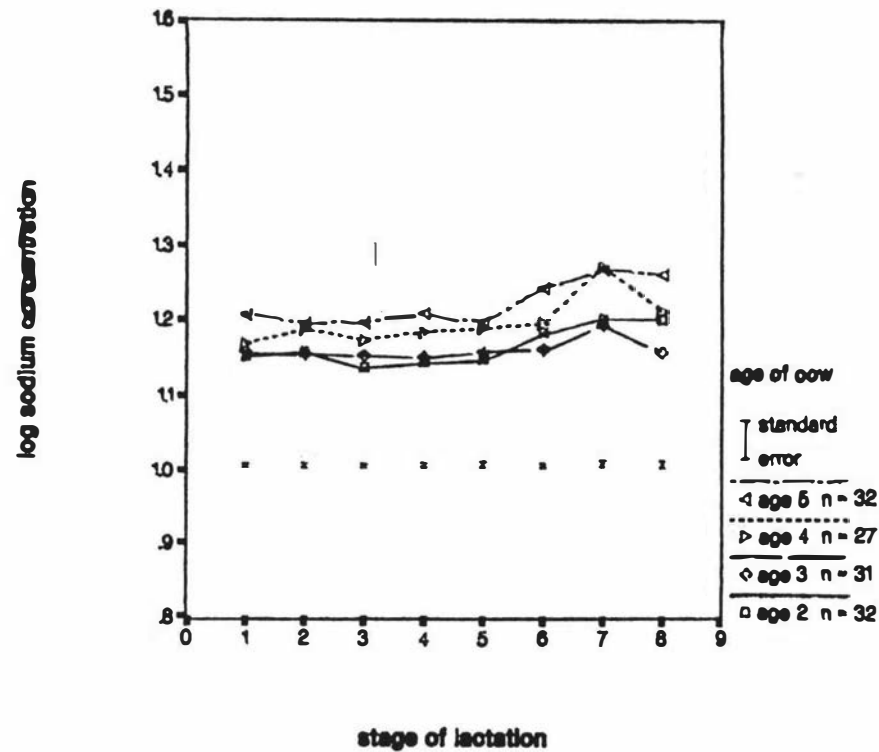
by bacteriological status



logsod = log (10) sodium concentration ( millimoles per litre )  
standard error = pooled monthly standard error

**graph 6.2aii**  
**logsod vs stage of lactation**

by age of cow

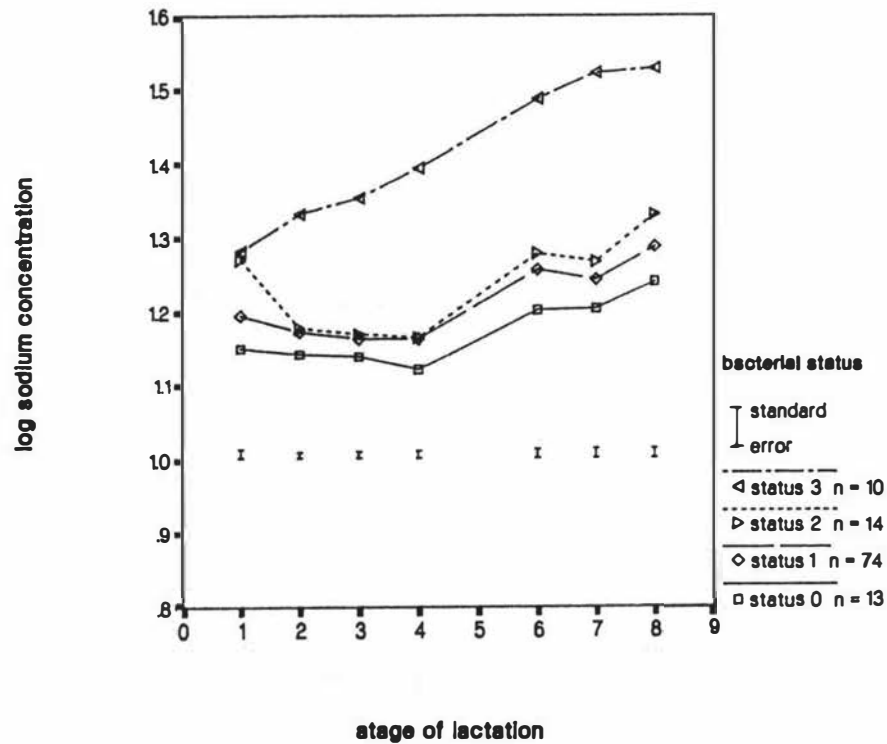


logsod = log (10) sodium concentration ( millimoles per litre )  
standard error = pooled monthly standard error

# SODIUM CONCENTRATION - HERD B

**graph 6.2bi**  
**logsod vs stage of lactation**

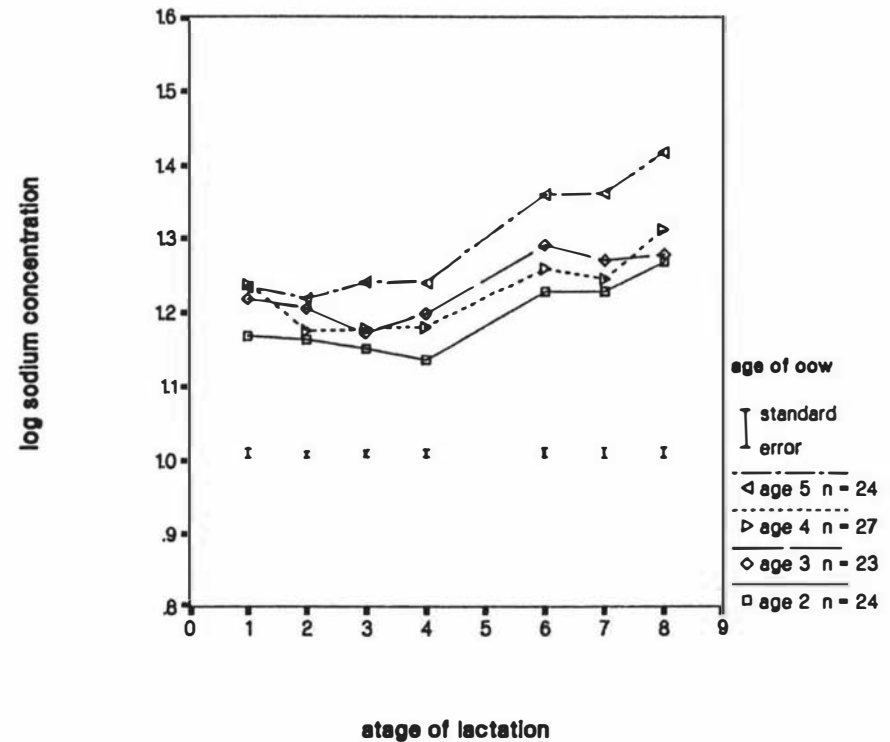
by bacteriological status



logsod = log (10) sodium concentration ( millimoles per litre )  
standard error = pooled monthly standard error

**graph 6.2bii**  
**logsod vs stage of lactation**

by age of cow

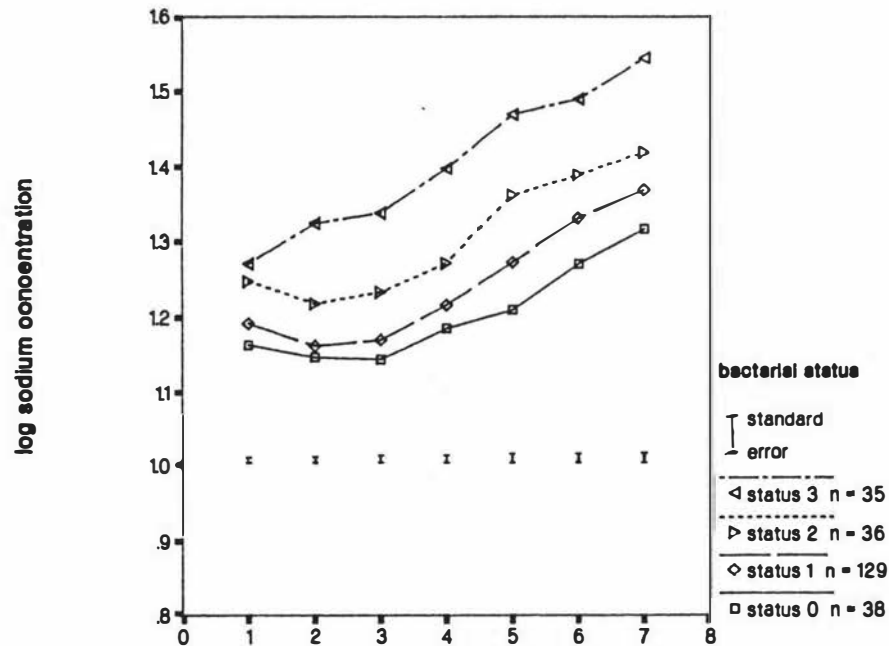


logsod = log (10) sodium concentration ( millimoles per litre )  
standard error = pooled monthly standard error

# SODIUM CONCENTRATION - HERD C

**graph 6.2ci**  
**logsod vs stage of lactation**

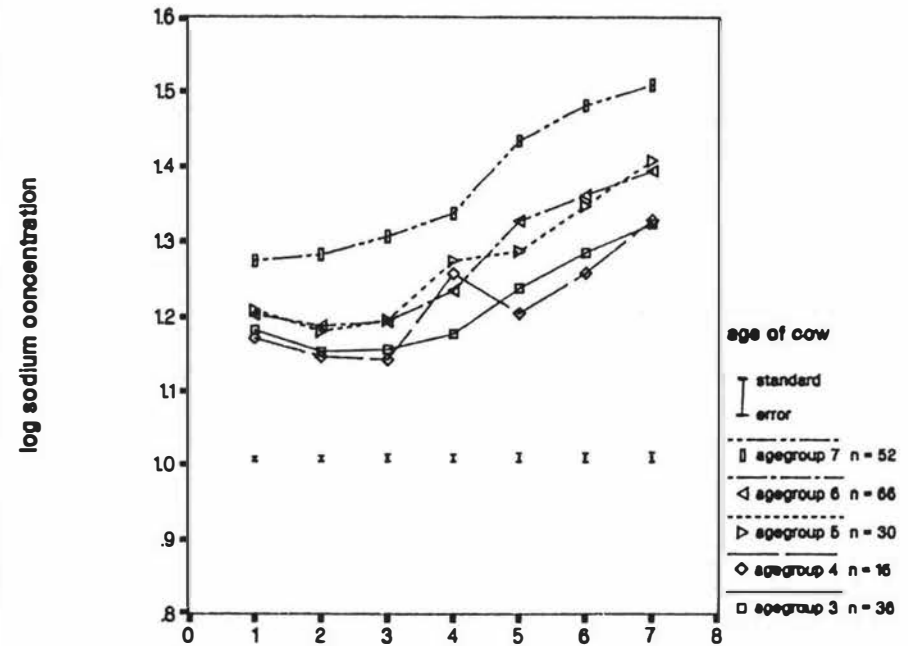
by bacteriological status



logsod =  $\log_{10}$  sodium concentration ( millimoles per litre )  
standard error = pooled monthly standard error

**graph 6.2cii**  
**logsod vs stage of lactation**

by age of cow

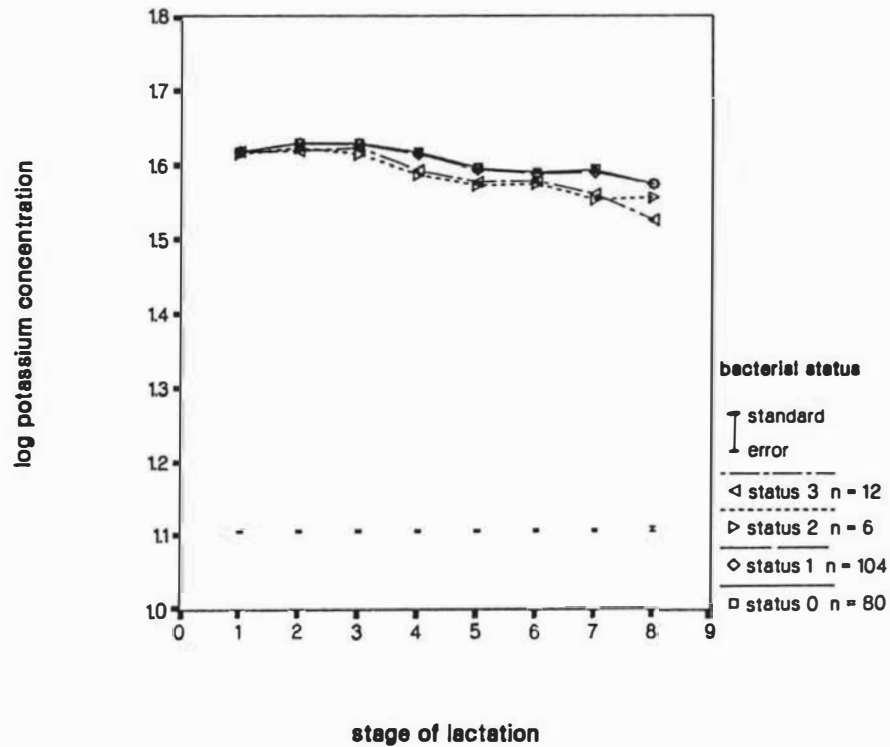


logsod =  $\log_{10}$  sodium concentration ( millimoles per litre )  
standard error = pooled monthly standard error

# POTASSIUM CONCENTRATION - HERD A

**graph 6.3ai**  
**logpot vs stage of lactation**

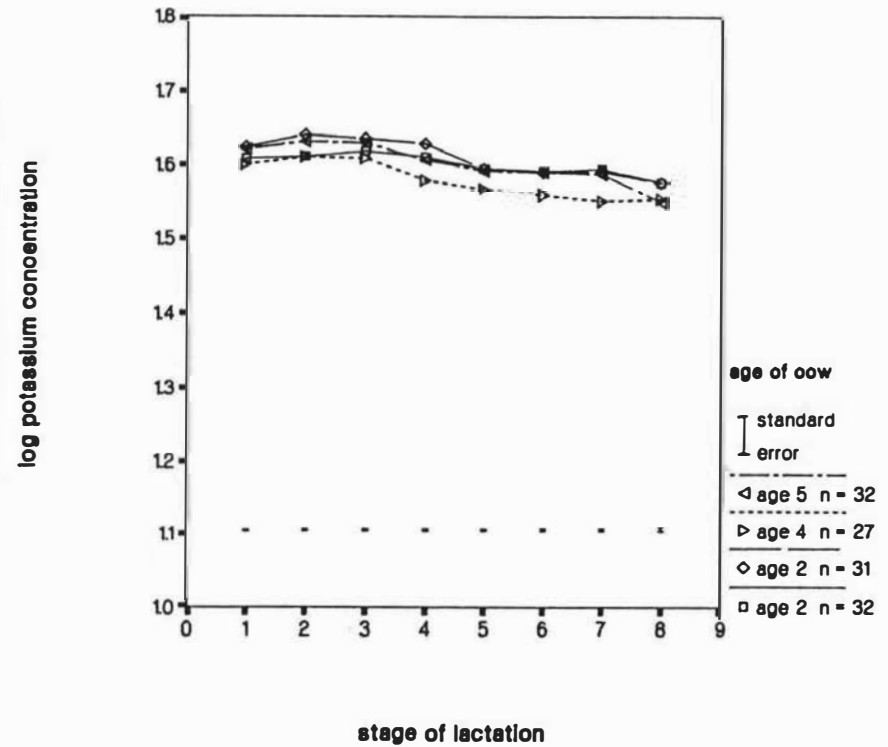
by bacteriological status



logpot =  $\log_{10}$  potassium concentration ( millimoles per litre )  
standard error = pooled monthly standard error

**graph 6.3aii**  
**logpot vs stage of lactation**

by age of cow

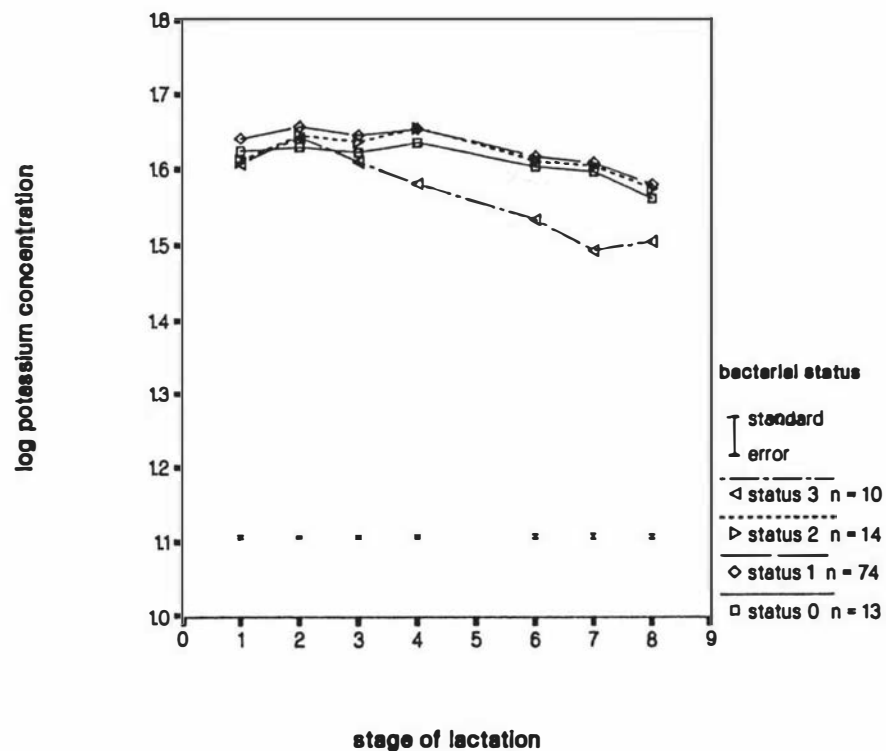


logpot =  $\log_{10}$  potassium concentration ( millimoles per litre )  
standard error = pooled monthly standard error

# POTASSIUM CONCENTRATION - HERD B

**graph 6.3bi**  
**logpot vs stage of lactation**

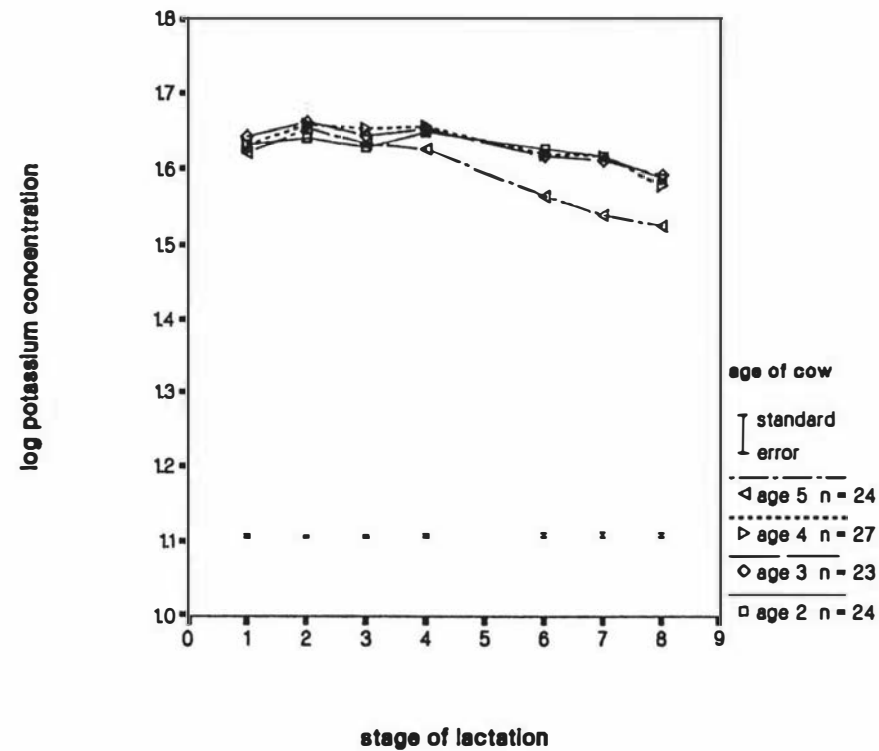
by bacteriological status



logpot = log (10) potassium concentration ( millimoles per litre )  
standard error = pooled monthly standard error

**graph 6.3bii**  
**logpot vs stage of lactation**

by age of cow

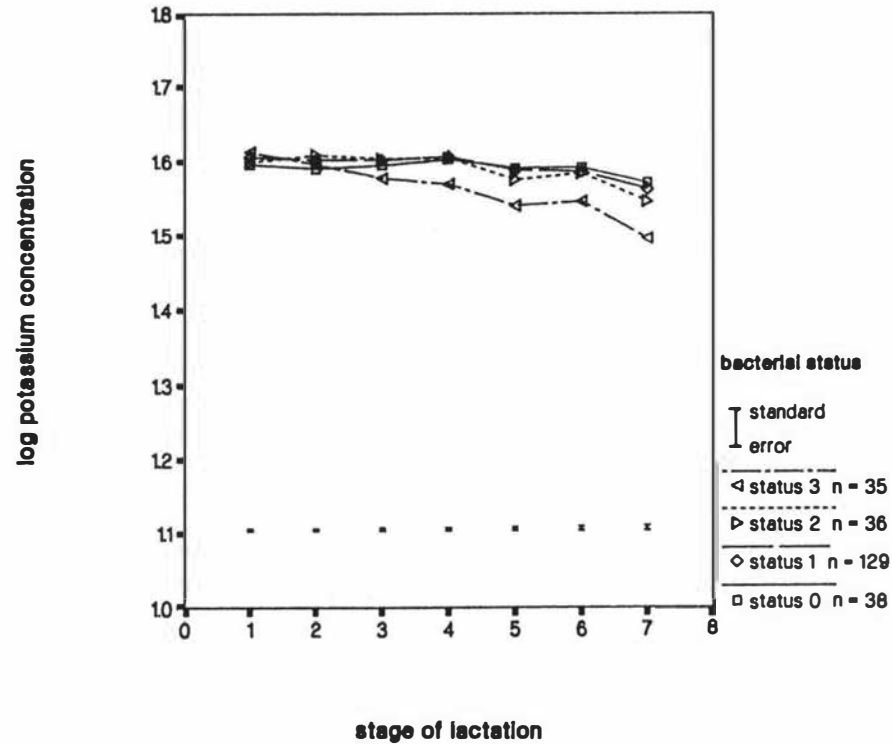


logpot = log (10) potassium concentration ( millimoles per litre )  
standard error = pooled monthly standard error

# POTASSIUM CONCENTRATION - HERD C

**graph 6.3ci**  
**logpot vs stage of lactation**

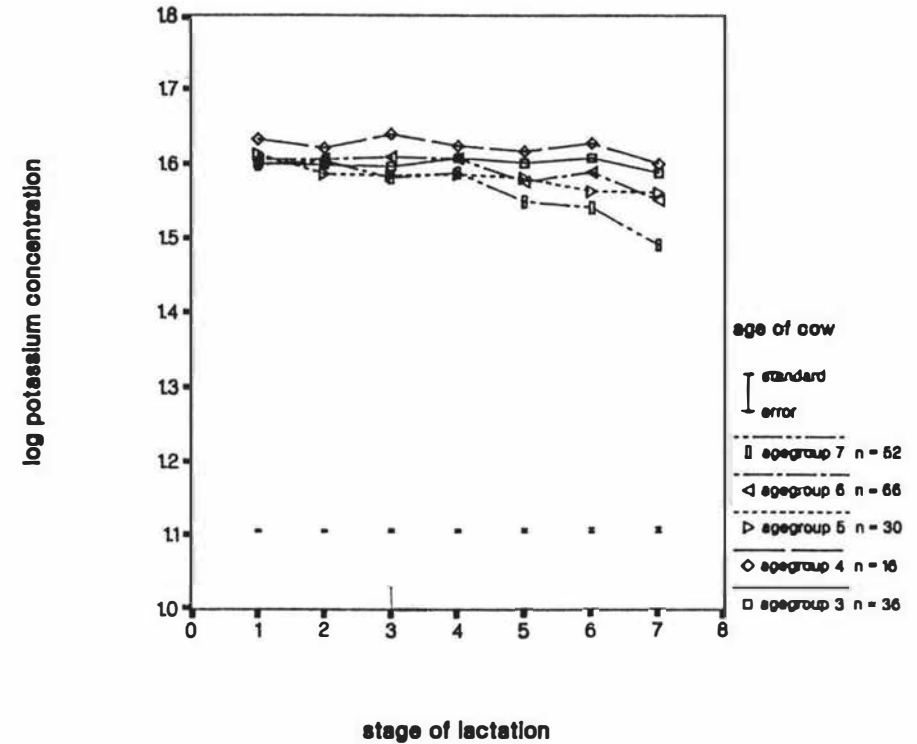
by bacteriological status



logpot = log (10) potassium concentration (millimoles per litre)  
standard error = pooled monthly standard error

**graph 6.3cii**  
**logpot vs stage of lactation**

by age of cow

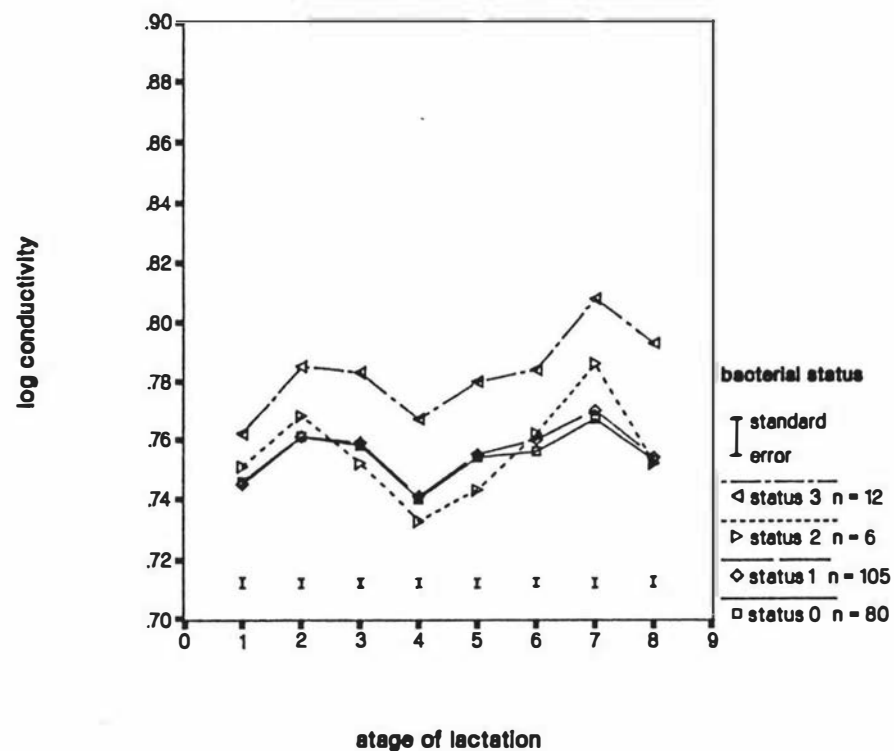


logpot = log (10) potassium concentration (millimoles per litre)  
standard error = pooled monthly standard error

# ELECTRICAL CONDUCTIVITY - HERD A

**graph 6.4ai**  
**logcond vs stage of lactation**

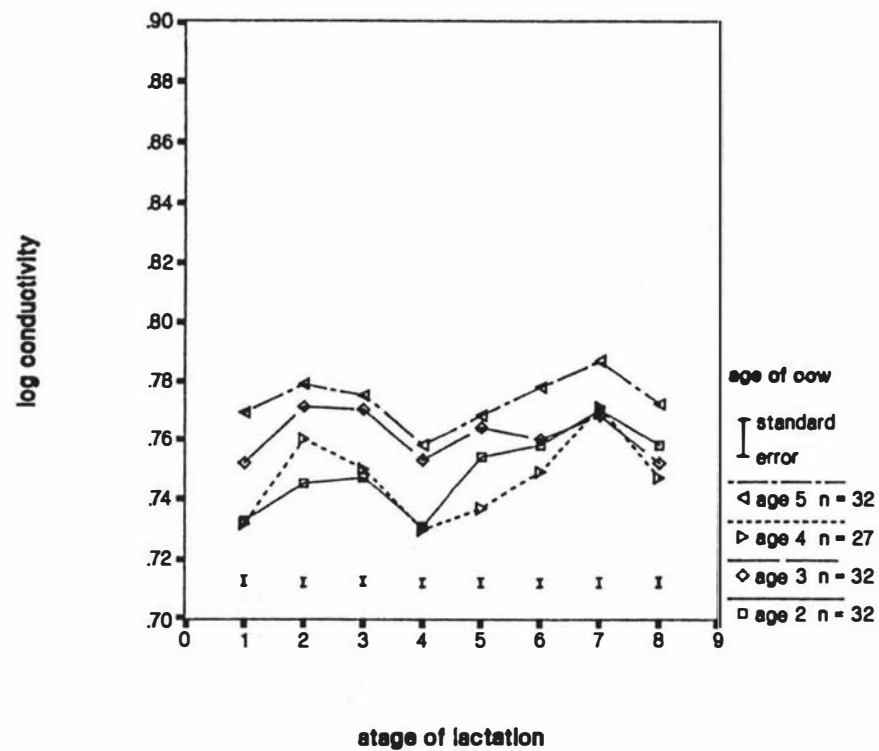
by bacteriological status



logcond = log (10) electrical conductivity ( mS/cm )  
standard error = pooled monthly standard error

**graph 6.4aii**  
**logcond vs stage of lactation**

by age of cow

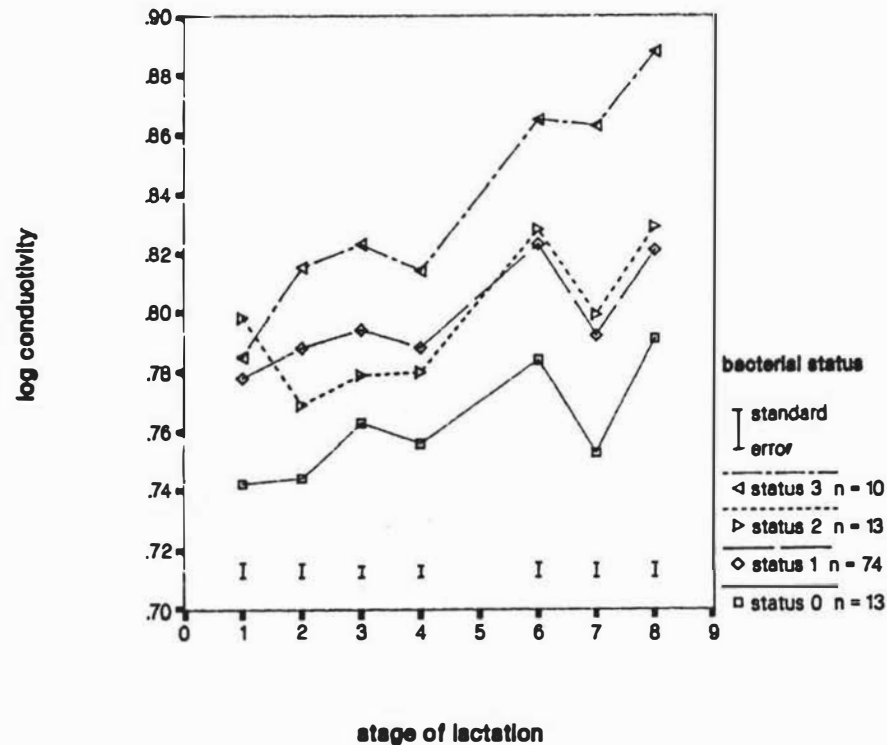


logcond = log (10) electrical conductivity ( mS/cm )  
standard error = pooled monthly standard error

# ELECTRICAL CONDUCTIVITY - HERD B

**graph 6.4bi**  
**logcond vs stage of lactation**

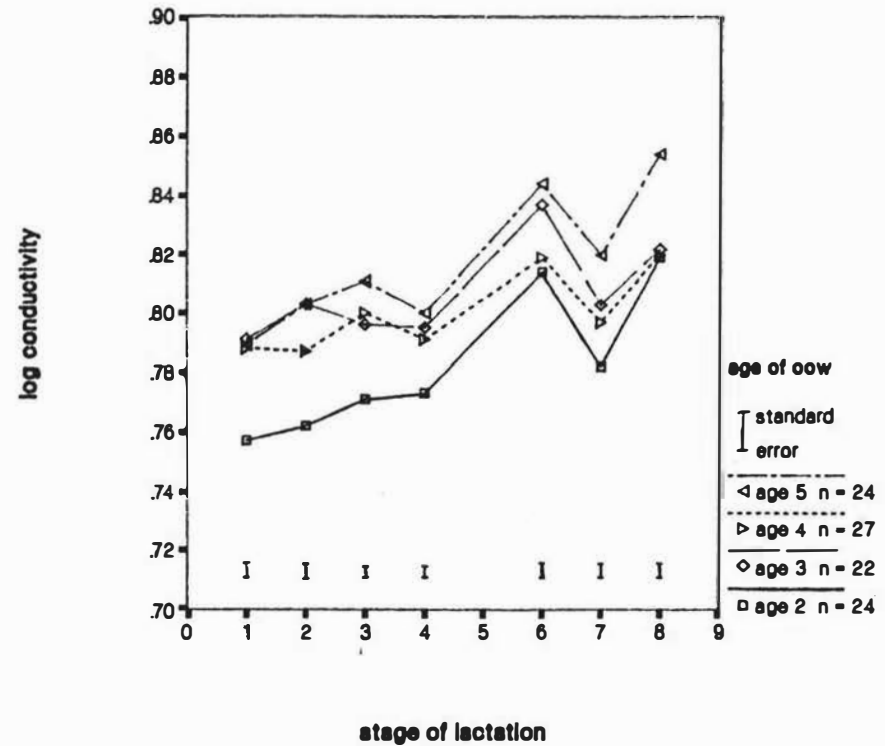
by bacteriological status



logcond = log (10) electrical conductivity ( mS/cm )  
standard error = pooled monthly standard error

**graph 6.4bii**  
**logcond vs stage of lactation**

by age of cow

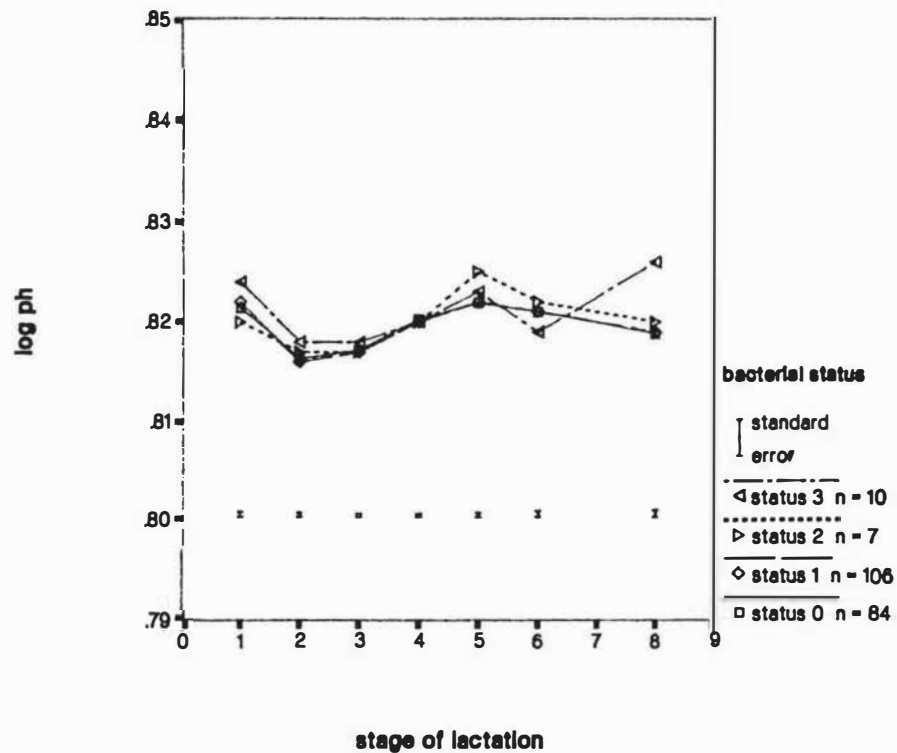


logcond = log (10) electrical conductivity ( mS/cm )  
standard error = pooled monthly standard error

# pH OF MILK - HERD A

**graph 6.5ai**  
**logph vs stage of lactation**

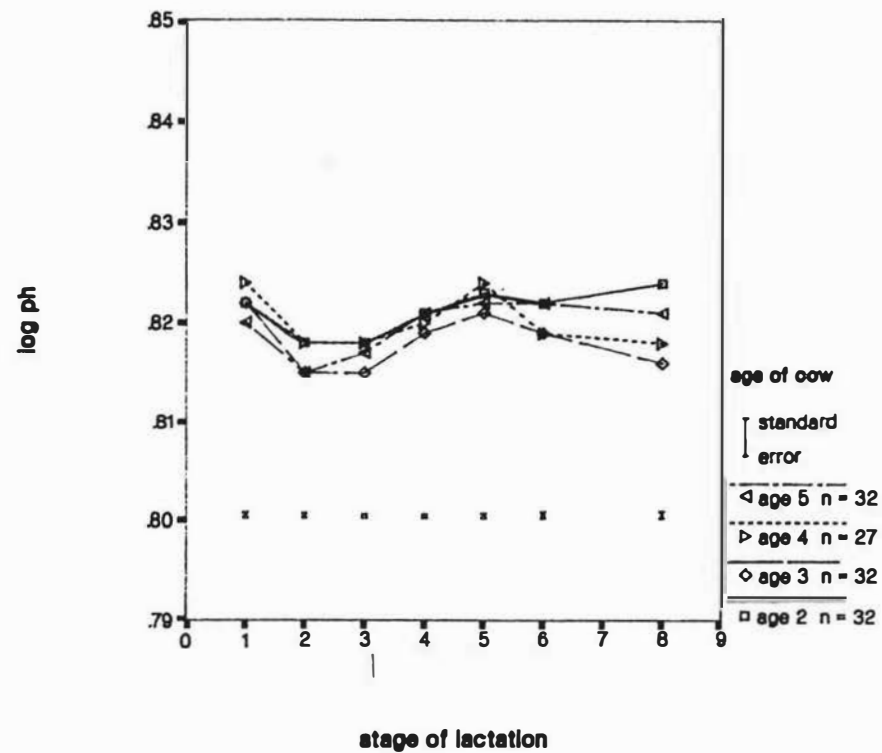
by bacteriological status



logph = log (10) ph  
standard error = pooled monthly standard error

**graph 6.5aii**  
**logph vs stage of lactation**

by age of cow

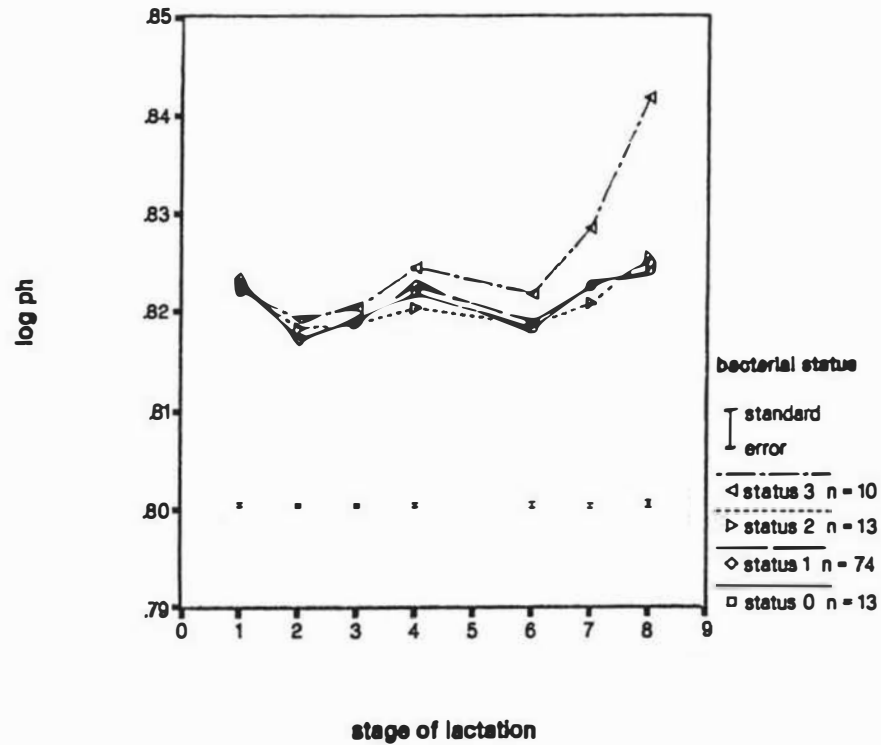


logph = log (10) ph  
standard error = pooled monthly standard error

# pH OF MILK - HERD B

**graph 6.5bi**  
**logph vs stage of lactation**

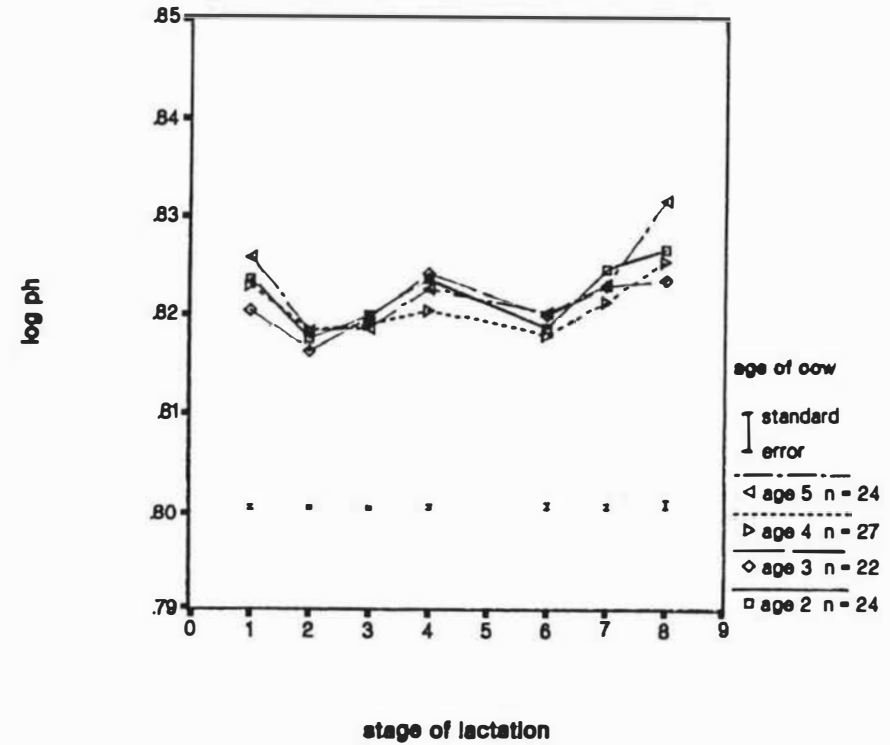
by bacteriological status



logph = log (10) ph  
standard error = pooled monthly standard error

**graph 6.5bii**  
**logph vs stage of lactation**

by age of cow

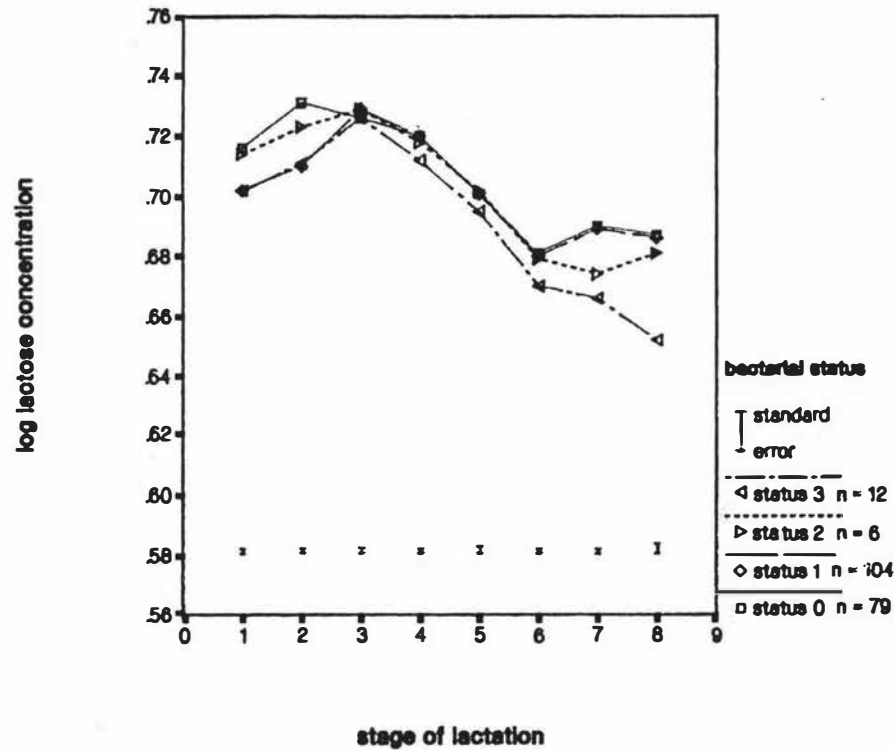


logph = log (10) ph  
standard error = pooled monthly standard error

# LACTOSE CONCENTRATION - HERD A

**graph 6.6ai**  
**loglact vs stage of lactation**

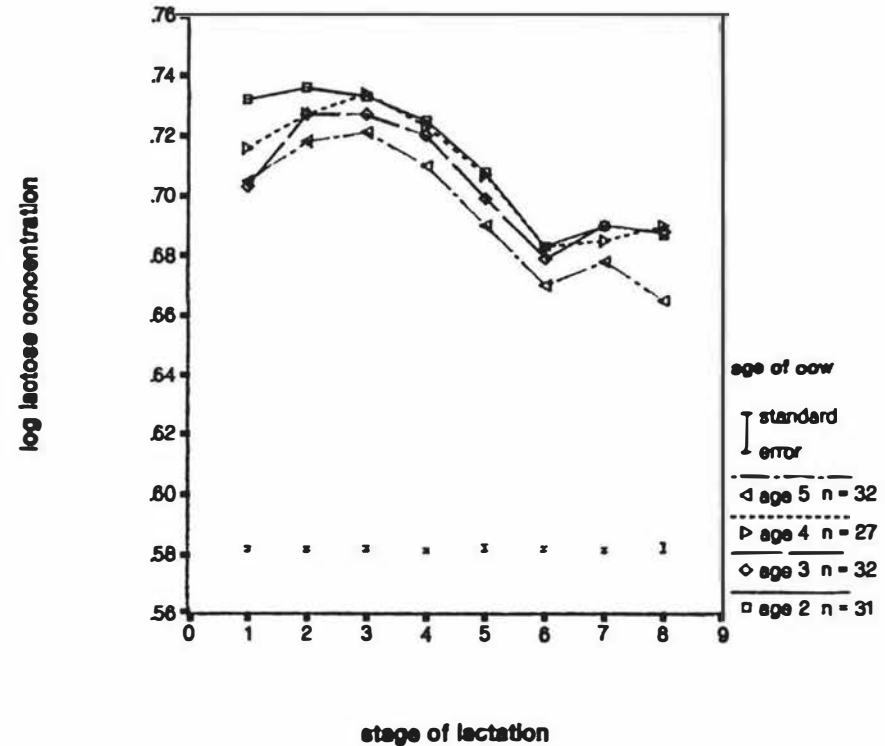
by bacteriological status



loglact =  $\log_{10}$  lactose concentration (%)  
standard error = pooled monthly standard error

**graph 6.6aii**  
**loglact vs stage of lactation**

by age of cow

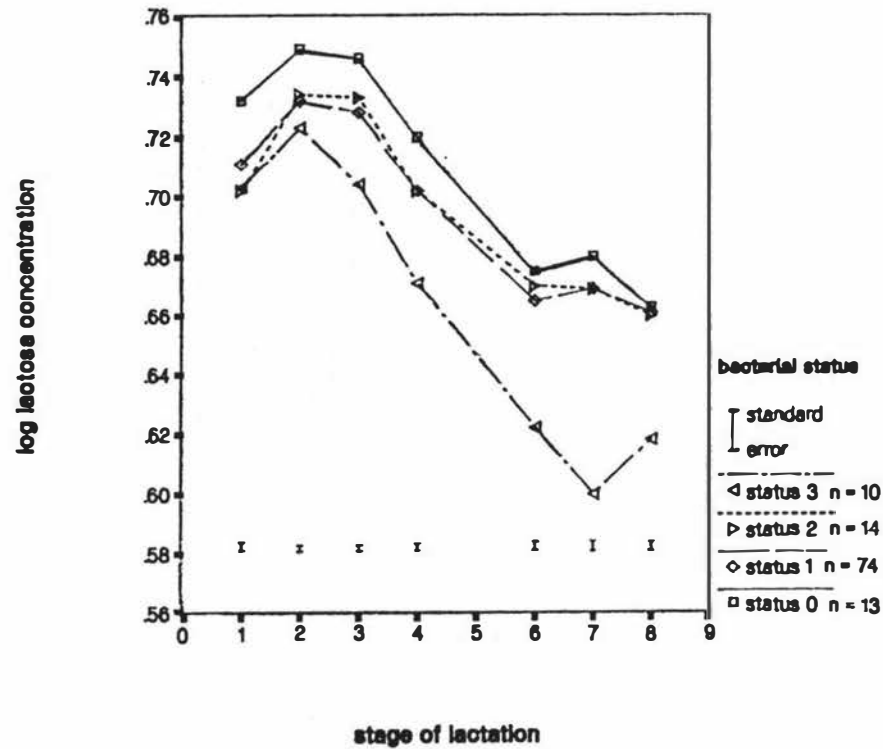


loglact =  $\log_{10}$  lactose concentration (%)  
standard error = pooled monthly standard error

# LACTOSE CONCENTRATION - HERD B

**graph 6.6bi**  
**loglact vs stage of lactation**

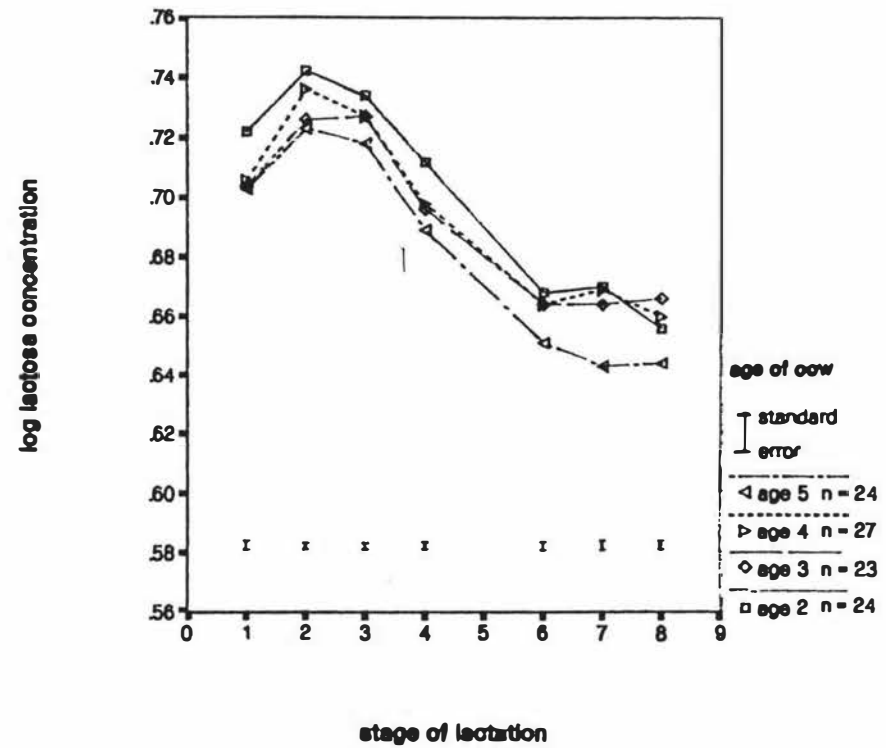
by bacteriological status



loglact =  $\log_{10}$  lactose concentration (%)  
standard error = pooled monthly standard error

**graph 6.6bii**  
**loglact vs stage of lactation**

by age of cow

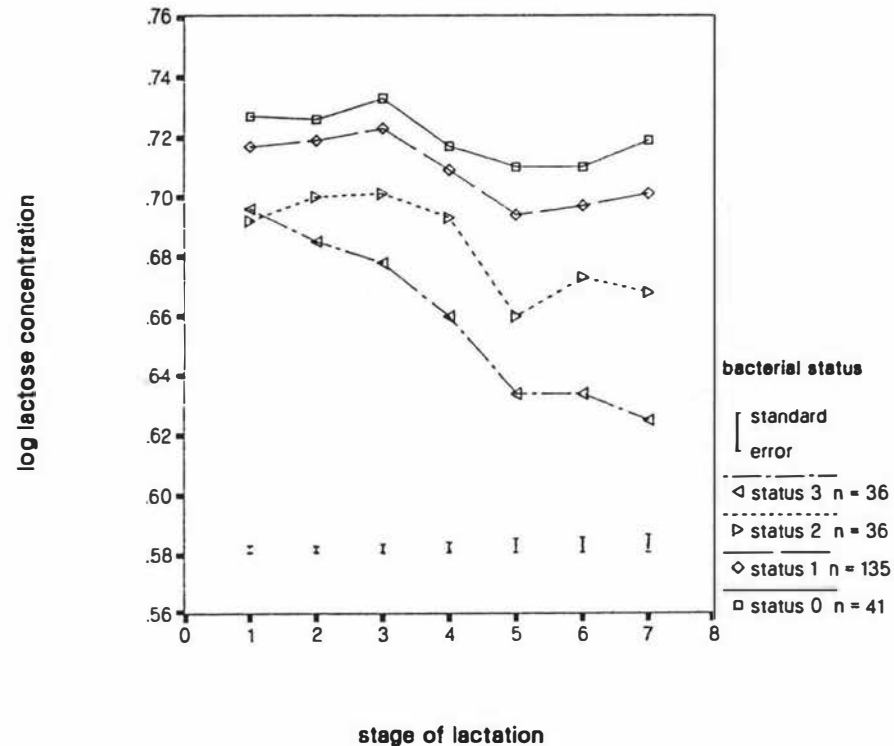


loglact =  $\log_{10}$  lactose concentration (%)  
standard error = pooled monthly standard error

# LACTOSE CONCENTRATION - HERD C

**graph 6.6ci**  
**loglact vs stage of lactation**

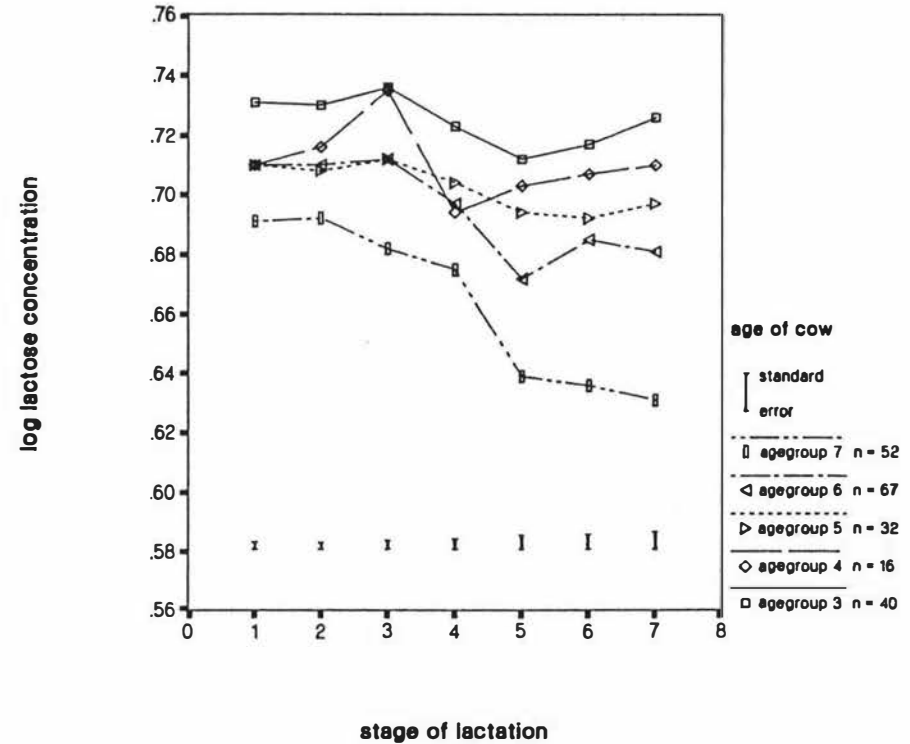
by bacteriological status



loglact = log (10) lactose concentration ( % )  
standard error = pooled monthly standard error

**graph 6.6cii**  
**loglact vs stage of lactation**

by age of cow

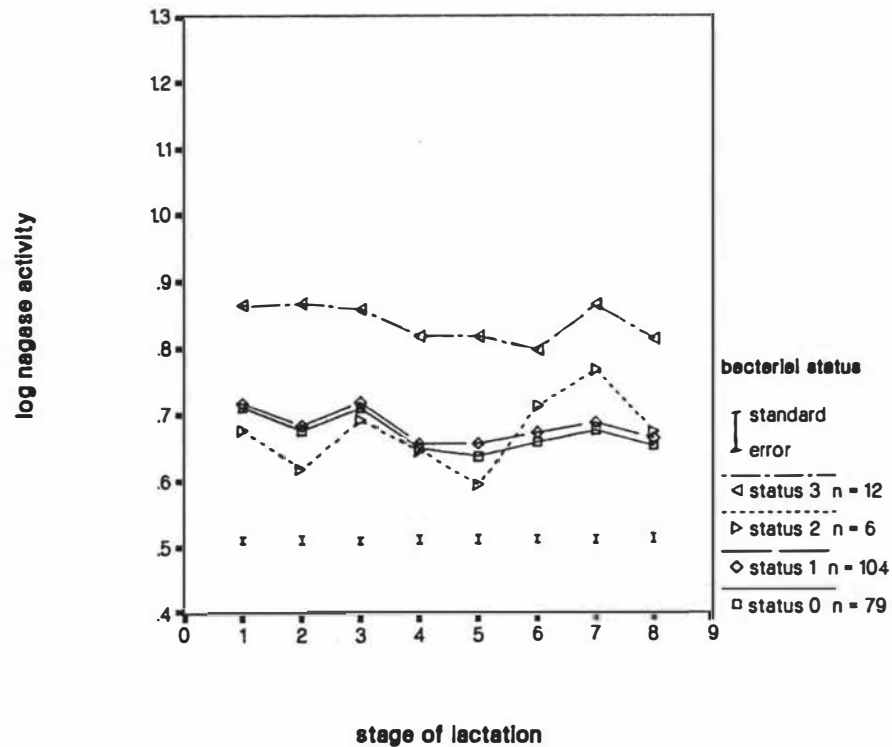


loglact = log (10) lactose concentration ( % )  
standard error = pooled monthly standard error

# NAGase ACTIVITY - HERD A

**graph 6.7ai**  
**lognag vs stage of lactation**

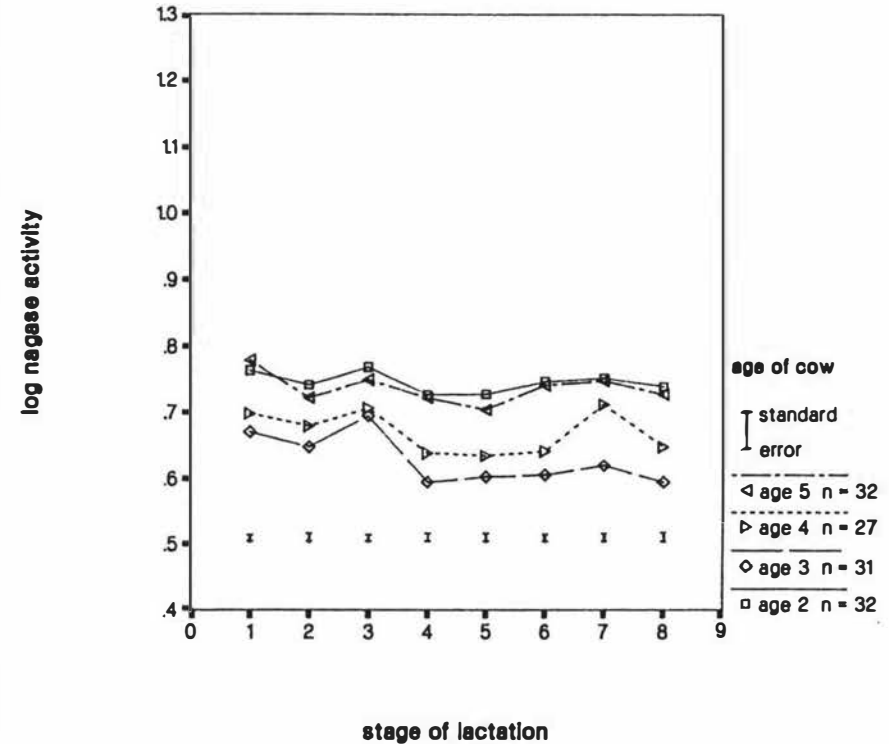
by bacteriological status



lognag = log (10) NAGase activity ( nmole/ml/minute )  
standard error = pooled monthly standard error

**graph 6.7aii**  
**lognag vs stage of lactation**

by age of cow

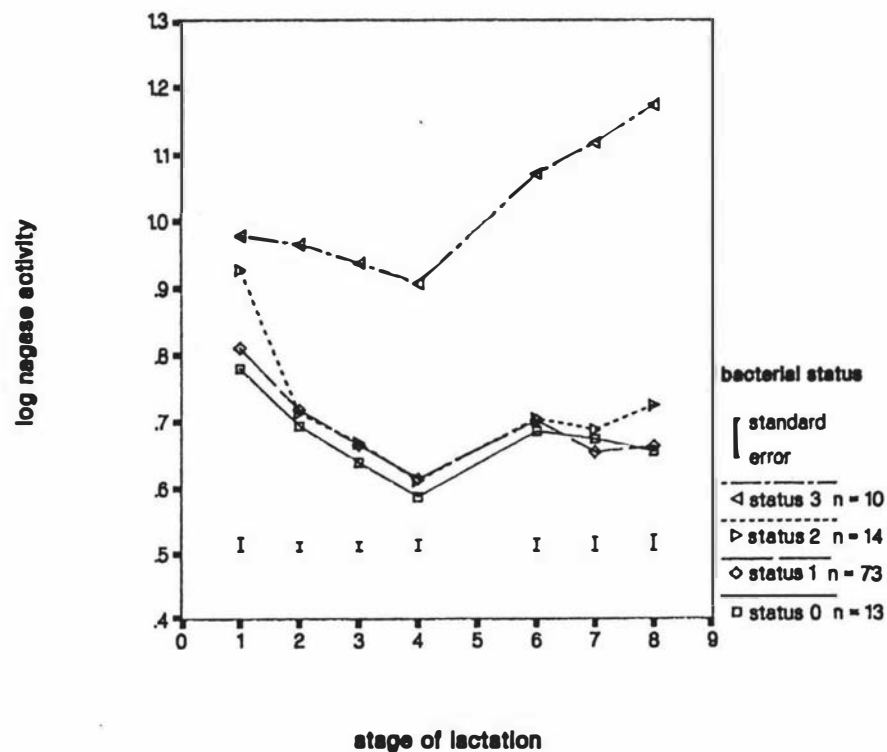


lognag = log (10) nase activity ( nmole/ml/minute )  
standard error = pooled monthly standard error

# NAGase ACTIVITY - HERD B

**graph 6.7bi**  
**lognag vs stage of lactation**

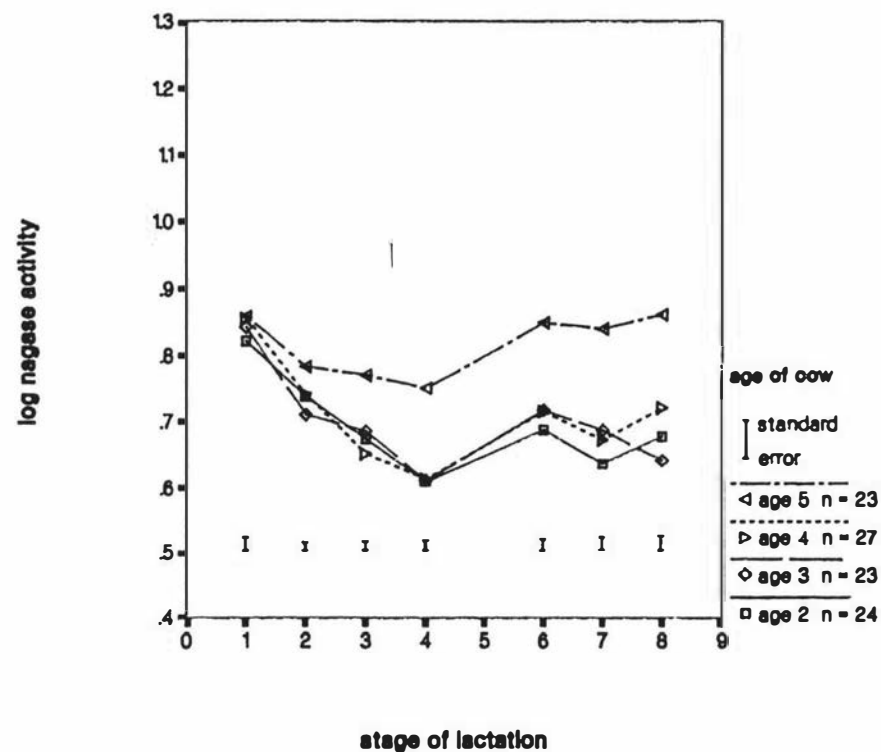
by bacteriological status



lognag = log (10) NAGase activity ( nmole/ml/minute )  
standard error = pooled monthly standard error

**graph 6.7bii**  
**lognag vs stage of lactation**

by age of cow

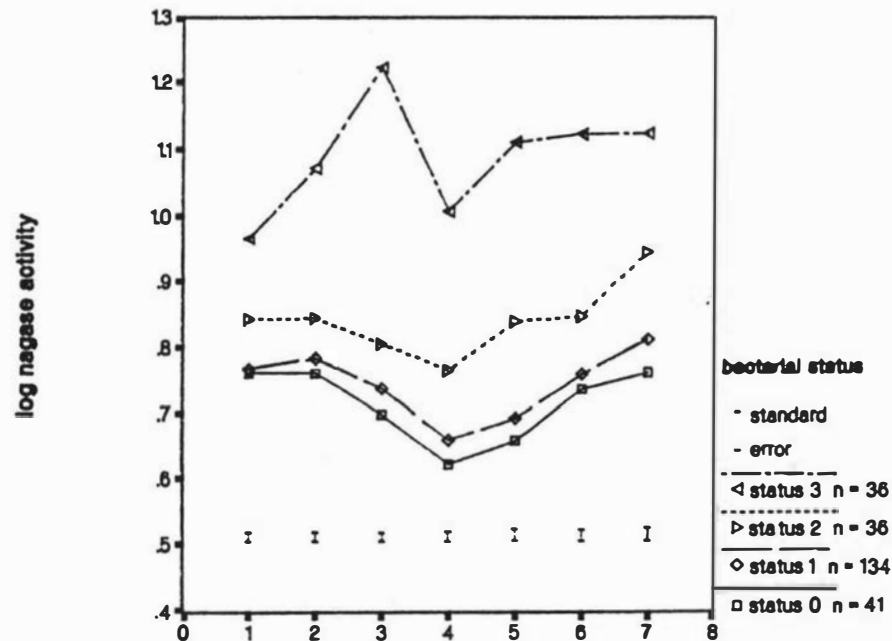


lognag = log (10) nage activity ( nmole/ml/minute )  
standard error = pooled monthly standard error

# NAGase ACTIVITY - HERD C

**graph 6.7ci**  
**lognag vs stage of lactation**

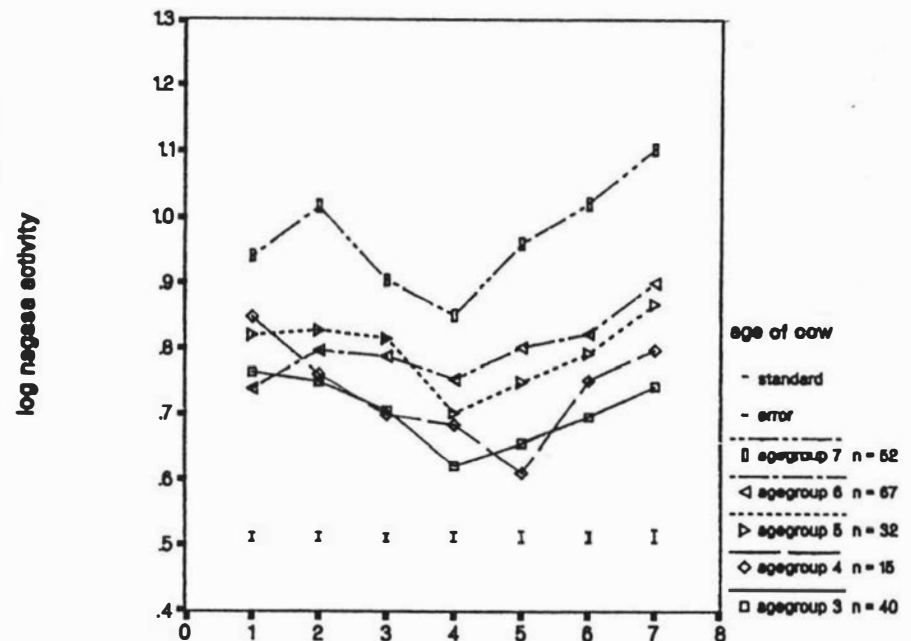
by bacteriological status



lognag = log (10) NAGase activity ( nmole/ml/minute )  
standard error = pooled monthly standard error

**graph 6.7cii**  
**lognag vs stage of lactation**

by age of cow

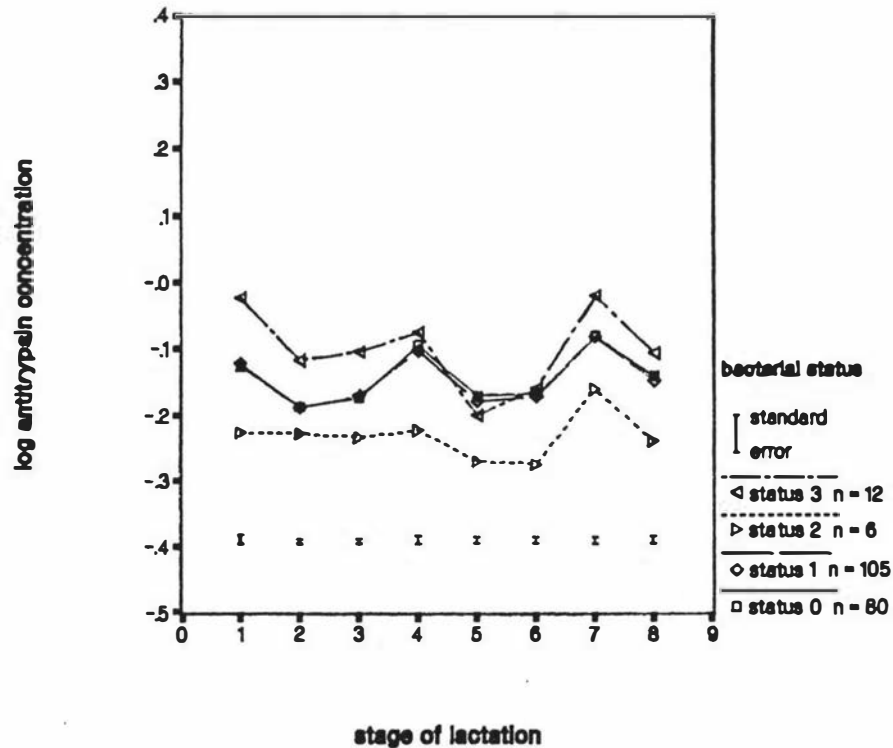


lognag = log (10) NAGase activity ( nmole/ml/minute )  
standard error = pooled monthly standard error

# ANTITRYPSIN CONCENTRATION - HERD A

**graph 6.8ai**  
**logant vs stage of lactation**

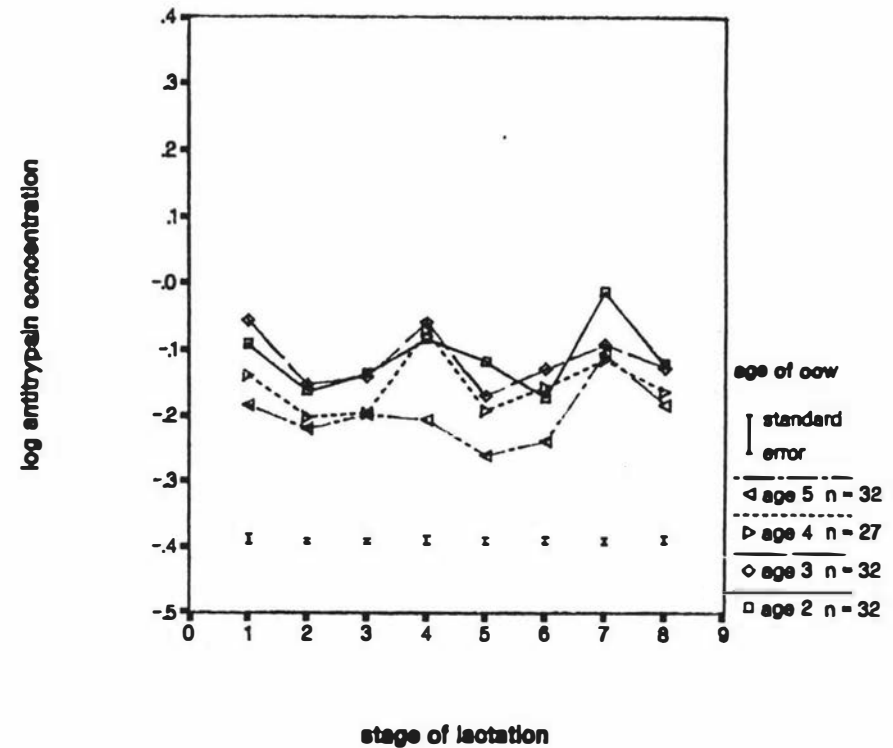
by bacteriological status



logant = log (10) antitrypsin concentration ( relative units )  
standard error = pooled monthly standard error

**graph 6.8aii**  
**logant vs stage of lactation**

by age of cow

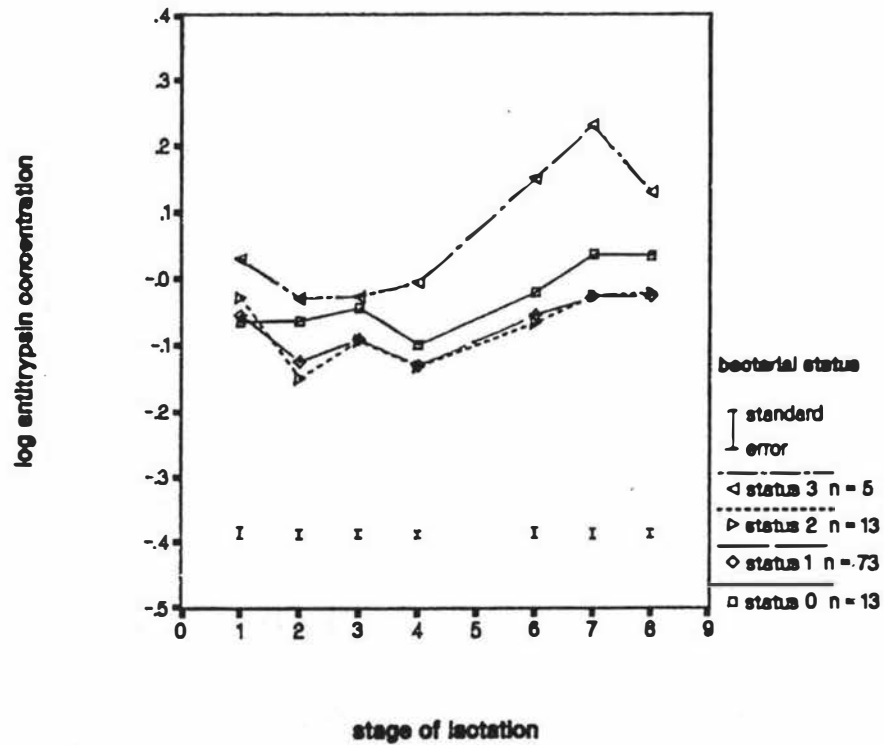


logant = log (10) antitrypsin concentration ( relative units )  
standard error = pooled monthly standard error

# ANTITRYPSIN CONCENTRATION - HERD B

**graph 6.8bi**  
**logant vs stage of lactation**

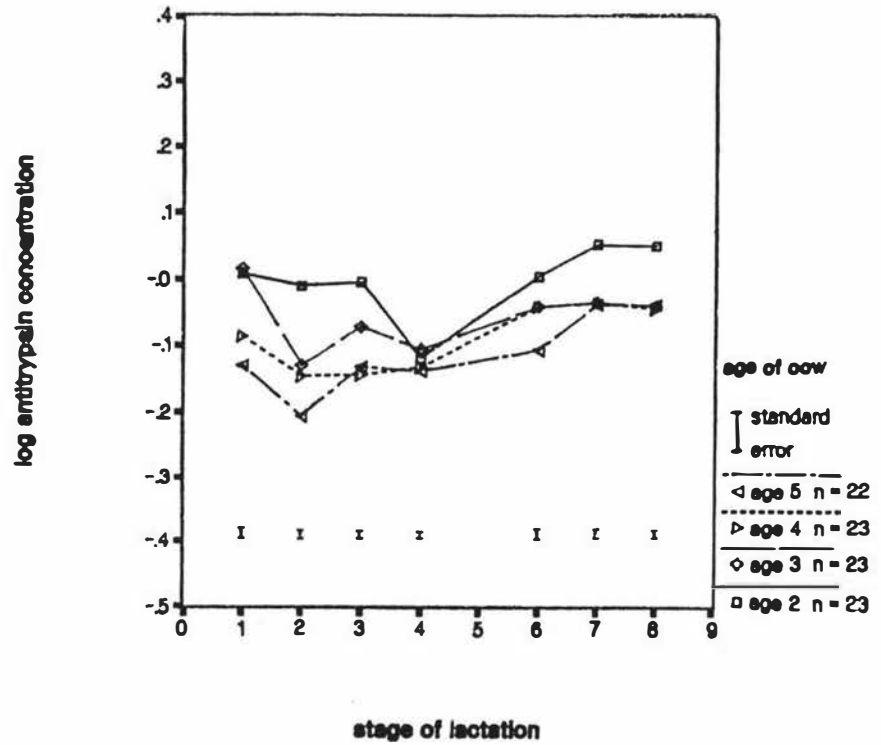
by bacteriological status



logant = log (10) antitrypsin concentration (relative units)  
standard error = pooled monthly standard error

**graph 6.8bii**  
**logant vs stage of lactation**

by age of cow

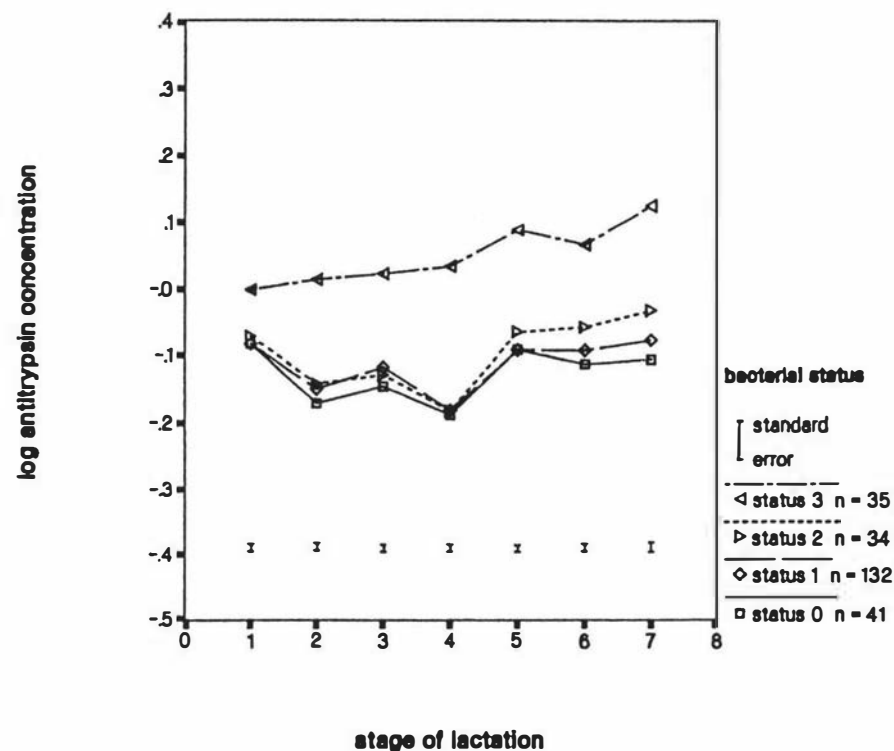


logant = log (10) antitrypsin concentration (relative units)  
standard error = pooled monthly standard error

# ANTITRYPSIN CONCENTRATION - HERD C

**graph 6.8ci**  
**logant vs stage of lactation**

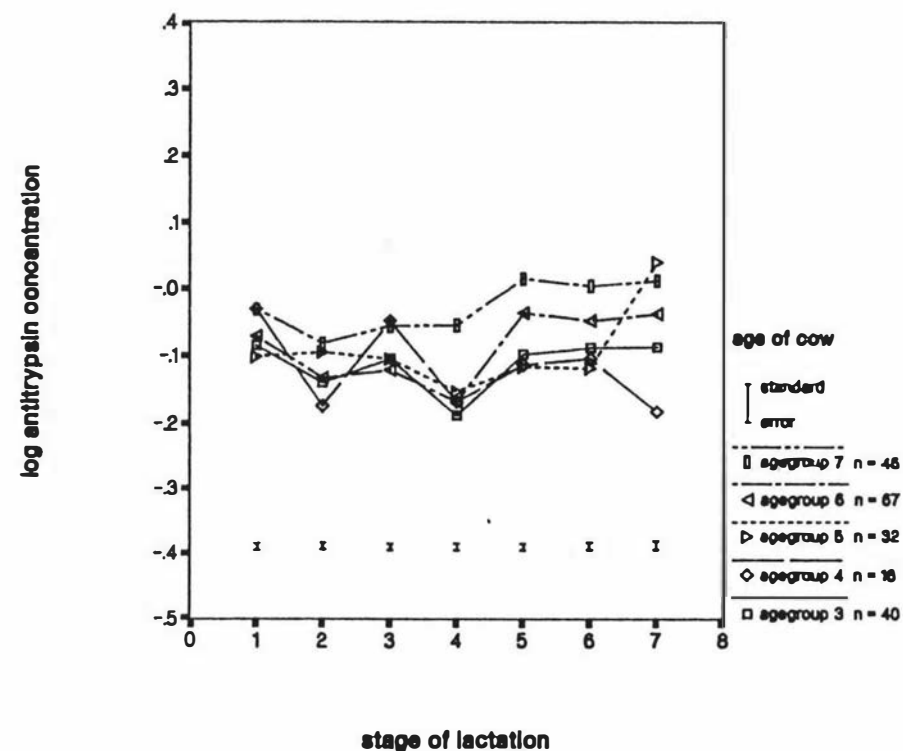
by bacteriological status



logant = log (10) antitrypsin concentration (relative units)  
standard error = pooled monthly standard error

**graph 6.8cii**  
**logant vs stage of lactation**

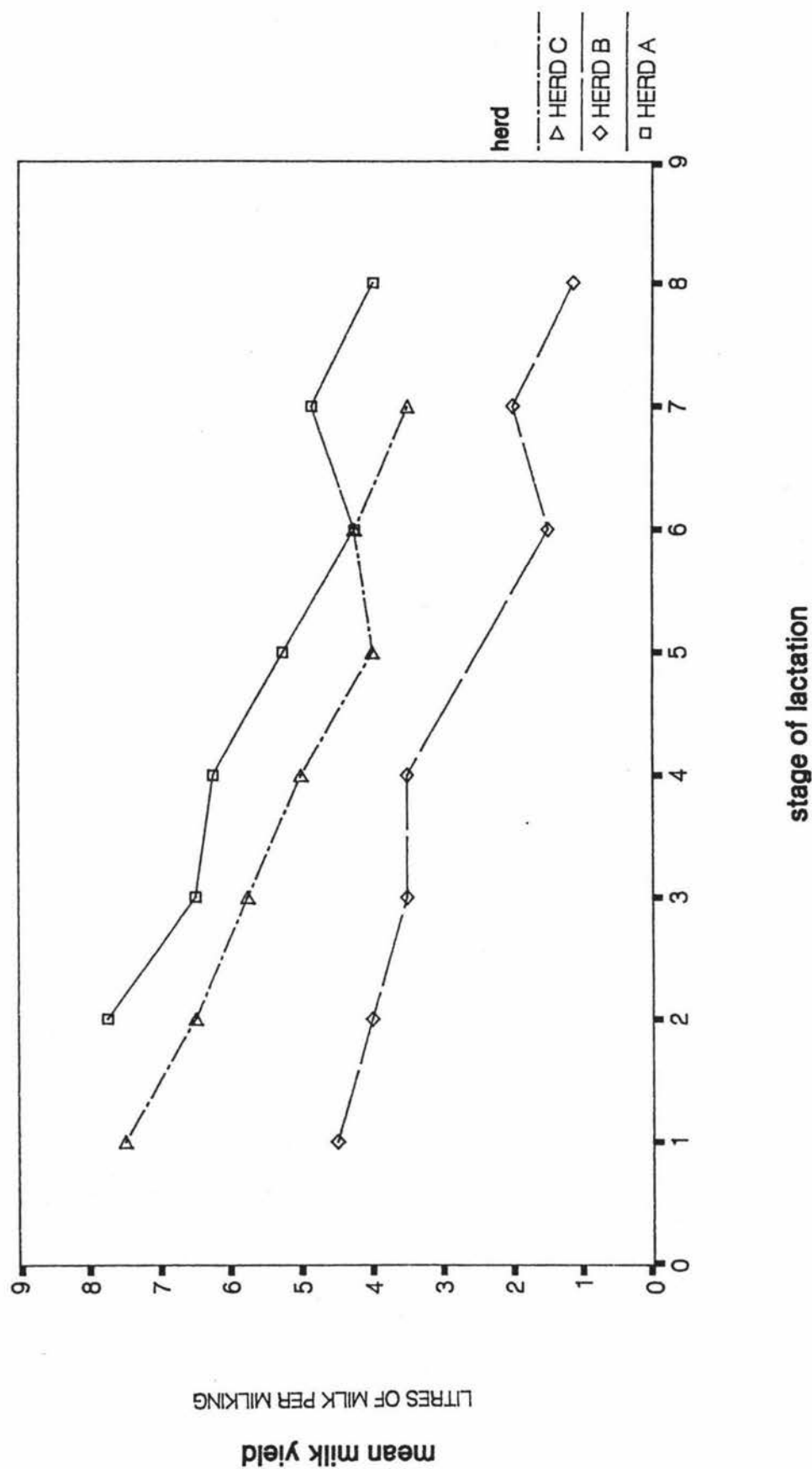
by age of cow



logant = log (10) antitrypsin concentration (relative units)  
standard error = pooled monthly standard error

# GRAPH 6.9 COMPOSITE MILK YIELD DURING LACTATION

WITHIN INDIVIDUAL HERDS



## CHAPTER SEVEN

### THE DIAGNOSIS OF INFECTION IN QUARTER SAMPLES

#### INTRODUCTION

A number of tests have been devised in an effort to diagnose subclinical bovine mastitis. In New Zealand, as in many other dairying countries, the somatic cell count is the most widely adopted method for diagnosing this disease.

Comparatively little information is available on the relationship between the somatic cell count of composite milk ( milk from all four quarters ) from cows, or from individual quarters, and the infection status of the cow or quarter, under New Zealand conditions of management.

During the present trial, a number of tests were assessed with regard to their ability to discriminate between quarters which were subclinically infected, and those quarters which were uninfected. The tests which were used included:

- (1) The somatic cell count
- (2) The sodium concentration
- (3) The potassium concentration
- (4) The electrical conductivity
- (5) The pH
- (6) The lactose concentration
- (7) The NAGase activity
- (8) The antitrypsin concentration

The present chapter describes the ability of each of these tests alone, or in combination with the somatic cell count, to distinguish between infected and uninfected quarters in cows from three New Zealand dairy herds.

## MATERIALS AND METHODS

The three dairy herds which were used and the details of the sampling procedure have been described previously ( chapter three ).

### (1) DEFINITION OF INFECTION

A quarter was deemed to be infected if two or more colonies of a recognized mastitis pathogen were isolated on one occasion from a 50 microlitre sample of milk which had been taken using aseptic techniques. It is recognized that this definition of infection is subject to some error, in that sampling on only one occasion may fail to detect infection where only small numbers of bacteria are present in the milk. Conversely, a small number of quarters may be classified as infected, when the organisms have come from external sources such as the skin of the teat.

A similar " one shot " diagnostic sysem has been used in a recent comparison of mastitis screening tests in Sweden ( Emanuelson et al, 1987 ).

Provided that teat dipping is used and that the samples are taken by trained staff, then the rate of a false prediction of the infection status of a quarter is relatively small ( Neave, 1975 ).

During the present study, the samples were taken by a small number of trained staff, following methods recommended by the International Dairy Federation ( Dodd, 1981 ). Teat spraying with an antiseptic was used in all three herds, although its use in herd B was intermittant during the season. The sampling and cultural methods which were used, ensured that both the false positive and false negative results were minimized.

While the rate of false prediction of infection status using a single sample may be low, a lower false prediction rate may be achieved if two or more samples are taken ( Neave, 1975 ). The use of 2 or 3 confirmatory tests on successive days was impractical during the current study, due to financial and time constraints. Some of the limitations were overcome by sampling the same cows on 6 to 8 occasions during the lactation. To confirm the diagnoses would have meant sampling a smaller number of cows.

## (2) CLASSIFICATION OF QUARTERS

Two bacteriological classification schemes were used:

### Bacteriological classification scheme 1

Quarters infected with a major pathogen were deemed to be infected, bacteriologically negative quarters, and quarters harbouring minor pathogens were regarded as uninfected.

### Bacteriological classification scheme 2

Quarters infected with a major or with a minor pathogen were deemed to be infected, bacteriologically negative quarters were regarded as uninfected.

The assay methods for each of the parameters have been described ( see chapter three ).

## (3) CRITICAL THRESHOLD FOR EACH PARAMETER

The ability of each parameter to predict the presence or absence of infection was calculated using a program which was written in BASIC and run on the Massey University Prime computer ( see appendix for the program listing ). Each parameter was analysed separately.

The critical threshold, ie the threshold at which the proportion of false negative and false positive results are equal, was determined by entering an initial threshold, set well below the expected critical threshold, and a final threshold, set well above the expected critical threshold. If the critical threshold was below the initial threshold, or above the final threshold, then new limits were set, and the program run again. Each quarter sample had been coded 0 or 1 on the basis of the bacteriological findings. This figure, plus the value of the parameter in question, eg the somatic cell count, were read into the computer from an internal file and the quarters were classified as follows:

- (1) If the quarter was infected and the value of the parameter at that test was above the threshold figure then the result was deemed to be a true positive result.
- (2) If the quarter was uninfected and the value of the parameter was below that of the threshold figure, then the result was deemed to be a true negative result.

- (3) If the quarter was infected but the value of the parameter was below the threshold figure, then the result was deemed to be a false negative result.
- (4) If the quarter was uninfected but the value of the parameter was above that of the threshold figure, then the result was deemed to be a false positive result.

This process was repeated for each sample, using the initial threshold figure. An increment was then automatically added to the initial threshold figure and the comparison of the results for each quarter was repeated at this new threshold. The whole process was repeated until the threshold figure reached the final threshold which had been entered ( see above ). At each threshold level, the proportion of false negative and false positive results were printed, the critical threshold being the point at which the false positive results, as a proportion of the total number of uninfected samples, and the false negative results, as a proportion of the total number of infected samples, are equal. The probability of misclassification is the proportion of false results which occurs at the critical threshold ( Sheldrake et al, 1983a ).

#### (4) STAGE OF LACTATION

For the purposes of the analysis, the lactation was divided into four stages as follows:

- Stage 1        includes samples taken during the second and third months of lactation and corresponds to stages 1 and 2 in chapter six.
- Stage 2        includes samples taken during the fourth and fifth months of lactation and corresponds to stages 3 and 4 in chapter six.
- Stage 3        includes samples taken during the sixth and seventh months of lactation and corresponds to stages 5 and 6 in chapter six.
- Stage 4        includes samples taken during the eighth and ninth months of lactation and corresponds to stages 7 and 8 in chapter six.

### (5) DISCRIMINANT FUNCTION ANALYSIS

The ability of each parameter to distinguish between infected and uninfected quarters was also determined using discriminant function analysis ( Statistical Analytical Systems, 1985 ). This procedure computes a linear or quadratic discriminant function for classifying observations into two or more groups on the basis of one or more numeric variables, by determination of the generalised squared distance between groups. This procedure allows the application of the classification to a second data set.

The discriminative power of a combination of the somatic cell count and each of the other parameters was determined using discriminant function analysis. All discriminant analyses were performed on log (10) transformed data, as the untransformed data were markedly skewed.

## RESULTS

### (1) RESULTS FOR TOTAL DATA SET BACTERIOLOGICAL CLASSIFICATION 1

Table 7.1i shows the results for the total data set, ie all three herds, at all four stages of the lactation, using bacteriological classification 1 ( see materials and methods section, chapter seven, for an explanation of bacteriological classification criteria ).

The critical threshold for the somatic cell count was 245,000 cells per ml of milk. At this threshold, the probability of misclassification was 20 %, therefore 80 % of samples were correctly classified as being either infected or uninfected by the somatic cell count.

The ability of the remaining parameters to distinguish between infected and uninfected quarters was lower, with only the sodium concentration and the NAGase activity showing a probability of misclassification of less than 30 % of samples. The potassium concentration and the pH of the milk proved to be of little value for discriminating between infected and uninfected quarters, as in each case the probability of misclassification exceeded 40 % of samples.

The overall infection rate, when the figures for the three herds were combined, and the criteria of bacteriological classification 1 were applied, was approximately 14.3 % of quarters. The infection rate was lower for the electrical conductivity and for the pH of milk than was the case for the other parameters. This is due to the fact that electrical conductivity readings and pH readings were not carried out on samples taken from herd C. The infection rate for major pathogens within herd C was relatively high ( see chapter five ). Consequently, the figures for the electrical conductivity and the pH of the milk are based on herds A and B only, a fact which must be borne in mind in later sections of this, and succeeding chapters.

(2) RESULTS WITHIN INDIVIDUAL HERDS,  
AT ALL STAGES OF LACTATION  
BACTERIOLOGICAL CLASSIFICATION 1

Table 7.1ii shows the results within individual herds, at all four stages of the lactation, using bacteriological classification 1.

Under this classification scheme, herd C showed the highest infection rate, approximately 18.7 % of quarters being infected. By contrast, the infection rate within herd A was 9.8 % of quarters.

The critical threshold for the somatic cell count varied markedly between the herds, ranging from 85,000 cells per ml within herd A, to 375,000 cells per ml within herd C. Differences between herds with respect to critical thresholds also occurred with the other parameters, but the differences were smaller than were those observed with the somatic cell count.

In general, the critical threshold for parameters which increase in concentration as a result of mastitis, eg the sodium concentration and the NAGase activity, was higher within herds B and C, than within herd A. With respect to those parameters which tend to decrease in concentration as a result of mastitis, eg lactose concentration and potassium concentration, no clear trend in critical threshold between herds occurred.

Within each of the three herds, the somatic cell count proved to be the parameter with the greatest ability to distinguish between infected and uninfected udder quarters.

The ability to correctly classify quarters differed between herds, the probability of misclassification ranging from 13.7 % in herd A, to 23.9 % in herd C. Nevertheless, more than 75 % of quarter foremilk samples were correctly classified by the somatic cell count in each of the three herds.

Within each herd, as within the total data set, the sodium concentration and the NAGase activity of the milk were moderately successful in distinguishing infected from uninfected quarters. The probability of misclassification for the sodium concentration of the milk ranged from 26.7 % to 32.8 % within herds B and C respectively while the probability of misclassification for the NAGase activity ranged from 25.3 % in herd C, to 30.4 % within herd A.

The probabilities of misclassification for the remaining parameters were higher, with over 30 % of quarter samples being misclassified within each herd. Differences between herds with respect to the probability of misclassification occurred for each parameter, but were particularly marked for the lactose concentration, with which 31.3 % and 47.2 % of the samples were misclassified in herds C and B respectively.

(3) RESULTS FOR THE TOTAL DATA SET,  
BY STAGE OF LACTATION  
BACTERIOLOGICAL CLASSIFICATION 1

Table 7.1iii gives the results for all three herds, by stage of lactation.

The critical threshold for the somatic cell count increased markedly from 105,000 cells per ml during stage 1, to 390,000 cells per ml during stage 3, after which there occurred a small decrease in the critical threshold to 345,000 cells per ml.

Other parameters also showed changes in critical threshold during the course of the lactation, although in no case were these changes as marked as those observed for the somatic cell count. In general, those parameters which increase in concentration as a result of mastitis, showed an increasing critical threshold during the lactation, while the critical threshold decreased during the course of the lactation for those parameters whose concentration in the milk decreases as a result of mastitis.

The ability of the somatic cell count to distinguish between infected and uninfected quarters, was greatest at stage 2 of the lactation, although the differences between stages with respect to diagnostic ability were not as great for the somatic cell count as for some other parameters. In general, the diagnostic ability of each parameter was greatest during either stage 2 or stage 4 of lactation. The greatest variation in diagnostic ability of the parameters during lactation was shown by the pH of the milk, whose probability of misclassification ranged from 50.0 % of samples during stage 2, to 38.9 % of samples during stage 4.

(4) RESULTS WITHIN INDIVIDUAL HERDS,  
BY STAGE OF LACTATION, SOMATIC CELL COUNT ONLY  
BACTERIOLOGICAL CLASSIFICATION 1

Table 7.1iv shows the results within individual herds, by stage of lactation for the somatic cell count only, using bacteriological classification 1.

Within each of the three herds, the critical threshold was higher at the fourth stage of lactation, than at the first stage. However, the pattern of change of the critical thresholds differed between herds. Within herd A, there was little change in critical threshold until the final two months of lactation, at which time a moderate increase occurred. A decrease in the critical threshold between stages 1 and 2, was followed by large increases in critical threshold during the remainder of lactation within herd B. The

critical threshold for herd C increased markedly between stages 1 to 3, but decreased slightly during the final stage of the lactation.

When the data from each of the three herds were combined, the ability of the somatic cell count to distinguish between infected and uninfected quarters was slightly greater at stages 2 and 4, than at stages 1 and 3 ( table 7.1iii ). Within individual herds, the pattern of change during the lactation with respect to the ability of the somatic cell count to distinguish infected from uninfected quarters differed, being greatest during stage 1 within herd A, but greatest during stage 4 of lactation within herds B and C.

(5) RESULTS FOR THE TOTAL DATA SET,  
AT ALL STAGES OF LACTATION,  
BACTERIOLOGICAL CLASSIFICATION 2

Table 7.1v shows the results for the total data set, at all four stages of the lactation, using bacteriological classification 2.

When bacteriological classification 2 was applied to the data, the critical threshold for the somatic cell count was 80,000 cells per ml of milk. At this threshold the probability of misclassification was 19.9 %. This means that 80.1 % of quarter foremilk samples were correctly classified as being taken from infected or uninfected quarters by the somatic cell count.

For those parameters which show an increase in concentration as a result of mastitis, the critical threshold figures which were calculated using the bacteriological classification 2 were lower than the respective figures when bacteriological classification 1 was used.

The somatic cell count was notably more successful in terms of correctly classifying milk samples according to the bacteriological status of the quarter than were any of the other parameters. The sodium concentration of the milk misclassified 29 % of samples, while the remaining parameters each misclassified more than 30 % of samples.

The diagnostic ability of the somatic cell count, the sodium concentration, the potassium concentration and the lactose concentration varied little between bacteriological classification schemes. The pH, the NAGase activity and the antitrypsin concentration of the milk showed a greater ability to distinguish between infected and uninfected quarters under bacteriological classification 1 than under bacteriological classification 2, while the opposite was true of the electrical conductivity of the milk samples.

(6) RESULTS WITHIN INDIVIDUAL HERDS,  
AT ALL STAGES OF LACTATION,  
BACTERIOLOGICAL CLASSIFICATION 2

Table 7.1vi shows the results within individual herds, during all four stages of lactation, using bacteriological classification 2.

The critical thresholds for the somatic cell count were similar for herds A and B, while in herd C, a higher critical threshold was estimated.

Within each herd, the critical threshold for the somatic cell count was lower under bacteriological classification 2, than under bacteriological classification 1 ( see table 7.1ii ). This feature was more marked within herds B and C, than within herd A.

The critical thresholds of the parameters other than the somatic cell count were generally lower and showed less variability between the herds when bacteriological classification 2 was used than was the case when bacteriological classification 1 was used.

The ability of the somatic cell count to distinguish between infected and uninfected quarters was similar under both classification schemes, being greatest within herd A and least within herd C. When bacteriological classification 2 was applied, the somatic cell count was able to correctly classify over 75 % of quarters within each of the three herds.

The sodium concentration, the potassium concentration and the pH showed little difference in their respective abilities to classify quarters according to bacteriological status under the two classification schemes. For the electrical conductivity, the ability to correctly classify quarters within herd B was greater when bacteriological classification 2 was used, while there was little difference between classification schemes with respect to the electrical conductivity within herd A. Both the NAGase activity and the antitrypsin concentration showed a greater ability to correctly classify quarters as infected or uninfected under bacteriological classification 1 than under bacteriological classification 2. This feature was particularly evident within herd B. The ability of the lactose concentration to correctly classify quarters, was greater under bacteriological classification 1 within herds A and C, although the opposite was true within herd B.

(7) RESULTS OF DISCRIMINANT FUNCTION ANALYSES,  
ON ALL THREE HERDS, USING A SINGLE PARAMETER  
BACTERIOLOGICAL CLASSIFICATION 1

Table 7.2i shows the results of the discriminant function analyses on data from all three herds using bacteriological classification 1.

(i) PREDICTIVE ABILITY FOR ALL QUARTERS

The first column shows the percentage of quarters which were correctly classified by each parameter, when that parameter alone was used in the model.

The somatic cell count correctly classified 79.6 % of quarter samples as being either infected or uninfected. The test with the greatest predictive ability of infection status was the NAGase activity, which correctly classified 85.0 % of samples, while the test with the lowest predictive ability was the electrical conductivity, which correctly classified 73.4 % of the quarter samples. The lactose concentration and the sodium concentration each showed a slightly greater predictive ability than did the somatic cell count for the total data set.

(ii) PREDICTIVE ABILITY FOR INFECTED QUARTERS

The second column shows the ability of each parameter to correctly classify the infected quarters as being infected. The somatic cell count correctly predicted the infection status of 80.9 % of infected samples. Much lower figures were recorded for each of the other parameters. The NAGase activity was the next best test, being able to correctly classify 51.4 % of samples from infected quarters, while the remaining parameters were able to correctly classify fewer than half of the infected quarters. The potassium concentration, the pH, and the lactose concentration had little predictive ability with respect to milk samples from infected quarters.

(iii) PREDICTIVE ABILITY FOR UNINFECTED QUARTERS

The third column shows the ability of each parameter to correctly classify the uninfected quarters as being uninfected. Each of the parameters showed a relatively high predictive ability with respect to uninfected quarters. The somatic cell count correctly classified 79.3 % of samples from uninfected quarters. The parameter with the greatest predictive ability with respect to uninfected quarters was the lactose concentration, while the lowest predictive ability was shown by the electrical

conductivity. However, each of the parameters correctly classified more than 75 % of the samples from uninfected quarters.

(8) RESULTS OF DISCRIMINANT FUNCTION ANALYSIS  
WITHIN INDIVIDUAL HERDS, USING A SINGLE PARAMETER  
BACTERIOLOGICAL CLASSIFICATION 1

Table 7.2ii shows the results of the discriminant function analysis within individual herds, using bacteriological classification 1.

(i) PREDICTIVE ABILITY FOR ALL QUARTERS

As was evident with regard to the threshold analysis ( see above ), the discriminative power of the somatic cell count was greatest within herd A and least within herd C. Within herd A, 89.2 % of all the samples were correctly classified as being taken from either infected or uninfected quarters.

With respect to the other parameters, the interaction between the parameter and the herd varied, with some parameters showing a higher predictive ability within herd A, others within herds B or C.

The predictive abilities of the sodium concentration and of the potassium concentration were lowest within herd C, and were highest within herd B. Both the NAGase activity and the antitrypsin concentration were better able to correctly classify quarters within herds B and C, than within herd A. There was little difference between herds with respect to the ability of either the lactose concentration or of the electrical conductivity to correctly classify quarters as being infected or uninfected.

(ii) PREDICTIVE ABILITY FOR INFECTED QUARTERS

The ability of the somatic cell count to correctly classify the infected quarters as being infected was greatest within herd A and least within herd C, although the absolute difference between herds was only 4.7 percentage units. The superiority of the somatic cell count over the other parameters, with regard to the classification of infected quarters, was evident in all three of the herds.

The sodium concentration, the potassium concentration, the lactose concentration, the NAGase activity, the electrical conductivity and the pH of the milk, each showed a greater ability to correctly classify infected quarters within herd B, than within herds A or C. There was little difference between the herds with respect to the ability of the antitrypsin concentration to predict the infection status of infected udder quarters.

### (iii) PREDICTIVE ABILITY FOR UNINFECTED QUARTERS

The predictive ability of the somatic cell count with respect to uninfected samples was notably higher within herd A, than within herd C. This finding did not hold true for any other parameter however. The predictive ability of the antitrypsin concentration with respect to uninfected quarters was much higher within herd C than within herd A.

There was comparatively little variation between herds with respect to the ability of the sodium concentration, potassium concentration, electrical conductivity, or the pH of the milk to correctly classify infected quarters, although it must be remembered that the latter two parameters were measured within herds A and B only. The ability of the lactose concentration to correctly classify uninfected quarters was greatest within herd C, whilst that of the NAGase activity was greatest within herd B.

## (9) RESULTS OF DISCRIMINANT FUNCTION ANALYSES ON ALL THREE HERDS USING THE SOMATIC CELL COUNT PLUS ONE OTHER PARAMETER BACTERIOLOGICAL CLASSIFICATION 1

Table 7.2iii shows the results of the discriminant function analyses on all three herds when the somatic cell count plus one of the other parameters are included in the model. For the sake of comparison, the results of the analysis using the somatic cell count alone ( table 7.2i ) have been included. The figures in parentheses indicate the difference in the ability of the particular two test system to predict the infection status of quarters over the ability of the somatic cell count alone, a positive result indicating an higher predictive ability, a negative figure indicating a lower predictive ability.

### (i) PREDICTIVE ABILITY FOR ALL QUARTERS

The somatic cell count alone was able to correctly classify 79.6 % of samples as coming from either an infected, or an uninfected quarter.

The greatest improvement in diagnostic ability was achieved when either the electrical conductivity or the pH of the milk was used with the somatic cell count with improvements of 6.6 and 7.4 percentage units respectively. However, these two figures are misleading, in that the electrical conductivity and pH of the milk were measured on samples from herds A and B only. From table 7.2ii it may be seen that the somatic cell count alone was able to predict whether or not a quarter is infected in 89.2 % and 84.7 % of samples within herds A and B respectively.

If the somatic cell count results of herds A and B were taken alone, disregarding those of herd C, then approximately 87.5 % of samples would have been correctly classified, hence there is no real improvement in the diagnostic ability of the somatic cell count combined with either the electrical conductivity or with the pH, over that achieved with the somatic cell count results alone.

The greatest real improvement occurred when the somatic cell count and the lactose concentration were combined, in which case 82.7 % of all the samples were correctly classified, an improvement of only 3.1 percentage units over the results when the somatic cell count alone was used.

In the case of the combination of the antitrypsin concentration with the somatic cell count, there was a small decrease in the ability of the two test system to classify the quarters as infected or uninfected over the results when the somatic cell count alone was used.

#### (ii) PREDICTIVE ABILITY FOR INFECTED QUARTERS

The somatic cell count alone could correctly classify 80.9 % of infected samples as coming from an infected quarter, when data from all three herds were included.

The comparable figure for herds A and B only, was approximately 82.5 % of samples correctly classified. This figure should be used in relation to any improvement in predictive ability when either the electrical conductivity or the pH is combined with the somatic cell count for diagnosis.

In no case was there an increase in predictive ability of the two test system over that of the somatic cell count alone when the samples came from an infected quarter. In the case of certain parameters, the predictive ability actually decreased, the greatest decrease being for the combination of somatic cell count with the lactose concentration, in which the difference between the two test system and the somatic cell count alone was 8.4 percentage units. This feature was in contrast to the increase in predictive ability for

the overall data, when the somatic cell count and the lactose were combined ( see section 9 i, above )

### (iii) PREDICTIVE ABILITY FOR UNINFECTED QUARTERS

The somatic cell count alone correctly classified 79.3 % of samples from uninfected quarters, when data were taken from all three herds. For herds A and B only, the comparable figure was approximately 88.0 % of uninfected quarters correctly classified and this figure should be used for the combination of the somatic cell count with either the electrical conductivity or the pH of the milk.

The greatest increase in predictive ability of the two test system over that of the somatic cell count alone, occurred when the somatic cell count was combined with the lactose concentration of the milk, in which case the increase amounted to 5.1 percentage units. Increases in predictive ability also occurred when the sodium concentration, the potassium concentration or the NAGase activity were combined with the somatic cell count. The predictive ability with respect to uninfected quarters, of the combination of antitrypsin concentration and somatic cell count, was lower than that of the somatic cell count alone.

## (10) RESULTS OF DISCRIMINANT FUNCTION ANALYSIS WITHIN INDIVIDUAL HERDS, USING THE SOMATIC CELL COUNT PLUS ONE OTHER PARAMETER BACTERIOLOGICAL CLASSIFICATION 1

Table 7.2iv shows the results of the discriminant function analyses within individual herds, when the somatic cell count plus one of the other parameters are included in the model.

### (i) PREDICTIVE ABILITY FOR ALL QUARTERS

Within herds A and C, the greatest improvement in classification of quarters overall, was achieved when the somatic cell count and the lactose concentration of the milk were combined. Within herd B, the combination of somatic cell count plus lactose concentration did improve the ability to classify quarters, but the greatest improvement for herd B over the results of the somatic cell count alone, occurred when the NAGase activity and the somatic cell count of the milk were combined.

## (ii) PREDICTIVE ABILITY FOR INFECTED QUARTERS

Within herd A, the addition of a second test to the discriminant function generally resulted in a small decrease in ability to classify infected samples correctly, in comparison with the results of the somatic cell count alone, the largest decrease occurring when the somatic cell count and the sodium concentration of the milk were combined. A small increase in the percentage of infected quarters which were correctly classified occurred when the electrical conductivity and the somatic cell count of the milk were combined.

In contrast, the addition of a second test to the discriminant function generally improved the classification of infected quarters within herd B, the greatest increase occurring when the somatic cell count and the NAGase activity of the milk were combined.

Within herd C, the addition of a second test to the discriminant function resulted in a decrease in the classification of infected quarters as infected, the greatest decrease occurring when the somatic cell count and the lactose concentration of the milk were combined.

## (iii) PREDICTIVE ABILITY FOR UNINFECTED QUARTERS

The addition of a second test to the discriminant function generally resulted in little change in the predictive ability with respect to the uninfected quarters within herd A.

Within herds B and C the addition of a second test caused an increase in the predictive ability with respect to uninfected quarters. The greatest increase in predictive ability within herd C occurred when the somatic cell count and the lactose concentration of the milk were combined, resulting in an increase of 12.8 percentage units over the predictive ability of the somatic cell count alone.

## (11) CORRELATION BETWEEN THE SOMATIC CELL COUNT AND THE OTHER PARAMETERS

Table 7.3 shows the correlation coefficients between log (10) transformed variables and the log (10) somatic cell count, both for the combined data and within individual herds.

The correlation between the somatic cell count and the sodium concentration of milk, and between the somatic cell count and the NAGase activity of milk was strong. Other

parameters showed weaker relationships with the somatic cell count, the weakest being that between the somatic cell count and the pH of the milk. A negative correlation occurred between the somatic cell count and the lactose concentration or the potassium concentration of the milk.

In general, correlations were stronger within the infected quarters than in uninfected quarters, but this feature varied between the parameters. There was less difference between infected and uninfected quarters with respect to correlation coefficient between somatic cell count and sodium concentration, than between the somatic cell count and the pH of the milk.

In general, the correlations between the somatic cell count and the other parameters were weaker within herd A than within herds B or C. An exception to this rule was the relationship between the somatic cell count and the NAGase activity, where herds A and B showed similar coefficients, while a higher coefficient was obtained within herd C.

## DISCUSSION

### (1) PREDICTIVE ABILITY FOR THE TOTAL DATA SET, AT ALL STAGES OF LACTATION

Of the parameters tested during the present study, the somatic cell count showed the greatest predictive ability for classifying quarters as infected or uninfected. This was true for both bacteriological classification schemes ( tables 7.1i and 7.1v ).

Under bacteriological classification 1, quarters which yielded a major pathogen were deemed to be infected, while quarters which yielded a minor pathogen, or were bacteriologically negative, were deemed to be uninfected. Under bacteriological classification 2, quarters which yielded either a major or a minor pathogen were deemed to be infected, whilst the uninfected group included only those quarters which were bacteriologically negative at the time of sampling.

The results, in terms of the predictive ability of the somatic cell count, were very similar between bacteriological classifications 1 and 2, with 20.0 % and 19.9 % of quarters being misclassified respectively. However, the critical threshold, ie the threshold at which the proportion of false positive and false negative results are equal, differed between bacteriological classification schemes. For classification 1, the somatic cell count critical threshold was 245,000 cells per ml, while that for classification 2 was 80,000 cells per ml. This finding suggests that the minor pathogens do exert an effect on the somatic cell count and that this effect may be important in terms of the setting of threshold levels for the detection of udder infection.

If the aim of a detection system is to identify quarters which are infected with either a major or a minor pathogen, then a lower threshold will be required than is the case when only those quarters which are infected with a major pathogen are sought.

The parameter which was ranked second in terms of its ability to correctly classify quarters was the NAGase activity. The NAGase activity misclassified 29.6 % and 38.1 % of quarters under bacteriological classifications 1 and 2 respectively. The lower predictive ability under bacteriological classification 2 suggests that the minor pathogens cause less change in the NAGase activity than in the somatic cell count of the milk.

The sodium concentration of the milk showed a moderate predictive ability, but each of the remaining parameters was a comparatively poor predictor of the presence or absence of infection within individual quarters. The two parameters which showed the lowest predictive ability under both bacteriological classification schemes were the pH and the potassium concentration of the milk.

Other workers have compared the ability of selected parameters to discriminate between infected and uninfected quarters. However, care must be taken when comparing the results of such studies with those of the present trial, since differences in the level of infection within herds, the species or strains of pathogen involved, and the definition of infection, may differ. Sheldrake *et al* ( 1983a ) compared the predictive ability of the somatic cell count, the electrical conductivity and the concentration of bovine serum albumin ( BSA ) for discriminating between quarters which were infected with Staphylococcus aureus, and those which were either uninfected, or were infected by a minor pathogen. The somatic cell count showed a lower probability of misclassification, ie a greater predictive ability than did either the electrical conductivity, or the BSA concentration ( Sheldrake *et al*, 1983a ).

Not all trials have shown the somatic cell count to be the test with the greatest ability to correctly classify quarters. Using a threshold which gave an equal percentage of false positive and false negative results Mattila *et al* ( 1986a ) reported that 24 % of quarters were misclassified when the NAGase activity was used, while the comparative figures for the somatic cell count and for the antitrypsin concentration were 36 % and 34 % of quarters respectively. The NAGase activity showed a greater predictive ability and the somatic cell count a lower predictive ability than was the case during the present trial. During an earlier study by the same authors, 11.9 % of bacteriologically negative samples exceeded the threshold level, ie gave false positive results, while 39 % of bacteriologically positive samples gave readings below the threshold value, ie false negative results, when the antitrypsin concentration of milk was used to distinguish between infected and uninfected quarters ( Mattila *et al*, 1985 ). The performance of the antitrypsin concentration in the classification of quarters during the study of Mattila *et al* ( 1985 ) was superior to the performance of the same parameter during the present trial, but was inferior to the performance of the somatic cell count during the present trial.

In the trial data presented, the pH of the milk had a comparatively low predictive ability for udder infection. Similar results have been reported by other workers ( Marschke and Kitchen, 1985 ).

A small number of studies have reported the performance of the sodium concentration, the potassium concentration, the lactose concentration and the electrical conductivity of the milk as indicators of udder infection. The results of these studies will be compared with those of the present trial, later in this chapter.

## (2) PREDICTIVE ABILITY WITHIN INDIVIDUAL HERDS

The critical threshold for the somatic cell count differed between herds. When bacteriological classification 1 was used, the critical threshold ranged from 85,000 cells per ml in herd A, to 375,000 cells per ml in herd C.

Other researchers have shown the critical threshold to vary between herds. Sheldrake et al ( 1983a ) reported a range in critical thresholds of 158,000 to 251,000 cells per ml, when the somatic cell count was used in an effort to distinguish between those quarters which were free of infection, and those which were infected by Staphylococcus aureus.

One factor which affects the critical threshold of a parameter in a particular herd is the number of quarters which are infected with minor pathogens. Within herd A, very few quarters were infected by minor pathogens, whereas within herds B and C, the prevalence of minor pathogens was higher and increased as the season progressed ( graphs 5.1a, 5.1b and 5.1c ).

Under bacteriological classification 1, the minor pathogens were included with the bacteriologically negative quarters into an "uninfected" group, while the "infected" group contained those quarters which were infected with major pathogens only. The minor pathogens cause a slight increase in the somatic cell count of the milk ( see chapter six ) and when included with the bacteriologically negative quarters, will increase the mean somatic cell count of the "uninfected" group. The greater the number of quarters which are infected with minor pathogens, the greater will be this increase in mean somatic cell count, thus the effect will be greater within herds B and C, than within herd A. If the mean somatic cell count of the "uninfected" quarters increases, then the threshold which is required to distinguish between infected and uninfected quarters will also increase. Thus the variation in the prevalence of minor pathogens explains at least some of the difference between herds with respect to the critical threshold, with classification 1. Under bacteriological classification 2, the minor pathogens were included with the major pathogens in the "infected" group. In this case, the effect of the minor pathogens will be to decrease the mean somatic cell count of the "infected" group. At the same time, the mean somatic cell count of the "uninfected" group, which contains only bacteriologically negative quarters, will also be lower than under bacteriological classification 1. However, the decrease in the mean somatic cell count of the infected group will be greater than that of the "uninfected" group because of the smaller absolute numbers of quarters which were infected with major pathogens, than of quarters which were bacteriologically negative. Thus under bacteriological classification 2, the greater the number of quarters which are infected with minor pathogens, the greater will be the effect on the somatic cell count of the "infected" group, with the result that the critical threshold will tend to decrease. The outcome of this may be seen in table 7.1vi, in which herds A and B show similar critical thresholds with respect to somatic cell count, while that for herd C is higher. In each herd, the critical threshold

under bacteriological classification 2 was lower than the respective figure under bacteriological classification 1.

The fact that the critical threshold of herd C was higher than that of herds A and B under both bacteriological classification schemes, suggests that factors other than the prevalence of minor pathogens may also affect the critical threshold. The variation in the critical threshold value between herds may reflect differences in the bacterial flora of the herds. The mean somatic cell count of quarters which are infected with various pathogens, which are commonly associated with mastitis, differ significantly ( Ward and Schultz, 1972 ).

It was noted in chapter five of this thesis, that the bacterial flora of the three herds which were sampled during the present trial differed in some respects. Furthermore, it is likely that within each bacterial species or bacterial grouping ( see chapter five for explanation ), differences occurred with respect to the pathogenicity of various strains.

The variation between herds with respect to the critical threshold may also be related to the previous exposure of the quarters to mammary pathogens. The somatic cell count of the milk may be elevated after the infection has been eliminated. In quarters which were uninfected and had never been treated for clinical mastitis the mean somatic cell count was 260,000 cells per ml, whereas within quarters which were currently uninfected, but had been treated at some stage during the life of the cow, the mean somatic cell count was 600,000 cells per ml ( Ward and Schultz, 1972 ). In contrast, the results of clinical trials suggest that the successful treatment of acute clinical mastitis results in a decrease in the somatic cell count of the milk to levels similar to those of uninfected control quarters within 14 days of elimination of the infection ( Pyörälä, 1988 ).

The apparent contradiction in the findings of Ward and Schultz ( 1972 ) and those of Pyörälä ( 1988 ) cannot be easily explained, but may be related to the duration of infection prior to the cure or natural elimination of the infection. It is possible that in some cases, the elimination of the infection is incomplete, particularly when the disease is caused by Staphylococcus aureus. Organisms surviving within an abscess might continue to cause the migration of leukocytes from the blood, while not being found in the milk from that quarter. Damaged tissue may itself may release chemotactic factors ( Schalm, 1977 ) and this could serve as a possible mechanism for an elevated somatic cell count after the elimination of the infectious agent, in some quarters.

While the critical threshold differed between herds, so too did the ability of the parameters to distinguish between the infected and uninfected quarters ( tables 7.1ii and 7.1vi ). The predictive ability was greatest within herd A, and lowest within herd C, this being true under both bacteriological classification schemes 1 and 2, suggesting that

there was a greater degree of overlap between the distributions of the somatic cell counts of infected and uninfected quarters, within herds B and C, than within herd A.

Part of this effect may be due to the minor pathogens. Under bacteriological classification 1, the "uninfected" group comprised bacteriologically negative quarters, and those which contained minor pathogens. The minor pathogens did cause a small increase in the somatic cell count and thus were more likely to exceed the threshold and be misclassified than were the bacteriologically negative quarters. As noted previously, the prevalence of minor pathogens within herds B and C were higher than the prevalence within herd A. However, the difference in prevalence of minor pathogens between herds B and C was relatively small, suggesting that other factors may be involved in the variation of predictive ability between herds. These factors might include both the effect of previous infections on the somatic cell count of the bacteriologically negative quarters, and the effect of current infection on the somatic cell count of the infected quarters. As noted previously, both the bacterial flora and the occurrence of mastitis prior to the commencement of the trial, differed between herds.

The probability of misclassification reported by Sheldrake *et al* ( 1983a ) ranged from 8 % to 20 % of quarters in the three herds which were studied by these authors. The herd with the highest probability of misclassification also showed the highest critical threshold, a finding similar to that of the present trial.

The differences between herds with respect to the critical thresholds of the other parameters were much smaller in scale than were those of the somatic cell count. In the case of parameters which increase as a result of mastitis, the critical thresholds within herds B and C tended to be higher than were the respective figures within herd A. In the case of parameters which decrease as a result of mastitis, no trend was apparent.

While the somatic cell count showed a greater ability to correctly classify quarters within herd A than within herds B or C, this was not necessarily true of the other parameters, some of which showed a greater predictive ability within herds B or C, than within herd A. This probably reflects the greater effect of particular species, or strains of bacteria on some parameters than on others. This is not unreasonable in view of the fact that organisms such as the staphylococci and streptococci are capable of producing a wide range of haemolysins, leukocidins, fibrinolysins, deoxyribonucleases and other toxins or catabolic enzymes ( Lennette, 1980 ). Not all of these pathogenicity factors are produced by every strain of bacteria.

The greatest absolute difference between the herds with respect to diagnostic ability occurred with the lactose concentration of the milk ( table 7.1ii ). Within herd C the probability of misclassification was 31.3 % of quarters, while within herd B the comparative figure was 47.2 %, a difference of 15.9 percentage units. On closer examination of the raw results ( not shown ), this lower probability of misclassification

was due largely to the lower number of false negative than of false positive results, the latter being similar between herds, suggesting that the lactose concentration of quarters which were infected by a major pathogen, were affected more within herd C, than within herd B.

### (3) PREDICTIVE ABILITY DURING THE LACTATION

The critical threshold for the somatic cell count increased dramatically during the lactation, from 105,000 cells per ml during early lactation, to 390,000 cells per ml during stage 3, thereafter decreasing slightly during the final two month period ( table 7.1iii ).

In view of the effect of the stage of lactation on the somatic cell count ( chapter six ), this increase was not unexpected.

Graphs 6.1ai, 6.1bi and 6.1ci show an increase in the mean somatic cell count of quarter milk samples during mid to late lactation within each of the three herds during the present trial. This effect occurred within both infected and uninfected quarters.

If the mean somatic cell count of both the infected and the uninfected quarters is increasing, then the critical threshold will also increase. The reasons for the changes in the somatic cell count within infected and uninfected quarters, during the season, have been discussed in chapter six.

While the critical threshold increased by a factor of three during the season, changes in the predictive ability of the somatic cell count during the season were small, the probability of misclassification being slightly lower during stages 2 and 4, than during stages 1 and 3.

### IMPLICATION FOR THE LIVESTOCK IMPROVEMENT CORPORATION

The consistency in the predictive ability of the somatic cell count during the lactation has implications for the timing of sampling for the somatic cell counting scheme which is offered by the Livestock Improvement Corporation of the New Zealand Dairy Board. Currently, sampling at monthly or bimonthly intervals throughout the dairy season is recommended, the lactation average somatic cell count being used as the basis for decisions regarding application of dry cow therapy or culling at the end of the season. The results of the present study show that the somatic cell count is capable of distinguishing between infected and uninfected quarters with a relatively high degree of reliability at all stages of lactation, although it must be remembered that samples were not taken within the first month of calving during the present trial.

The changes, during lactation, in the critical thresholds of the parameters other than the somatic cell count were smaller in scale. There was a trend towards a higher predictive ability during stages 2 and 4, than during stages 1 and 3, although the absolute differences were often small. The pH of milk showed a difference in predictive ability of 11.1 percentage units, the greatest predictive ability, ie the lowest probability of misclassification occurring during the final stage of the lactation.

Graphs 6.5ai and 6.5bi show that the increase in the pH of the milk during mid to late lactation, was greater for infected than for uninfected quarters, within herds A and B respectively. pH readings were not determined on milk samples from herd C. The greater increase in the pH of the milk from infected quarters, than that from uninfected quarters, as the season progressed, allowed for a more reliable distinction to be made between infected and uninfected quarters towards the end of the lactation, than earlier in the lactation, when the pH of the milk was used as the diagnostic test.

#### (4) PREDICTIVE ABILITY OF THE SOMATIC CELL COUNT WITHIN INDIVIDUAL HERDS DURING THE LACTATION

The increase in the somatic cell count critical threshold during lactation occurred in all three herds ( table 7.1iv ), although the timing of the increase differed between herds.

Within herd A, the somatic cell count critical threshold showed little change until the final stage of the lactation. This finding is reflected in graph 6.1ai, in which there is little change in the mean somatic cell count until the final two months, at which time the mean somatic cell count increases slightly. The probability of misclassification was lowest during the first stage of the lactation, ie during the second and third months post-calving, within herd A.

Within herd B, there was an initial decrease in the somatic cell count critical threshold, which was followed by increases between stages 2 to 3 and 3 to 4. Graph 6.1bi shows a decrease in the mean somatic cell count for both infected and for uninfected quarters at the third sampling within herd B. The probability of misclassification was greatest during stage 2 of lactation, ie months 4 and 5 post-calving, and least at the end of the lactation, a finding which might not have been predicted from graph 6.1bi, since the apparent separation of the infected and uninfected quarters, with respect to the distribution of the somatic cell count, during the period which coincides with stage 2 is greater than is the case later in the season. It must be remembered however, that the somatic cell count scales on graphs 6.1ai, 6.1bi and 6.1ci are logarithmic, so that a decrease in the mean somatic cell count of the status 3 quarters is quantitatively greater than is the same apparent decrease in the mean somatic cell count of the quarters within status 1 or status 2.

Within herd C the critical threshold increased markedly between stages 1 to 3, but decreased slightly during the final two month period. Evidently, the somatic cell count of some of the uninfected quarters increased during the season. The isolation rate for major pathogens was relatively high within herd C. It is likely therefore, that some of the increase was due to the effect of previously infected quarters from which pathogens could no longer be isolated. Alternatively, the increase may have been due to the increasing prevalence of minor pathogens, which, as has been mentioned previously, were included with the bacteriologically negative quarters under bacteriological classification 1. The probability of misclassification decreased during the lactation within herd C, from 25.3 % of quarters during the first stage, to 14.5 % of quarters during the final stage. This suggests a better separation of the relative distributions of the somatic cell count for infected and for uninfected quarters, towards the end of the dairy season. Thus when the data from the three herds were combined, little change in the predictive ability of the somatic cell count occurred during the season. However the predictive ability was greatest at the end of the season within 2 of the 3 herds.

#### IMPLICATIONS FOR THE LIVESTOCK IMPROVEMENT CORPORATION

Under the present system of somatic cell counting which is offered by the Livestock Improvement Corporation, a set threshold is used and samples are taken at regular intervals during the season. By taking into account the changes in somatic cell count which occur within both infected and uninfected quarters, ie by using a higher threshold level later in the season, the diagnostic ability of the somatic cell count may be increased, particularly in herds with a relatively high incidence of mastitis.

During the final two months of lactation, the probabilities of misclassification for the three herds A, B and C were 14.1 %, 9.6 % and 14.5 % respectively. This means that the somatic cell count could correctly predict the infection status of more than 85 % of samples in each of the three herds, at a single sampling.

Had a set threshold been used throughout the season, rather than the critical threshold appropriate for that stage of lactation, then the percentage of false positive results would have increased, while the percentage of false negative results would have decreased, as the lactation progressed.

The critical threshold is defined at the start of the current chapter, as the point at which the proportion of false negative and false positive results are equal. It is possible to increase the total number of quarters which are correctly classified by using higher threshold settings. As the threshold is increased, the percentage of uninfected quarters which are correctly classified increases, while the percentage of infected quarters which are correctly classified decreases. A point will be reached at which all the uninfected quarters will be correctly classified, although many of the infected quarters will be

misclassified as the somatic cell count falls below the threshold. Since the number of "uninfected" quarters, ie those quarters which were bacteriologically negative or were infected with minor pathogens, outnumbered the "infected" quarters, the use of a very high threshold would give a high overall accuracy. This would be at the expense of an increase in the percentage of the infected quarters which would not be identified.

The critical threshold for the somatic cell count differed between herds ( table 7.1ii ). When the data from the three herds were combined, the critical threshold for the somatic cell count was approximately 250,000 cells per ml ( table 7.1i ). The effect of application of a common 250,000 cells per ml threshold level to the percentage of false negative and false positive results in each of the three herds is shown in the table 7.4.

In the case of herd A, the application of the common threshold causes an increase in the percentage of false negative results, and a decrease in the percentage of false positive results. Fewer of the infected quarters would be detected when the common threshold is higher than the particular critical threshold. The critical threshold for the somatic cell count within herd B is closer to the common threshold, hence the differences in the classification of quarters are smaller. Within herd C, the critical threshold is higher than the common threshold, so that the percentage of false negative results decreases while the percentage of false positive results increases, when the common threshold is applied.

The threshold level which is chosen in practice, will depend ultimately on the relative economic consequences of either failing to identify infected quarters, or misclassifying uninfected quarters as being infected ( McDermott *et al.* 1982 ).

Failure to detect infected quarters is likely to result in the following consequences:

- (1) A continuing loss of milk yield in at least some of the affected quarters.
- (2) The possibility of the infection spreading to other quarters, or to other cows within the herd.
- (3) A continuing contribution of mastitis causing bacteria to the total bacterial content of the milk, which may cause the milk to be downgraded.
- (4) A proportion of the infected quarters may become clinically affected at a future point in time.

The misclassification of uninfected quarters as being infected, is likely to result in:

- (1) The selection of an excessive number of cows for dry cow therapy.
- (2) The unnecessary culling of uninfected cows.

The financial losses which have been attributed to mastitis are substantial ( Janzen, 1970; Dobbins, 1977; Stevenson and McVeagh, 1983 ). The use of dry cow therapy has been shown to reduce the prevalence of clinical mastitis during the following season, in high prevalence herds ( Cagienard, 1983 ).

The cost of dry cow therapy, which under New Zealand conditions is generally administered to all four quarters of the selected cows within the herd, is approximately \$10 per cow. Although a detailed analysis of the cost effectiveness of dry cow therapy is beyond the scope of this thesis, it would seem prudent to err on the side of safety, by weighting the conditions of diagnosis in favour of a high rate of detection of infected quarters.

#### (5) DISCRIMINATION BETWEEN INFECTED AND UNINFECTED QUARTERS WITHIN THE COMBINED DATA SET

When discriminant function analyses were used to classify quarters according to bacteriological findings, the test with the greatest overall discriminative ability was the NAGase activity ( table 7.2i ). The somatic cell count correctly diagnosed 79.6 % of the quarter samples. The parameter with the lowest discriminative ability was the electrical conductivity. The absolute differences between parameters with respect to discriminative ability were relatively small. However, the ability of the parameters to detect infected quarters varied markedly, with the somatic cell count showing the greatest ability.

The sensitivity of a diagnostic test is defined as " the probability of a diagnostic test correctly identifying as positive, those animals which are truly positive " ( Sheldrake and Hoare, 1981 ). Thus, during the present trial, the somatic cell count showed a much higher sensitivity than did any other parameter.

Other workers have shown the sensitivity of the somatic cell count to be higher than that of the electrical conductivity of the milk ( Sheldrake and Hoare, 1981 ). A low sensitivity is associated with the pH of milk ( Marschke and Kitchen, 1985 ) and with the concentrations of sodium, potassium, lactose, and bovine serum albumin ( Fernando *et al*, 1985; Kangasniemi *et al*, 1986 ). A low sensitivity has also been claimed for the somatic cell count of milk ( Fernando *et al*, 1985 ).

The reason for the contrasting results with respect to the sensitivity of the somatic cell count, between the study of Fernando *et al* ( 1985 ) and those of the present study is not clear. Fernando *et al* ( 1985 ) carried out the bacteriology on strict foremilk samples, while the bacteriological results of the present trial are based on foremilk taken after the first few squirts of strict foremilk have been discarded. The low sensitivity for the somatic cell count which has been claimed by Fernando *et al* ( 1985 ) may be the result

of a proportion of the bacteriologically positive samples being streak canal infections, rather than infections situated deeper within the gland.

The high sensitivity of the somatic cell count during the present trial suggests that this parameter was better able to detect the less severely affected quarters than was any other parameter. Apart from the somatic cell count, the only parameter which detected more than half of the infected quarters, was the NAGase activity of the milk. The results of the present trial are in agreement with those of Kangasniemi *et al* ( 1986 ), who ranked diagnostic tests in the following order of decreasing sensitivity for udder infection: somatic cell count, NAGase activity, sodium concentration, BSA concentration, electrical conductivity and potassium concentration.

The specificity of a diagnostic test is defined as " the probability of a test correctly identifying as negative, those animals which are truly negative ", ( Sheldrake and Hoare, 1981 ).

The specificity of the parameters varied from 77.5 % to 93.5 % of samples during the present trial ( table 7.2i ). Thus each parameter was able to correctly classify at least 3 out of every 4 samples from uninfected quarters.

Higher figures for specificity than for sensitivity have been reported for the somatic cell count, sodium concentration, potassium concentration, electrical conductivity, and the lactose concentration of milk ( Fernando *et al*, 1985 ), and for the pH of milk ( Marschke and Kitchen, 1985 ).

The results of the present trial suggest that the uninfected quarters tend to show a narrower distribution with respect to the concentration of each parameter, than do the infected quarters.

#### (6) DISCRIMINATION BETWEEN INFECTED AND UNINFECTED QUARTERS WITHIN INDIVIDUAL HERDS

The results of the discriminant function analyses within individual herds, were similar to those of the threshold program in that the overall discriminative ability of the somatic cell count was greatest within herd A, and least within herd C. The greater discriminative ability within herd A, can be attributed to the difference in specificity, rather than to the difference in sensitivity between herds.

The somatic cell count consistently detected infected quarters, while the detection of uninfected quarters was greatest within herd A. The lower discriminative ability of the somatic cell count for uninfected quarters within herd C, may have been due to the

effects of the minor pathogens, or alternatively to the effects of previous infection on the somatic cell count of uninfected quarters within herd C.

In general, the detection of infected quarters by the parameters other than the somatic cell count was greater within herd B, than within herds A or C. This suggests that the major pathogens generally caused a greater degree of tissue damage within herd B, than within the other two herds.

There was no clear pattern between herds with respect to the ability of the parameters to detect the uninfected quarters, but in most cases, the detection rate was high within each of the three herds, and absolute differences between herds were small. An exception to this finding was that of the antitrypsin concentration which showed a markedly greater ability to detect uninfected quarters within herd C than within herd A. The reason for the large difference between herds in this respect is unclear, since no other parameters showed a similar trend.

#### (7) THE USE OF A MULTIPLE TEST SYSTEM FOR THE DIAGNOSIS OF SUBCLINICAL INFECTION

The suggestion has been made that the combination of two or more parameters may improve the accuracy with which subclinical mastitis is diagnosed, in comparison with the accuracy when one parameter is used ( Kitchen *et al*, 1980; Mattila and Sandholm, 1985; Mattila *et al*, 1986a; DuPreez, 1987 ). The rationale behind the use of a two test system, is that the various parameters which are available, may be selected to reflect different facets of the infection, the damage to the gland which has been caused by the pathogenic bacteria, or the ensuing inflammatory response.

The sodium concentration, the potassium concentration, the pH, and the electrical conductivity of the milk reflect the degree of leakage between adjacent secretory cells, following damage to the intercellular junctions. The NAGase activity of the milk is a measure of the degree of damage to the secretory tissue of the mammary gland, while the antitrypsin concentration reflects the increased vascular permeability which occurs during periods of inflammation. The lactose concentration shows to what extent the secretory capability of the epithelial cells has been affected, and also reflects leakage between the cells.

Although the concentrations of each of the parameters may change during the course of the infection, the changes do not necessarily occur at precisely the same time, or at the same rate ( Schalm, 1977 ). In addition, differences exist in terms of the rate at which the concentration of each parameter returns to normal, or preinfection levels, following the elimination of the infection ( Pyörälä, 1988 ).

By taking into account several different aspects of the infectious and inflammatory processes, the discrimination between infected and uninfected quarters might, theoretically, be improved.

Of the parameters which were studied during the present trial, that with the greatest discriminative ability was the somatic cell count. Since the somatic cell count is currently accepted as the standard method of diagnosing mastitis within New Zealand, the ability of pairwise combinations of the somatic cell count with each of the remaining parameters was tested in an effort to determine whether the addition of results of a second parameter, to those of the somatic cell count, improves the discriminative ability with regard to infection.

The results of these analyses, which are given in table 7.2iii, show that in general, a small improvement in the discriminative ability resulted from the addition of a second test to the model. The largest improvement occurred when the somatic cell count and the lactose concentration were combined. However, the addition of a second test generally resulted in a decrease in the sensitivity over that achieved when the somatic cell count alone was included in the model. The greatest decrease in sensitivity occurred when the somatic cell count and the lactose concentration were combined.

While the sensitivity decreased, the specificity of the system generally increased on addition of a second test to the model. Once again, the greatest increase in specificity occurred when the somatic cell count and the lactose concentration were included in the model.

While the increase in specificity was smaller than the decrease in sensitivity in terms of percentage units, there was a slight increase in the overall discriminative ability of the two test system over that of the somatic cell count alone. This increase in the overall discriminative ability was due to the greater number of uninfected than of infected quarters within the data set.

The greater sensitivity of the somatic cell count when compared with any of the other parameters which were selected for use during the present trial, suggests that the somatic cell count is able to detect the less severely affected quarters. These less severe infections apparently evoke an infiltration of leukocytes from the blood, but do not cause a substantial change in the concentrations of parameters which indicate damage to, or leakage between secretory cells of the mammary gland. Hence the use of a model which includes the somatic cell count plus one of the alternative parameters results in a decrease in the percentage of infected quarters which are correctly classified.

Conversely, the greater specificity of certain of the alternative parameters enabled a larger percentage of uninfected quarters to be correctly classified using the two

parameter model than was the case when the somatic cell count alone was included in the model.

Very little advantage accrued with the addition of a second test within herds A or B, the changes in sensitivity and specificity generally were small. Within herd C, the use of the somatic cell count plus either the lactose concentration, or the NAGase activity, resulted in larger changes in discriminative ability than occurred when the remaining parameters were included in the model. However, the increase in the discriminative ability resulted from an increase in specificity, while the sensitivity decreased over that obtained when the somatic cell count alone was included in the model.

Whether the marginal improvement in the discriminative ability of the two test system over that of the somatic cell count alone, justifies the adoption of the two test system on a commercial scale, will depend upon whether the economic benefits of doing so, outweigh the costs. This, in turn, depends upon the economic consequences of failing to detect infected quarters, and the consequences of misclassifying uninfected quarters as being infected. Any savings which might result from the decreased application of dry cow therapy or of culling programmes must be weighed against the increased costs which result from infected cows remaining within the herd.

Other workers have investigated the use of multiple test systems. Emanuelson *et al* ( 1987 ) used logistic regression to predict the infection status of individual quarters. The combination of the somatic cell count plus the antitrypsin concentration, or the somatic cell count plus the electrical conductivity resulted in a marginal improvement in predictive ability over that achieved using the somatic cell count alone. No improvement in predictive ability occurred when the somatic cell count and the NAGase activity of the milk were combined. The lack of improvement in discriminative ability which resulted from the inclusion of the somatic cell count and the NAGase activity of the milk, reflects the fact that the two parameters are highly correlated ( table 7.3 ).

The present study results confirm the findings of Emanuelson *et al* ( 1987 ), that the use of the models containing two parameters offer little advantage over the use of those containing the somatic cell count alone.

### SUMMARY AND CONCLUSIONS

- (1) When the absolute values of a single parameter were used to determine the infection status of an individual udder quarter, the probability of misclassification for the somatic cell count ranged from 14 % in herd A, to 24 % within herd C.

The probabilities of misclassification were lower for the somatic cell count, than for the sodium concentration, potassium concentration, electrical conductivity, pH, lactose concentration, NAGase activity or the antitrypsin concentration of the milk.

- (2) With respect to the somatic cell count and to a lesser extent with other parameters, the probability of misclassification of quarters, and the critical threshold of each herd, varied between herds. This variation in probability of misclassification and in critical thresholds was probably due to a combination of the following:

- (i) Differences between herds with respect to the bacterial flora and to the pathogenicity of individual major pathogens.
- (ii) Differences between herds with respect to the level of infection with minor pathogens.
- (iii) Differences between herds with respect to the effect of previous infection, on the current levels of the parameter in question.

- (3) The critical threshold for the somatic cell count changed during the lactation, increasing from 105,000 to 345,000 cells per ml as lactation progressed. The changes in critical threshold during the lactation were probably due to a combination of the following:

- (i) Differences between stages of lactation with respect to the severity of infection caused by major pathogens.
- (ii) Differences between stages of lactation with respect to the level of infection with minor pathogens.
- (iii) Differences between stages of lactation with respect to the effects of previous infections on the current levels of the parameter in question.

- (4) The level at which the threshold value is set, affects the proportion of infected and of uninfected quarters which are correctly classified by the parameter. Increasing the threshold value increases the proportion of uninfected quarters which are correctly classified, but decreases the proportion of infected quarters which are correctly classified.
- (5) The threshold value which is adopted in practice, will depend upon the economic consequences of:
  - (i) Failing to detect infected quarters.
  - (ii) Misclassifying uninfected quarters as being infected.

The present data suggest that, in order to achieve consistent accuracy in the identification of infected and uninfected quarters, lower threshold values should be used in herds with a low incidence of infection, and a higher threshold used in herds with a high incidence of infection. The relationship between the bulk milk somatic cell count and the level of infection might be used to select an appropriate threshold value for a particular herd.

- (6) Each of the parameters which were studied during the present trial showed a high specificity, with relatively few false positive results being recorded.
- (7) The sensitivity of each parameter was generally lower than was the corresponding specificity. The somatic cell count showed a higher sensitivity than did any other parameter.
- (8) The addition of a second test to a model containing the somatic cell count resulted in only a marginal improvement in the discriminative ability of the model. This improvement in discriminative ability resulted from an increase in the specificity of the system, the addition of the second test did not improve the sensitivity.

TABLE 7.11RESULTS FOR TOTAL DATA - BACTERIOLOGICAL CLASSIFICATION 1

| NAME OF<br>PARAMETER                               | CRITICAL<br>THRESHOLD | PROBABILITY OF<br>MISCLASSIFICATION | PERCENTAGE<br>INFECTED |
|----------------------------------------------------|-----------------------|-------------------------------------|------------------------|
| SOMATIC CELL<br>COUNT<br>( cells / ml )            | 245,000               | 20.0 %                              | 14.4 %                 |
| SODIUM<br>CONCENTRATION<br>( mMoles / litre )      | 17.8                  | 29.6 %                              | 14.3 %                 |
| POTASSIUM<br>CONCENTRATION<br>( mMoles / litre )   | 40.0                  | 44.4 %                              | 14.3 %                 |
| ELECTRICAL<br>CONDUCTIVITY<br>( mSiemens / cm )    | 6.02                  | 39.5 %                              | 10.3 %                 |
| pH                                                 | 6.62                  | 42.0 %                              | 10.3 %                 |
| LACTOSE<br>CONCENTRATION<br>( % )                  | 4.99                  | 38.0 %                              | 14.3 %                 |
| NAGase<br>ACTIVITY<br>( nM/ ml/ min )              | 6.10                  | 27.4 %                              | 14.4 %                 |
| ANTITRYPSIN<br>CONCENTRATION<br>( Relative units ) | 0.855                 | 35.2 %                              | 14.2 %                 |

TABLE 7.111RESULTS WITHIN INDIVIDUAL HERDS - BACTERIOLOGICAL CLASSIFICATION 1

| PARAMETER<br>NAME                                  | HERD        | CRITICAL<br>THRESHOLD           | PROBABILITY OF<br>MISCLASSIFICATION | PERCENTAGE<br>INFECTED    |
|----------------------------------------------------|-------------|---------------------------------|-------------------------------------|---------------------------|
| SOMATIC CELL<br>COUNT<br>( cells / ml )            | A<br>B<br>C | 85,000<br>200,000<br>375,000    | 13.7 %<br>17.0 %<br>23.9 %          | 9.8 %<br>11.1 %<br>18.8 % |
| SODIUM<br>CONCENTRATION<br>( mMoles / litre )      | A<br>B<br>C | 15.7<br>18.4<br>19.2            | 31.6 %<br>26.7 %<br>32.8 %          | 9.8 %<br>11.1 %<br>18.7 % |
| POTASSIUM<br>CONCENTRATION<br>( mMoles / litre )   | A<br>B<br>C | 40.0<br>41.8<br>39.2            | 43.9 %<br>41.8 %<br>45.7 %          | 9.8 %<br>11.1 %<br>18.7 % |
| ELECTRICAL<br>CONDUCTIVITY<br>( mSiemens / cm )    | A<br>B<br>C | 5.80<br>6.40<br>ND <sup>1</sup> | 40.8 %<br>42.0 %<br>ND              | 9.8 %<br>11.1 %<br>ND     |
| pH                                                 | A<br>B<br>C | 6.61<br>6.63<br>ND              | 42.6 %<br>47.1 %<br>ND              | 9.8 %<br>11.1 %<br>ND     |
| LACTOSE<br>CONCENTRATION<br>( % )                  | A<br>B<br>C | 5.00<br>4.90<br>5.01            | 40.8 %<br>47.2 %<br>31.3 %          | 9.8 %<br>11.1 %<br>18.7 % |
| NAGase<br>ACTIVITY<br>( nM / ml / min )            | A<br>B<br>C | 5.45<br>5.65<br>7.00            | 30.4 %<br>26.3 %<br>25.3 %          | 9.8 %<br>11.1 %<br>18.7 % |
| ANTITRYPSIN<br>CONCENTRATION<br>( Relative units ) | A<br>B<br>C | 0.725<br>0.935<br>0.885         | 43.0 %<br>36.5 %<br>33.6 %          | 9.8 %<br>10.8 %<br>18.6 % |

1 ND = No data

TABLE 7.1111RESULTS BY STAGE OF LACTATION - BACTERIOLOGICAL CLASSIFICATION 1

| PARAMETER<br>NAME                                  | STAGE            | CRITICAL<br>THRESHOLD                    | PROBABILITY OF<br>MISCLASSIFICATION  | PERCENTAGE<br>INFECTED               |
|----------------------------------------------------|------------------|------------------------------------------|--------------------------------------|--------------------------------------|
| SOMATIC CELL<br>COUNT<br>( cells / ml )            | 1<br>2<br>3<br>4 | 105,000<br>225,000<br>390,000<br>345,000 | 20.2 %<br>16.9 %<br>20.1 %<br>17.5 % | 14.7 %<br>14.0 %<br>14.6 %<br>14.2 % |
| SODIUM<br>CONCENTRATION<br>( mMoles / litre )      | 1<br>2<br>3<br>4 | 16.0<br>16.4<br>19.8<br>20.6             | 28.4 %<br>21.7 %<br>28.3 %<br>28.4 % | 14.8 %<br>13.9 %<br>14.5 %<br>14.1 % |
| POTASSIUM<br>CONCENTRATION<br>( mMoles / litre )   | 1<br>2<br>3<br>4 | 41.4<br>40.8<br>38.9<br>37.4             | 47.0 %<br>41.5 %<br>43.3 %<br>37.3 % | 14.8 %<br>13.9 %<br>14.5 %<br>14.1 % |
| ELECTRICAL<br>CONDUCTIVITY<br>( mSiemens / cm )    | 1<br>2<br>3<br>4 | 5.86<br>5.95<br>5.96<br>6.25             | 42.8 %<br>40.7 %<br>44.5 %<br>37.9 % | 9.0 %<br>10.0 %<br>10.8 %<br>11.6 %  |
| pH                                                 | 1<br>2<br>3<br>4 | 6.62<br>6.60<br>6.64<br>6.66             | 41.2 %<br>50.0 %<br>47.2 %<br>38.9 % | 10.0 %<br>10.0 %<br>10.7 %<br>11.9 % |
| LACTOSE<br>CONCENTRATION<br>( % )                  | 1<br>2<br>3<br>4 | 5.20<br>5.15<br>4.78<br>4.74             | 38.2 %<br>35.6 %<br>34.8 %<br>36.7 % | 14.7 %<br>14.0 %<br>14.6 %<br>14.1 % |
| NAGase<br>ACTIVITY<br>( nM / ml / min )            | 1<br>2<br>3<br>4 | 6.30<br>5.90<br>6.20<br>5.90             | 30.5 %<br>25.1 %<br>27.1 %<br>27.4 % | 14.7 %<br>14.0 %<br>14.6 %<br>14.1 % |
| ANTITRYPSIN<br>CONCENTRATION<br>( Relative units ) | 1<br>2<br>3<br>4 | 0.810<br>0.820<br>0.865<br>0.935         | 36.6 %<br>32.7 %<br>36.0 %<br>37.6 % | 14.6 %<br>14.0 %<br>14.4 %<br>13.8 % |

TABLE 7.1ivRESULTS OF SOMATIC CELL COUNT WITHIN HERDS, BY STAGE OF LACTATIONBACTERIOLOGICAL CLASSIFICATION 1

| HERD | STAGE | CRITICAL <sup>1</sup><br>THRESHOLD | PROBABILITY OF<br>MISCLASSIFICATION |
|------|-------|------------------------------------|-------------------------------------|
| A    | 1     | 95,000                             | 9.7 %                               |
|      | 2     | 80,000                             | 11.2 %                              |
|      | 3     | 70,000                             | 14.7 %                              |
|      | 4     | 120,000                            | 14.1 %                              |
| B    | 1     | 120,000                            | 18.3 %                              |
|      | 2     | 75,000                             | 25.0 %                              |
|      | 3     | 300,000                            | 11.1 %                              |
|      | 4     | 525,000                            | 9.6 %                               |
| C    | 1     | 125,000                            | 25.3 %                              |
|      | 2     | 320,000                            | 22.5 %                              |
|      | 3     | 765,000                            | 15.3 %                              |
|      | 4     | 715,000                            | 14.5 %                              |

1 ( cells / ml of milk )

TABLE 7.1v

RESULTS FOR TOTAL DATA - BACTERIOLOGICAL CLASSIFICATION 2

| NAME OF<br>PARAMETER                               | CRITICAL<br>THRESHOLD | PROBABILITY OF<br>MISCLASSIFICATION | PERCENTAGE<br>INFECTED |
|----------------------------------------------------|-----------------------|-------------------------------------|------------------------|
| SOMATIC CELL<br>COUNT<br>( cells / ml )            | 80,000                | 19.9 %                              | 42.8 %                 |
| SODIUM<br>CONCENTRATION<br>( mMoles / litre )      | 16.4                  | 29.0 %                              | 42.9 %                 |
| POTASSIUM<br>CONCENTRATION<br>( mMoles / litre )   | 40.4                  | 45.3 %                              | 42.9 %                 |
| ELECTRICAL<br>CONDUCTIVITY<br>( mSiemens / cm )    | 5.96                  | 33.4 %                              | 35.4 %                 |
| pH                                                 | 6.62                  | 45.0 %                              | 35.9 %                 |
| LACTOSE<br>CONCENTRATION<br>( % )                  | 5.07                  | 39.1 %                              | 42.8 %                 |
| NAGase<br>ACTIVITY<br>( nM / ml / min )            | 5.35                  | 38.1 %                              | 42.8 %                 |
| ANTITRYPSIN<br>CONCENTRATION<br>( Relative units ) | 0.785                 | 40.6 %                              | 42.7 %                 |

TABLE 7.1viRESULTS WITHIN INDIVIDUAL HERDS - BACTERIOLOGICAL CLASSIFICATION 2

| PARAMETER<br>NAME                                  | HERD        | CRITICAL<br>THRESHOLD           | PROBABILITY OF<br>MISCLASSIFICATION | PERCENTAGE<br>INFECTED     |
|----------------------------------------------------|-------------|---------------------------------|-------------------------------------|----------------------------|
| SOMATIC CELL<br>COUNT<br>( cells / ml )            | A<br>B<br>C | 60,000<br>50,000<br>165,000     | 13.5 %<br>19.7 %<br>24.3 %          | 20.0 %<br>57.4 %<br>50.7 % |
| SODIUM<br>CONCENTRATION<br>( mMoles / litre )      | A<br>B<br>C | 15.6<br>15.9<br>17.5            | 29.9 %<br>27.8 %<br>30.3 %          | 20.0 %<br>57.4 %<br>50.9 % |
| POTASSIUM<br>CONCENTRATION<br>( mMoles / litre )   | A<br>B<br>C | 40.1<br>42.6<br>39.6            | 44.9 %<br>42.1 %<br>43.9 %          | 20.0 %<br>57.4 %<br>50.9 % |
| ELECTRICAL<br>CONDUCTIVITY<br>( mSiemens / cm )    | A<br>B<br>C | 5.76<br>6.21<br>ND <sup>1</sup> | 40.5 %<br>38.0 %<br>ND              | 20.0 %<br>57.4 %<br>ND     |
| pH                                                 | A<br>B<br>C | 6.62<br>6.63<br>ND              | 42.4 %<br>48.8 %<br>ND              | 19.9 %<br>57.4 %<br>ND     |
| LACTOSE<br>CONCENTRATION<br>( % )                  | A<br>B<br>C | 5.04<br>4.96<br>5.12            | 44.5 %<br>38.0 %<br>36.3 %          | 20.0 %<br>57.4 %<br>50.8 % |
| NAGase<br>ACTIVITY<br>( nM / ml / min )            | A<br>B<br>C | 5.15<br>5.00<br>5.85            | 37.0 %<br>45.4 %<br>36.5 %          | 20.0 %<br>57.4 %<br>50.8 % |
| ANTITRYPSIN<br>CONCENTRATION<br>( Relative units ) | A<br>B<br>C | 0.705<br>0.855<br>0.810         | 46.9 %<br>48.2 %<br>39.4 %          | 20.0 %<br>57.3 %<br>50.7 % |

1 ND = No data

TABLE 7.21

RESULTS OF DISCRIMINANT FUNCTION ANALYSIS FOR ALL HERDS

BACTERIOLOGICAL CLASSIFICATION 1

| PARAMETER                 | PERCENTAGE OF CORRECT DIAGNOSES |                  |                    | NUMBER OF OBSERVATIONS |
|---------------------------|---------------------------------|------------------|--------------------|------------------------|
|                           | TOTAL SAMPLES                   | INFECTED SAMPLES | UNINFECTED SAMPLES |                        |
| SOMATIC CELL COUNT        | 79.6 %                          | 80.9 %           | 79.3 %             | 3210                   |
| SODIUM CONCENTRATION      | 81.9 %                          | 43.4 %           | 88.4 %             | 3206                   |
| POTASSIUM CONCENTRATION   | 78.6 %                          | 25.3 %           | 87.5 %             | 3206                   |
| ELECTRICAL CONDUCTIVITY   | 73.4 %                          | 37.8 %           | 77.5 %             | 1669                   |
| pH                        | 77.1 %                          | 26.5 %           | 83.0 %             | 1606                   |
| LACTOSE CONCENTRATION     | 83.7 %                          | 25.0 %           | 93.5 %             | 3214                   |
| NAGase ACTIVITY           | 85.0 %                          | 51.4 %           | 90.6 %             | 3212                   |
| ANTITRYPSIN CONCENTRATION | 76.3 %                          | 43.7 %           | 81.7 %             | 3191                   |

TABLE 7.211RESULTS OF DISCRIMINANT FUNCTION ANALYSIS FOR INDIVIDUAL HERDS

| PARAMETER                 | HERD | PERCENTAGE OF CORRECT DIAGNOSES |                  |                    | NUMBER OF OBSERVATIONS |
|---------------------------|------|---------------------------------|------------------|--------------------|------------------------|
|                           |      | TOTAL SAMPLES                   | INFECTED SAMPLES | UNINFECTED SAMPLES |                        |
| SOMATIC CELL COUNT        | A    | 89.2 %                          | 83.3 %           | 89.9 %             | 984                    |
|                           | B    | 84.7 %                          | 81.6 %           | 85.1 %             | 686                    |
|                           | C    | 73.6 %                          | 78.6 %           | 72.5 %             | 1540                   |
| SODIUM CONCENTRATION      | A    | 84.2 %                          | 41.7 %           | 88.8 %             | 983                    |
|                           | B    | 85.9 %                          | 51.3 %           | 90.2 %             | 686                    |
|                           | C    | 78.4 %                          | 47.0 %           | 85.6 %             | 1537                   |
| POTASSIUM CONCENTRATION   | A    | 78.9 %                          | 21.9 %           | 85.1 %             | 983                    |
|                           | B    | 82.9 %                          | 30.3 %           | 89.5 %             | 686                    |
|                           | C    | 76.5 %                          | 24.4 %           | 88.5 %             | 1537                   |
| ELECTRICAL CONDUCTIVITY   | A    | 73.2 %                          | 38.5 %           | 76.9 %             | 984                    |
|                           | B    | 72.7 %                          | 44.7 %           | 76.2 %             | 685                    |
|                           | C    | ND <sup>1</sup>                 | ND               | ND                 |                        |
| pH                        | A    | 75.4 %                          | 28.9 %           | 80.4 %             | 921                    |
|                           | B    | 78.4 %                          | 32.9 %           | 84.1 %             | 685                    |
|                           | C    | ND                              | ND               | ND                 |                        |
| LACTOSE CONCENTRATION     | A    | 78.1 %                          | 31.3 %           | 83.2 %             | 983                    |
|                           | B    | 79.6 %                          | 36.8 %           | 84.9 %             | 686                    |
|                           | C    | 80.6 %                          | 23.2 %           | 93.8 %             | 1545                   |
| NAGase ACTIVITY           | A    | 77.9 %                          | 43.8 %           | 81.6 %             | 983                    |
|                           | B    | 89.3 %                          | 59.2 %           | 93.1 %             | 685                    |
|                           | C    | 83.0 %                          | 55.4 %           | 89.4 %             | 1544                   |
| ANTITRYPSIN CONCENTRATION | A    | 57.9 %                          | 51.0 %           | 58.7 %             | 984                    |
|                           | B    | 72.8 %                          | 49.3 %           | 75.6 %             | 679                    |
|                           | C    | 76.4 %                          | 49.7 %           | 82.5 %             | 1538                   |

1 ND = No data

TABLE 7.2111RESULTS OF DISCRIMINANT FUNCTION ANALYSIS FOR ALL THREE HERDSSOMATIC CELL COUNT PLUS ONE OTHER PARAMETER

| PERCENTAGE OF CORRECT DIAGNOSES    |                              |                     |                       |             |
|------------------------------------|------------------------------|---------------------|-----------------------|-------------|
| PARAMETER                          | TOTAL<br>SAMPLES             | INFECTED<br>SAMPLES | UNINFECTED<br>SAMPLES | # OF<br>OBS |
| SCC ALONE<br>HERDS A+B+C           | 79.6 %                       | 80.9 %              | 79.3 %                | 3210        |
| SCC ALONE<br>HERDS A+B ONLY        | 87.5 %                       | 82.5 %              | 88.0 %                | 1670        |
| SCC +<br>SODIUM                    | 80.9 % ( +1.3 ) <sup>1</sup> | 78.2 % ( -2.7 )     | 81.3 % ( +2.0 )       | 3201        |
| SCC +<br>POTASSIUM                 | 81.4 % ( +1.8 )              | 77.3 % ( -3.6 )     | 82.1 % ( +2.8 )       | 3201        |
| SCC +<br>CONDUCTIVITY <sup>2</sup> | 86.2 % ( -1.3 )              | 82.0 % ( -0.5 )     | 86.7 % ( -1.3 )       | 1669        |
| SCC +<br>pH <sup>2</sup>           | 87.0 % ( -0.5 )              | 81.3 % ( -1.2 )     | 87.6 % ( -0.4 )       | 1605        |
| SCC +<br>LACTOSE                   | 82.7 % ( +3.1 )              | 72.5 % ( -8.4 )     | 84.4 % ( +5.1 )       | 3209        |
| SCC +<br>NAGase                    | 81.0 % ( +1.4 )              | 76.1 % ( -4.8 )     | 81.8 % ( +2.5 )       | 3207        |
| SCC +<br>ANTITRYPSIN               | 79.1 % ( -0.5 )              | 80.7 % ( -0.2 )     | 78.8 % ( -0.4 )       | 3196        |

1 Figures in parentheses represent the difference in discriminative ability between the somatic cell count alone and the somatic cell count plus the parameter concerned ( significance not tested ).

2 Electrical conductivity and pH readings were not taken on samples from herd C - see text for explanation.

TABLE 7.21vRESULTS OF DISCRIMINANT FUNCTION ANALYSIS WITHIN INDIVIDUAL HERDSSOMATIC CELL COUNT PLUS ONE OTHER PARAMETER

| PARAMETER             | HERD | PERCENTAGE OF CORRECT DIAGNOSES |                     |                       |             |
|-----------------------|------|---------------------------------|---------------------|-----------------------|-------------|
|                       |      | TOTAL<br>SAMPLES                | INFECTED<br>SAMPLES | UNINFECTED<br>SAMPLES | # OF<br>OBS |
| SCC<br>ALONE          | A    | 89.2 %                          | 83.3 %              | 89.9 %                | 984         |
|                       | B    | 84.7 %                          | 81.6 %              | 85.1 %                | 686         |
|                       | C    | 73.6 %                          | 78.6 %              | 72.5 %                | 1540        |
| SCC +<br>SODIUM       | A    | 89.0 % ( -0.2 ) <sup>1</sup>    | 80.2 % ( -3.1 )     | 90.0 % ( +0.1 )       | 983         |
|                       | B    | 87.5 % ( +2.8 )                 | 82.9 % ( +1.3 )     | 88.0 % ( +2.9 )       | 686         |
|                       | C    | 75.1 % ( +1.5 )                 | 76.0 % ( -2.6 )     | 74.9 % ( +2.4 )       | 1532        |
| SCC +<br>POTASSIUM    | A    | 89.5 % ( +0.3 )                 | 81.3 % ( -2.0 )     | 90.4 % ( +0.5 )       | 983         |
|                       | B    | 85.3 % ( +0.6 )                 | 82.9 % ( +1.3 )     | 85.6 % ( +0.5 )       | 686         |
|                       | C    | 79.0 % ( +5.4 )                 | 76.7 % ( -1.9 )     | 79.5 % ( +7.0 )       | 1532        |
| SCC +<br>CONDUCTIVITY | A    | 89.8 % ( +0.6 )                 | 84.4 % ( +1.1 )     | 90.4 % ( +0.5 )       | 984         |
|                       | B    | 84.2 % ( -0.5 )                 | 82.9 % ( +1.3 )     | 84.4 % ( -0.7 )       | 685         |
|                       | C    | ND <sup>2</sup>                 | ND                  | ND                    |             |
| SCC +<br>pH           | A    | 89.0 % ( -0.2 )                 | 83.3 % ( +0.0 )     | 89.7 % ( -0.2 )       | 921         |
|                       | B    | 85.8 % ( +1.1 )                 | 81.6 % ( +0.0 )     | 86.4 % ( +1.3 )       | 685         |
|                       | C    | ND                              | ND                  | ND                    |             |
| SCC +<br>LACTOSE      | A    | 90.2 % ( +1.0 )                 | 82.3 % ( -1.0 )     | 91.1 % ( +1.2 )       | 983         |
|                       | B    | 87.8 % ( +3.1 )                 | 84.2 % ( +2.6 )     | 88.2 % ( +3.1 )       | 686         |
|                       | C    | 81.9 % ( +8.3 )                 | 67.5 % ( -11.1 )    | 85.3 % ( +12.8 )      | 1540        |
| SCC +<br>NAGase       | A    | 88.1 % ( -1.1 )                 | 82.3 % ( -1.0 )     | 88.7 % ( -1.2 )       | 983         |
|                       | B    | 88.9 % ( +4.2 )                 | 84.2 % ( +2.6 )     | 89.5 % ( +4.4 )       | 685         |
|                       | C    | 80.3 % ( +6.7 )                 | 73.0 % ( -5.6 )     | 82.0 % ( +9.5 )       | 1539        |
| SCC +<br>ANTITRYPSIN  | A    | 88.3 % ( -0.9 )                 | 82.3 % ( -1.0 )     | 89.0 % ( -0.9 )       | 984         |
|                       | B    | 84.8 % ( +0.1 )                 | 80.8 % ( -0.8 )     | 85.3 % ( +0.2 )       | 679         |
|                       | C    | 74.5 % ( +0.9 )                 | 76.2 % ( -2.4 )     | 74.1 % ( +1.6 )       | 1533        |

1 Figures in parentheses represent the difference in discriminative ability between the somatic cell count alone and the somatic cell count plus the parameter concerned ( significance not tested ).

2 ND = No data.

TABLE 7.3

CORRELATION BETWEEN SOMATIC CELL COUNT AND OTHER PARAMETERS

| HERD   | STATUS     | SOD  | POT   | CON  | PH   | LAC   | NAG  | ANT  |
|--------|------------|------|-------|------|------|-------|------|------|
| TOTAL  | UNINFECTED | 0.66 | -0.34 | 0.28 | 0.16 | -0.42 | 0.54 | 0.30 |
|        | INFECTED   | 0.67 | -0.43 | 0.47 | 0.46 | -0.44 | 0.70 | 0.62 |
|        | OVERALL    | 0.65 | -0.36 | 0.31 | 0.23 | -0.40 | 0.57 | 0.36 |
| HERD A | UNINFECTED | 0.52 | -0.20 | 0.14 | 0.10 | -0.19 | 0.45 | 0.18 |
|        | INFECTED   | 0.55 | -0.40 | 0.26 | 0.45 | -0.28 | 0.68 | 0.53 |
|        | OVERALL    | 0.52 | -0.25 | 0.16 | 0.17 | -0.21 | 0.49 | 0.23 |
| HERD B | UNINFECTED | 0.63 | -0.41 | 0.19 | 0.18 | -0.43 | 0.41 | 0.23 |
|        | INFECTED   | 0.67 | -0.50 | 0.50 | 0.46 | -0.54 | 0.67 | 0.54 |
|        | OVERALL    | 0.63 | -0.43 | 0.25 | 0.24 | -0.45 | 0.47 | 0.28 |
| HERD C | UNINFECTED | 0.64 | -0.33 | ND   | ND   | -0.51 | 0.55 | 0.34 |
|        | INFECTED   | 0.66 | -0.42 | ND   | ND   | -0.43 | 0.70 | 0.62 |
|        | OVERALL    | 0.64 | -0.35 | ND   | ND   | -0.46 | 0.58 | 0.40 |

SOD = SODIUM CONCENTRATION  
 POT = POTASSIUM CONCENTRATION  
 CON = ELECTRICAL CONDUCTIVITY

LAC = LACTOSE CONCENTRATION  
 NAG = NAGase ACTIVITY  
 ANT = ANTITRYPSIN CONCENTRATION

TABLE 7.4

THE EFFECT OF USING CRITICAL THRESHOLD VALUES,  
OR SET THRESHOLD VALUES, ON THE DIAGNOSIS OF INFECTION  
WITHIN INDIVIDUAL UDDER QUARTERS

| HERD | THRESHOLD            | % FALSE<br>NEGATIVE<br>RESULTS | % FALSE<br>POSITIVE<br>RESULTS |
|------|----------------------|--------------------------------|--------------------------------|
| A    | 85,000 ( critical )  | 13.7 %                         | 13.7 %                         |
|      | 250,000 ( set )      | 30.2 %                         | 3.6 %                          |
| B    | 200,000 ( critical ) | 17.0 %                         | 17.0 %                         |
|      | 250,000 ( set )      | 18.4 %                         | 13.8 %                         |
| C    | 375,000 ( critical ) | 23.9 %                         | 23.9 %                         |
|      | 250,000 ( set )      | 17.0 %                         | 34.0 %                         |

### THE EFFECT OF AGE OF COW, STAGE OF LACTATION, AND BACTERIOLOGICAL STATUS ON THE LEVEL OF EACH SELECTED PARAMETER IN COMPOSITE MILK SAMPLES

#### INTRODUCTION

The effects of age of cow, stage of lactation and bacteriological status on the level of each of the selected parameters were presented in chapter six , for foremilk samples taken from individual quarters. In many cases, these factors and their interactions exerted statistically significant effects on the levels of the parameters which were studied ( table 6.1 ). In the present chapter, the corresponding data for composite milk samples are studied. The composite milk samples were obtained during the whole period of milk removal from each cow, using a milk meter ( see chapter three ). The composition of these samples, is therefore representative of all the milk removed from all four quarters. In contrast, the quarter samples were foremilk samples, taken prior to the milking, and as such, represent only one fraction of the milk from each individual quarter.

A number of studies, including the present study, ( chapter four ), have shown that levels of the various parameters in the milk change during milking ( Paape and Tucker, 1966; Schalm and Ziv-Silberman, 1968; Fernando and Spahr, 1983; Fernando *et al*, 1985; Berning *et al*, 1987b; Marschke *et al*, 1987; Fox *et al*, 1988 ). It is possible that the effects of age of cow, the stage of lactation, and the bacteriological status of the udder on the somatic cell count and on the other parameters, may also differ with the stage of the milking process at which the sample is taken. It may not, therefore, be valid to apply the results of chapter six to the current composite sample data set.

Physiological factors such as the age of the cow, or the stage of lactation, should affect all four quarters of a cow equally. However, infection of the udder does not necessarily occur in all four udder quarters. Therefore while infection of a particular quarter might have a statistically significant effect on the level of a parameter in the milk from that quarter, the dilution of milk from infected quarters by that from uninfected quarters, will tend to reduce this effect in the composite milk sample from that cow.

In New Zealand, as in many other countries, the composite milk sample is currently used for the diagnosis of subclinical mastitis. The effect of infection in one or more quarters, and the effects of the physiological factors on the levels of the selected parameters in composite milk samples must be understood if the results of the diagnostic tests are to be interpreted correctly.

## MATERIALS AND METHODS

### (1) STATISTICAL ANALYSIS

Data from each of the three herds were analysed separately using SAS ( Statistical Analytical Systems, 1985 ). Repeated measures analysis was used to study the effects of the age of the cow, the stage of lactation, the bacteriological status of the udder and the interactions of these factors on each parameter in composite samples using the GLM ( General Linear Models ) procedure.

Under repeated measures analysis if a data point is missing, then that whole record is removed from the analysis, thus a particular cow was excluded from consideration if data were not available for the full season.

When the effect of the age of the cow, the stage of the lactation, or the bacteriological status on the level of a parameter was found to be statistically significant, contrast analysis was used as a multiple comparison technique, to determine which levels of the factor differed.

### (2) BACTERIOLOGICAL STATUS

Within each herd, the cows were divided into three groups on the basis of the frequency of isolation of pathogenic bacteria from individual udder quarters during the lactation.

- Status 1    cows were those from which no major pathogens were isolated at any stage of the season.
- Status 2    cows were those from which major pathogens were isolated from at least one quarter, on three or fewer occasions.
- Status 3    cows were those from which major pathogens were isolated from at least one quarter, on four or more occasions.

The status 1 cows included those from which no bacteria were isolated at any stage of the lactation, from any of the four quarters, as well as cows from which minor pathogens were isolated. Likewise, each status 2 or status 3 cow may, or may not, have harboured minor pathogens. The relatively small number of cows which remained totally uninfected throughout the trial period, and those from which minor pathogens only could be isolated, precluded the further splitting of cows according to the isolation of minor pathogens.

It is recognized, therefore, that the minor pathogens may have exerted some effect on the results, particularly with regard to the status 1 cows. However, it was shown in chapter six, that while the presence of minor pathogens did affect the level of certain of the parameters in the milk of individual udder quarters, this effect was small in comparison with that of the major pathogens. Furthermore, the changes which occurred in the level of each parameter as the lactation progressed, tended to be similar for those quarters which were infected with minor pathogens, and those quarters which were uninfected. The dilution of milk from quarters which are infected with minor pathogens, by milk from uninfected quarters is likely to further reduce the effect of minor pathogens on the level of a parameter in composite milk samples.

Because the number of cows in the present study was relatively small, the effect of the number of infected quarters within the udder on the level of each parameter in composite milk was not investigated.

### (3) AGE OF COW

Within herds A and B, the ages of the cows which were included in the present trial ranged from 2 to 5 years. The numbers of 2, 3, 4 and 5 year old cows were approximately equal within herds A and B.

In herd C, the cows ranged from 3 to 10 years of age, but for the purposes of the analysis, the cows were grouped as follows:

- Age group 3      ( three year old cows )
- Age group 4      ( four year old cows )
- Age group 5      ( five year old cows )
- Age group 6      ( cows aged six years and older ).

The number of cows in each age group differed.

### (4) STAGE OF LACTATION

Samples were not taken during the first month of lactation, but each cow was sampled monthly thereafter, giving a maximum of eight monthly samples, which represent eight stages of lactation. These composite samples were collected at the same milking as the individual quarter samples.

No composite samples were taken from herd A during stage 1, due to the lack of sampling equipment. No samples were taken from herd B during month 5.

## RESULTS AND DISCUSSION

The results of the analysis of variance are given in table 8.1. All graphs and tables are located at the end of the chapter.

The results of the contrast analysis between each bacteriological status are given in table 8.2. The results of contrast analyses between consecutive stages of lactation are given separately for herds A, B and C in tables 8.3a, 8.3b and 8.3c respectively.

Table 8.4 gives a breakdown of the number of infected quarters within the infected cows.

Charts 8.1i to 8.8i show the effects of bacteriological status on the level of the selected parameters in composite milk samples within each of the three herds.

Charts 8.1ii to 8.8ii show the effects of the age of the cow on the level of the selected parameters in composite milk samples within each of the three herds.

Graphs 8.1iii to 8.8iii show the effects of the stage of the lactation on the level of the selected parameters in composite milk samples within each of the three herds.

### (1) SOMATIC CELL COUNT

#### (a) EFFECTS OF THE BACTERIOLOGICAL STATUS

The effect of the bacteriological status of the udder on the somatic cell count of composite milk samples was highly significant in each of the three herds ( table 8.1 ). Within each herd, the mean somatic cell count of the status 3 cows was higher than that of cows within status 1 or status 2. The mean somatic cell counts of status 1 and status 2 cows did not differ significantly within herds A or B. Within herd C, the mean somatic cell count of status 2 cows was higher than that of status 1 cows (  $p < 0.05$  ).

The association between infection of the udder and the increase in the somatic cell count of the milk, has been recognized since the late 19th century, ( Giesecke and Van den Heever, 1974 ).

While the relationship between the bacteriological status of a quarter, and the somatic cell count of milk from that quarter is relatively straightforward, the relationship between infection of udder quarters and the somatic cell count of composite samples is complicated by the fact that the cow may be infected in 1, 2, 3, or 4 quarters ( table

8.4 ). Thus the high cell count milk from infected quarters will be diluted by lower cell count milk from uninfected quarters within the infected udder.

This dilution effect has been well illustrated. When 1, 2, 3 or 4 quarters within the udder yielded a positive leukocyte test, the percentages of positive leukocyte tests in the corresponding composite sample were 42.7 %, 72.0 %, 82.4 % and 90.3 % respectively ( Munch-Petersen and Mulgrave, 1969 ). For each additional infected quarter, there is an approximate doubling of the somatic cell count of the composite milk sample ( Natzke *et al*, 1972a ).

Clearly, the effect of bacterial infection of the udder on the somatic cell count, and also on all the other parameters which may be used for mastitis diagnosis, will depend upon not only the severity of infection and the response of the cow to that infection, but also to the number of udder quarters which are infected at the time.

Table 8.4 shows the percentage of composite samples which came from udders with 1, 2, 3, or 4 quarters infected with a major pathogen, by herd and overall. The data includes infected udders only. It may be seen that in the majority of cows which were infected, the infection was confined to one quarter. In less than 20 % of infected cows did composite samples come from udders in which more than two quarters were infected. In herd A over 96 % of samples from infected cows were infected in either 1 or 2 quarters only. These figures suggest that in the present trial, the effect of the bacteriological status of the udder on the levels of the parameters in composite milk should be markedly smaller than the effect on individual quarter samples.

In the case of the somatic cell count of the composite milk samples, the effect of the bacteriological status was highly significant in each herd (  $p < 0.01$  ). This is due to the magnitude of the effect of infection on somatic cell count at the quarter level, and suggests that the composite milk somatic cell count will be a useful parameter for the diagnosis of subclinical infection, even when infection is limited to a single quarter within the udder.

#### (b) EFFECTS OF THE STAGE OF LACTATION

The effect of the stage of lactation on the somatic cell count of composite milk samples was significant within herd A (  $p < 0.05$  ) and highly significant within herd B (  $p < 0.01$  ), while within herd C, the stage of lactation effect just failed to reach significance at the 5 % level ( table 8.1 ). In general, an increase in the somatic cell count occurred as the lactation progressed.

The mean somatic cell counts of cows within each herd, at each stage of the lactation are shown in graph 8.1iii.

Within herd A, none of the individual monthly differences in mean somatic cell count reached significance, but between stages 5 to 8 there was a trend towards an increase in the somatic cell count in composite milk samples.

Within herd B, no significant change in the mean somatic cell count of composite samples occurred during the first four stages of lactation. Between stages 4 to 7, significant monthly increases in somatic cell count were recorded, with no further change in somatic cell count occurring during the final stage of lactation.

While the overall effect of the stage of lactation did not reach significance at the 5 % level within herd C, significant increases in the mean somatic cell count occurred between stages 2 to 3, and stages 3 to 4. Nonsignificant increases in somatic cell count were recorded later in the lactation for cows within herd C.

In general, the changes seen during the season in composite samples were similar to those seen in quarter samples ( graphs 6.1ai, 6.1bi and 6.1ci for herds A, B, and C respectively ). The similarity in the findings between quarter samples and composite samples with regard to changes in the somatic cell count during the lactation were not unexpected, since in general, similar changes occurred in the somatic cell counts of infected and of uninfected quarters.

As noted earlier, the composite sample reflects the milk from the entire milking process, whereas the quarter samples were of foremilk only. It would appear that the difference between the sample types, in respect of the stage of the milking at which the sample is taken, had little effect on the pattern of change in the somatic cell count during the lactation.

The somatic cell count increased during mid to late lactation in all three herds. This is consistent with the findings of Kennedy *et al* ( 1982 ), and also Ng-Kwai-Hang *et al* ( 1984 ), although Emanuelson and Persson ( 1984 ) found no increase after their results had been adjusted for changes in milk yield.

The reasons for the observed changes in the somatic cell count during the course of the lactation have been discussed previously with respect to quarter samples ( chapter six ), the same explanations being applicable to composite milk samples.

During the present trial, little change in the somatic cell count occurred during the final stage of lactation, particularly within herds A and B. This is probably due to environmental changes, since the stage of lactation effects, and those of the season are confounded.

No samples were taken during the first month post-partum, therefore the high somatic cell counts which have been reported early in the season by other workers ( Natzke et al 1972a , Kennedy et al 1982, and Ng-Kwai-Hang et al, 1984 ) would not have been observed during the present trial.

### (c) EFFECTS OF THE AGE OF THE COW

The mean somatic cell count for cows within each age group in each of the three herds is shown in chart 8.1ii.

Although there was a trend towards increasing somatic cell count with increasing age of the cow, the increase did not reach significance at the 5 % level in any of the three herds ( table 8.1 ).

Some researchers have found a small increase in somatic cell count with increasing age of the cow. In cows which are uninfected at the time of sampling there is a slight increase in the somatic cell count, although cows which have remained uninfected for a period of three years show no significant increase in somatic cell count with age, suggesting that the increase with age may be attributed to the effects of previous infections ( Natzke et al, 1972a ).

Other researchers have found age to have a greater effect. Syrstad and Røn ( 1979 ) reported that age has a highly significant effect on the somatic cell count of composite milk samples. For cows younger than six years, there is a small but linear increase in somatic cell count as the age of the cow increases, whereas for older cows the increase is more pronounced ( Kennedy et al, 1982; Ng- Kwai-Hang et al, 1984 ).

The failure of the increases in the somatic cell count to reach significance during the present trial may be partly due to the relatively small number of cows which were included in the analysis. Had the analysis been carried out on the three herds combined, significant age effects might have been seen. The age range within herds A and B was small, the oldest cows being five years of age and this may also have contributed to the lack of statistical significance within herds A and B.

The low numbers of animals prevented the separate analysis of the effect of age on the somatic cell count of uninfected cows. From the work of Natzke et al ( 1972a ) it would appear that the increase in cell count with age is due to the effect of infection or recently eliminated infection, rather than to a physiological increase. Neither the effect of the interaction between the bacteriological status and the stage of lactation, nor that between the bacteriological status and the age of the cow on the somatic cell count of the composite milk samples reached significance at the 5 % level.

A highly significant effect of the interaction between the age of the cow and the stage of lactation on the somatic cell count occurred within herd B, suggesting that the effect of the stage of lactation on the somatic cell count differed between cows within the various age groups. This was due mainly to the mid to late lactation increase in somatic cell count being smaller for the two year old cows, ie heifers, than for the older cows, so that for the last 2 to 3 months of testing the two year old cows showed lower somatic cell counts than did the other age groups.

## (2) SODIUM CONCENTRATION

### (a) EFFECTS OF THE BACTERIOLOGICAL STATUS

The mean sodium concentration of samples in each bacteriological status, within the three herds is given in chart 8.2i. The effect of the bacteriological status on the sodium concentration of composite milk samples was significant (  $p < 0.05$  or less ) in each of the three herds ( table 8.1 ).

Within each herd, the mean sodium concentration of composite milk samples from status 3 cows was higher than that from status 1 cows (  $p < 0.05$  or less ). Within herd B, the mean sodium concentration of composite milk samples from status 2 cows was higher than that from status 1 cows (  $p < 0.05$  ), while within herd C, the mean sodium concentration of milk from status 3 cows was higher than that from status 2 cows (  $p < 0.05$  ).

The results of the present study show that infection of the udder by major pathogens causes a statistically significant increase in the mean sodium concentration of the composite milk samples. Similar findings have been reported by other researchers ( Fernando *et al*, 1985; Daniel and Pavithran, 1985 ).

The reason for the increase in sodium concentration which is associated with bacterial infection of the udder was discussed in chapter six. It is clear that the scale of increase of sodium concentration in infected quarters was large enough to overcome the dilution effects of milk from uninfected quarters, and to significantly raise the sodium concentration of composite milk samples.

Dilution of milk from infected quarters by that from uninfected quarters within the udder might be expected to reduce the effect of infection on the sodium concentration of composite milk samples. However, the sodium concentration in strippings milk is higher than that of foremilk samples ( chapter four ). The composite sample contains milk which is representative of the entire milking process. Thus the fact that bacterial

infection causes a significant increase in the sodium concentration of composite milk samples may be due in part to the nature of the sample.

#### (b) EFFECTS OF THE STAGE OF LACTATION

The mean sodium concentration of composite samples from each herd during the lactation is given in graph 8.2iii. The effect of the stage of lactation on the sodium concentration of composite milk samples was significant in each of the three herds ( table 8.1 ).

Within herd A, there was no significant change in the mean sodium concentration before the fourth stage of lactation. Between stages 4 to 5 there was a significant decrease in the sodium concentration (  $p < 0.05$  ), followed by increases in the mean sodium concentration of the composite samples between stages 5 to 7 of the lactation (  $p < 0.01$  ). The decrease in sodium concentration during the final stage did not quite reach significance at the 5 % level.

Within herd B, no significant change in the mean sodium concentration occurred before the fourth stage of lactation. The increase in sodium concentration between stages 4 to 6 was highly significant (  $p < 0.01$  ) and there was a further increase in the mean sodium concentration during the final stage of the lactation (  $p < 0.01$  ).

Within herd C, significant increases in the mean concentration of sodium in composite milk samples occurred for each month between stages 3 and 7 (  $p < 0.05$  or less ). In general, the shape of the graph for composite data resembled that for the quarter samples within each herd ( graphs 6.2aii, 6.2bii and 6.2cii for herds A, B and C respectively ).

The sodium concentration of composite milk samples is high at parturition, reaches the normal lactation levels by day 7 post-partum, and increases late in the lactation ( Daniel and Pavithran, 1985 ). Safwate *et al* ( 1981 ) reported a decrease in milk sodium during the first four weeks of lactation. This was followed by an increase between weeks 5 to 7 and the sodium concentration increased only slowly thereafter until the end of the lactation.

The relatively small number of cows in the present study precluded the analysis of uninfected vs infected cows at each stage of lactation in the three herds, thus it is not clear whether these increases occurred in cows which were free of infection. However, data from chapter six, relating to quarter samples showed that similar changes occurred both in quarters which were infected, and in those which were uninfected, indicating that the stage of lactation effects are physiological and are probably related to the milk yield, and to natural involutionary change within the mammary tissue.

### (c) EFFECTS OF THE AGE OF THE COW

The mean sodium concentration for each age group within each of the three herds is shown in chart 8.2ii. While there was a trend towards an increase in sodium concentration with age in each herd, this increase did not reach significance at the 5 % level in any of the three herds ( table 8.1 ).

The number of samples was insufficient to show to what extent the trend of increasing mean sodium concentration with increasing age, was due to infection, or to physiological factors. The incidence of infection did increase with age during the present trial ( see chapter five ), and as has been shown in the present chapter, infection with a major pathogen exerts a significant effect on the concentration of sodium in composite milk samples.

None of the interactions between the effects of the bacteriological status, the stage of lactation or the age of the cow on the sodium concentration of the composite milk samples were significant at the 5 % level ( table 8.1 ).

### (3) POTASSIUM CONCENTRATION

#### (a) EFFECTS OF THE BACTERIOLOGICAL STATUS

The mean potassium concentration of the composite milk samples within each bacteriological status , in each of the three herds is given in chart 8.3i.

The bacteriological status did not exert a significant effect on the concentration of potassium in the composite milk samples within any of the three herds ( table 8.1 ).

When quarter samples were examined ( chapter six ), the effect of the bacteriological status on the potassium concentration was significant within herd B only. The effect of infection on milk potassium concentration at the level of the individual udder quarter, was clearly too small to be detected in the composite milk samples within herd B.

Other workers have shown very little difference in the potassium concentration of milk from infected cows and that from uninfected cows ( Fernando et al, 1985 ).

The difference between the potassium concentration in foremilk and in strippings milk is smaller than the corresponding difference in the sodium concentration ( chapter four ). Thus the decrease in potassium concentration which occurs during the milking process in infected quarters, is insufficient to overcome the dilution effects of the milk

from uninfected quarters, with the result that the potassium concentration of the composite milk sample does not decrease significantly following infection.

### (b) EFFECTS OF THE STAGE OF LACTATION

The mean potassium concentration of the composite milk samples for each herd during the lactation is shown in graph 8.3iii.

The stage of lactation at which the sample was taken exerted a significant effect on the potassium concentration within herd B (  $p < 0.05$  ), and a highly significant effect on herd A cows (  $p < 0.01$  ) while in herd C, stage of lactation effects on the potassium concentration of composite milk samples just failed to reach significance at the 5 % level ( table 8.1 ).

Contrast analysis was used to determine which months differed significantly ( tables 8.3a, 8.3b and 8.3c for herds A, B, and C respectively ).

Within herd A, none of the consecutive monthly differences reached significance, but there was a trend towards decreasing potassium concentration as the season progressed, particularly between stages 2 to 6, and during the final stage of the season.

In herd B, the potassium concentration fluctuated during the first four stages, before decreasing significantly between stages 4 to 6 of lactation (  $p < 0.01$  ). A further decrease in potassium concentration of the composite milk samples occurred during the final stage of lactation (  $p < 0.01$  ).

While the effect of stage of lactation on the potassium concentration was not statistically significant overall within herd C, individual monthly differences did reach significance on several occasions, with a decrease in the potassium concentration occurring between stages 4 to 5, and stages 6 to 7 of the lactation.

Other workers have reported that the potassium concentration of composite milk samples increases rapidly following parturition, reaches a maximum during mid-lactation, and decreases later in the lactation ( Daniel and Pavithran, 1985 ). However, Safwate *et al* ( 1981 ) found that the potassium concentration remained stable from day 1 of lactation until day 70, and then decreased until the end of lactation.

Samples were not taken from cows in their first month of lactation during the present trial, so the concentration of potassium in composite milk samples immediately following parturition could not be ascertained.

Too few animals were included in the present study to determine whether similar lactational changes in potassium concentration occurred both in infected and in uninfected cows. Data for quarter samples ( chapter six ) suggest that similar, although more marked, changes occur during the season in quarters which are infected compared with those which are free of major or minor pathogens.

The reasons for the changes in the potassium concentration during the season within individual quarters ( chapter six ) are applicable to those changes which occur within composite milk samples also.

### (c) EFFECTS OF THE AGE OF THE COW

The mean potassium concentration for cows within each age group, in each of the three herds is shown in chart 8.3ii.

As was the case with both the somatic cell count and the sodium concentration, the effect of age on potassium concentration of the composite milk samples did not reach significance in any of the three herds ( table 8.1 ).

Although the number of cows within each age group was low, and this may have contributed to a failure to reach significance, there was no clear trend towards a decreasing potassium concentration of composite milk samples as the age of the cows increased. Some evidence of a decrease in potassium concentration with increasing age was obtained with quarter samples ( graphs 6.3aii, 6.3bii and 6.3cii ), but the effect of the age of the cow on the potassium concentration was too small to be seen in the composite milk samples.

None of the interactions between the effects of the bacteriological status, the stage of lactation, or the age of the cow on the potassium concentration of composite milk samples reached significance at the 5 % level ( table 8.1 ).

#### (4) ELECTRICAL CONDUCTIVITY

##### (a) EFFECTS OF THE BACTERIOLOGICAL STATUS

The mean electrical conductivity readings for the cows in each bacteriological status within herds A and B are shown in chart 8.4i. The measurement of electrical conductivity was not carried out on samples taken from herd C.

In neither herd A nor herd B was the main effect of the bacteriological status on electrical conductivity significant at the 5 % level ( table 8.1 ). While significance was not reached, there was a slight trend towards higher conductivity readings for status 3 cows, than for cows of either status 1 or status 2.

Composite strict foremilk samples from cows which are infected with major pathogens show significantly higher conductivity readings than do cows which are uninfected ( Batra and McAlister, 1984 ). Similar results have been reported with respect to composite milk samples ( Fernando *et al*, 1985 ).

While the concentration of sodium in the foremilk is lower than that in the strippings milk, particularly within infected quarters ( chapter four ), the same is not true of the electrical conductivity. The electrical conductivity tends to be lower in strippings milk than in foremilk samples within uninfected quarters, although higher in strippings milk than in foremilk within infected quarters.

It would appear that the dilution effect of the milk from uninfected quarters was large enough to mask the increase in conductivity in milk from infected quarters. As with the somatic cell count, this may be due in part to the fact that infected cows tended to be infected in either 1 or 2 quarters, with very few cows having all four quarters infected simultaneously ( table 8.4 ).

While most infected cows were infected in only 1 or 2 quarters, it must also be remembered that the infection of a quarter is usually associated with reduced production from that quarter ( Morris, 1973 ). Therefore the contribution of the infected quarter to the level of any of the parameters in the composite sample is decreased. The existence of yield compensation ( Woolford *et al*, 1983 ) will decrease the contribution of infected quarters to the composite milk sample still further.

##### (b) EFFECTS OF THE STAGE OF LACTATION

The mean electrical conductivity readings of cows within herds A and B during the lactation are given in graph 8.4iii. The effect of the stage of lactation on the electrical

conductivity of the composite milk samples was highly significant within herd A, (  $p < 0.01$  ), and significant within herd B (  $p < 0.05$  ) ( table 8.1 ).

Within herd A, a highly significant decrease in the mean electrical conductivity occurred between stages 3 to 4 of lactation. The electrical conductivity then increased gradually until stage 7, with a further decrease occurring during the final month (  $p < 0.01$  ).

Within herd B, no significant change in electrical conductivity occurred during the first four stages of the lactation. The electrical conductivity increased significantly between stages 4 to 6, but decreased during the following stage (  $p < 0.01$  ). A further increase in the electrical conductivity of the composite milk samples occurred during the final stage of the lactation within herd B (  $p < 0.01$  ).

Within both herds A and B, the changes in electrical conductivity in composite samples follow a similar pattern to the changes which occurred within individual quarter samples ( graphs 6.4ai and 6.4bi ). The decrease in the electrical conductivity of composite milk samples between stages 6 to 7 of lactation within herd B, may have been due to the increase in milk yield which occurred during this period. The increase in the milk yield was due to an increase in the availability of feed following a break in the drought.

Although little published data is available regarding lactational changes in conductivity in composite milk samples, the references cited regarding the respective concentrations of sodium and potassium ions show that changes in the ionic content of the milk do occur as the lactation proceeds. The changes which occur during the lactation with respect to the chloride concentration of the milk, resemble the changes in the concentration of sodium ( Daniel and Pavithran, 1985 ).

Since two of the three ions which are largely responsible for determining the electrical conductivity of the milk increase in concentration late in lactation, while the concentration of the third ion decreases in concentration, it is logical to expect that the electrical conductivity itself will increase during the course of the lactation. Data regarding lactational changes in the conductivity of quarter samples ( Sheldrake *et al* 1983b ) support this view.

### (c) EFFECT OF THE AGE OF THE COW

The mean electrical conductivity readings for cows within each age group in each of herds A and B are shown in chart 8.4ii.

In neither herd A nor herd B was the effect of the age of the cow on the electrical conductivity of the composite milk samples significant ( table 8.1 ).

Analysis of the data for individual quarters did show an association between electrical conductivity and the age of the cow, with higher electrical conductivity readings being recorded for milk from the quarters of the older cows than of their younger herdmates. As was the case for the other parameters, analysis of the data for quarters did not determine whether the increase in conductivity with age was due to the higher infection rate, or to physiological factors.

The pattern of change with age was similar between quarter and composite samples, although the scale of difference between the age groups was less for the composite samples. This together with the lower number of composite samples than of quarter samples may have contributed to a lack of statistically significant differences in electrical conductivity between the age groups in composite milk samples.

None of the interactions between the effects of the bacteriological status, the stage of lactation or the age of the cow on the electrical conductivity of composite milk samples reached significance at the 5 % level.

## (5) pH OF MILK

### (a) EFFECTS OF THE BACTERIOLOGICAL STATUS

The mean pH for cows within each bacteriological status in herds A and B is shown in chart 8.5i.

The pH readings of milk samples from herd C were not determined. In neither herd A nor herd B was the effect of bacteriological status on the pH of composite milk samples significant at the 5 % level.

The failure to reach significance is unlikely to be due merely to the small number of animals which were included in the analysis, since there was no obvious trend towards a higher pH within the infected cows than within their uninfected herdmates.

The effect of bacteriological status on the pH of individual quarter foremilk samples was significant only within herd B, in which status 3 quarters showed a significantly higher mean pH than did quarters within status 1 or status 2. The absolute differences in pH between status 3 quarters, and status 1 or status 2 quarters were small however, and dilution effects appear to have masked these differences in the composite samples.

### (b) EFFECTS OF THE STAGE OF LACTATION

The mean pH readings of the composite milk samples from herds A and B during the lactation are given in graph 8.5iii.

Within herd A, the effect of the stage of lactation on the pH of composite milk samples was not statistically significant, but significance was reached within herd B (  $p < 0.05$  ).

Despite the lack of a statistically significant effect of stage of lactation overall, the mean pH of composite milk samples increased between stages 3 to 4 of lactation within herd A (  $p < 0.01$  ).

Within herd B, the initial decrease in the mean pH was followed by an increase, although that between stages 2 to 3 was not statistically significant. Between stages 4 to 6, the mean pH decreased (  $p < 0.01$  ). During the final two stages of the lactation, the pH of the composite samples increased (  $p < 0.05$  ).

As with the other parameters examined, the changes which occurred during the lactation in the pH of composite samples show similar patterns to those observed in individual quarter foremilk samples ( chapter six ).

Other researchers have reported an increase in the pH of milk towards the end of lactation ( Kossila, 1967 ). An increase was seen in herd B in the present trial during the final two months of testing, although this was preceded by a decrease during mid-lactation.

As was discussed in chapter six, the increase in pH which occurs late in the lactation is likely to be associated with increased leakage between adjacent secretory cells, allowing ions to flow down their respective concentration gradients between the extracellular fluid and the milk, or vice versa. The increased leakage is associated with involution of areas of the mammary gland ( see chapter six ).

### (c) EFFECT OF THE AGE OF THE COW

The mean pH readings of cows within each age group in herds A and B are given in chart 8.5ii.

In neither herd A nor herd B was the effect of age of the cow on the pH of the composite milk samples significant at the 5 % level. Within herd A, analysis of the data for individual udder quarters revealed that milk from the two year old cows showed

significantly higher pH readings at certain stages of the lactation than did that from older cows. While the effect of the age of the cow on the pH of composite milk samples was not statistically significant, chart 8.5ii shows that higher pH readings were recorded in the milk from the two year old cows, than in the milk of older cows. The reason for the higher pH readings in the younger cows is not clear, since the infection rate increased with age, and infection caused a nonsignificant increase in the pH of the composite milk samples.

## (6) LACTOSE CONCENTRATION

### (a) EFFECTS OF THE BACTERIOLOGICAL STATUS

The mean lactose concentration for cows within each bacteriological status in each of the three herds is given in chart 8.6i. Within herds A and B, the effects of bacteriological status on the lactose concentration of composite samples were not statistically significant.

Within herd C, the effect of bacteriological status on the lactose concentration of composite milk samples was significant (  $p < 0.05$  ), the mean lactose concentration of cows within status 3 being lower than that of cows within status 1 (  $p < 0.05$  ). The difference between the lactose concentration of composite milk samples from cows of status 2 and cows of status 1 did not quite reach significance ( table 8.2 ).

A lower concentration of lactose in the milk of infected cows than of uninfected cows has been shown in previous studies ( Fernando *et al.*, 1985; Wiesner, 1985 ).

Several possible reasons for the decrease in the lactose concentration of infected quarter samples have been suggested ( chapter six ) and these explanations are applicable to the lactose concentration in the composite milk samples.

In only 1 of the 3 herds which were studied during the present trial, were the changes in lactose concentration at the level of the individual udder quarter, large enough to cause a significant decrease in the lactose concentration of composite milk samples.

### (b) EFFECTS OF THE STAGE OF LACTATION

The mean lactose concentration of the composite milk samples from each herd during the lactation is shown in graph 8.6iii. The effect of the stage of lactation on the lactose concentration of composite milk samples was highly significant within each of the three herds (  $p < 0.01$  or less ), ( table 8.1 ).

Within herd A, the mean lactose concentration of the composite milk samples did not change significantly between stages 2 to 3 of lactation, but decreased sharply between stages 3 to 6 (  $p < 0.01$  ), before increasing between stages 6 to 7 (  $p < 0.01$  ). No change in the mean lactose concentration occurred during the final stage of lactation within herd A.

Within herd B, the mean lactose concentration of the composite milk samples increased between stages 1 to 2 (  $p < 0.01$  ). The decrease in the lactose concentration between stages 2 to 3 did not reach significance, while the decreases between stages 3 to 4 and stages 4 to 6 were significant (  $p < 0.01$  ). No significant change to the mean lactose concentration of the composite milk samples occurred after stage 6 of lactation within herd B.

The changes in lactose concentration of the composite milk samples of herd C were generally smaller than were those of herds A or B. Significant decreases in lactose concentration occurred between stages 3 to 4 and stages 4 to 5 of lactation (  $p < 0.01$  ) although these decreases were followed by an increase in the lactose concentration between stages 5 to 6 (  $p < 0.05$  ).

The lactose concentration of composite milk samples has been shown to decrease during mid to late lactation ( White and Davies, 1958; Rook and Campling, 1965 ). The lactose concentration of milk is low following parturition, but reaches normal lactation values by day 7 of the lactation ( Daniel and Pavithran, 1985 ).

While samples were not taken during the first month of lactation, the results of the present study agree with those reports which are cited with respect to the decrease in the lactose concentration of the composite milk samples during mid to late lactation.

As with the other parameters which have been considered above, the pattern of change in lactose concentration in composite samples, during the course of the lactation followed the pattern seen in quarter samples ( graphs 6.6aii, 6.6bii and 6.6cii ).

### (c) EFFECTS OF THE AGE OF THE COW

The mean lactose concentration for cows in each age group within each herd is given in chart 8.6ii.

The effect of the age of the cow on the lactose concentration of composite milk samples was not significant within any of the three herds ( table 8.1 ).

While significance was not reached, there was some evidence of a decrease in lactose concentration with age, in that the oldest age group in each herd showed a lower lactose concentration than did any of the other age groups, this being particularly marked within herd C.

The difference between the mean lactose concentration of the composite samples from cows within the oldest age group, and those of cows from the younger age groups was not tested for statistical significance.

It is not clear whether the slight trend towards a lower lactose concentration in the oldest cows was due to a physiological increase, or to the increase in the infection rate in these older cows ( chapter five ).

The effect of the age of the cow on the lactose concentration of individual quarter foremilk samples was highly significant within each of the three herds ( chapter six ). The decrease in the lactose concentration of quarter foremilk samples with the increasing age of the cow may have been due to the higher infection rate of the older cows. However the dilution of milk from infected quarters by that from uninfected quarters, probably prevented the apparent decrease in lactose concentration with the increasing age of the cow from reaching significance in composite milk samples.

Other workers have shown the lactose concentration of composite milk to decrease with increasing lactation number ( Rook and Campling, 1965; Mijnen et al, 1982 ).

The lack of statistical significance with regard to the effect of the age of the cow on the lactose concentration of composite milk samples during the present trial may be due in part to the low number of cows which were included in the analysis.

None of the interactions between the effects of the bacteriological status, the stage of lactation, or the age of the cow on the lactose concentration of the composite milk samples reached significance at the 5 % level during the present trial.

## (7) NAGase ACTIVITY

### (a) EFFECTS OF THE BACTERIOLOGICAL STATUS

The mean NAGase activity for cows within each bacteriological status in the three herds is given in chart 8.7i.

The effect of bacteriological status on NAGase activity was highly significant (  $p < 0.01$  or less ) in herds B and C, but failed to reach significance at the 5 % level within herd A.

Within herd B, status 3 cows showed a significantly higher mean NAGase activity than did status 1 cows (  $p < 0.01$  ). The difference between the mean NAGase activities of status 2 and 3 cows just failed to reach significance at the 5 % level within herd B.

Within herd C, the status 3 cows showed a mean NAGase activity which was significantly higher than that of either status 1 or status 2 cows (  $p < 0.01$  ). In neither herd B nor herd C did the difference between the mean NAGase activity of the status 1 and status 2 cows reach significance at the 5 % level.

In 2 of the 3 herds which were studied, persistent infection with a major pathogen caused a significant increase in milk NAGase activity over that of cows which were never infected during the season.

A number of studies have shown that the NAGase activity of infected quarters is higher than that of uninfected quarters ( Mattila and Sandholm, 1985; Mattila *et al*, 1986b; Emanuelson *et al*, 1987; Nagahata *et al*, 1987; Miller and Paape, 1988 ) and this was confirmed in chapter six of this thesis. If any quarter foremilk NAGase activity is two fold greater than that of the quarter with the lowest NAGase activity, ie interquarter ratio is greater than 2.0, then the response carries through to the corresponding composite sample ( Kitchen *et al*, 1984a ).

The lower mean NAGase activity of infected quarters within herd A than within herds B and C may explain the failure of the presence of infection to cause a statistically significant increase in the NAGase activity of the composite milk samples within herd A.

### (b) EFFECTS OF THE STAGE OF LACTATION

The mean NAGase activity for cows from each of the three herds during the lactation is given in graph 8.7iii.

The effect of the stage of lactation on the NAGase activity of composite milk samples was significant in each of the three herds (  $p < 0.05$  or less ).

Within herd A, an initial increase in NAGase activity occurred between stages 2 to 3 of lactation (  $p < 0.01$  ), followed by significant decreases in the mean NAGase activity during the next two stages (  $p < 0.01$  ). The NAGase activity increased significantly between stages 5 to 6 (  $p < 0.01$  ), but neither the increase between stages 6 to 7 nor the decrease during the final month of testing reached significance at the 5 % level.

Within herd B, the mean NAGase activity of the composite samples decreased significantly during the first four stages of lactation (  $p < 0.01$  or less ). The NAGase activity increased significantly between stages 4 to 6 (  $p < 0.01$  ) but decreased between stages 6 to 7 (  $p < 0.05$  ), while the increase in NAGase activity during the final stage did not reach significance at the 5 % level.

Within herd C, a small decrease in the NAGase activity occurred between stages 2 to 3 of lactation (  $p < 0.05$  ), but there was no further significant change in the NAGase activity until the increase between stages 6 to 7 of lactation (  $p < 0.05$  ).

The reason for the large decrease in the mean NAGase activity of composite milk samples during the first half of lactation within herd B is not clear. It was not possible to analyse each bacteriological status individually during the lactation, due to low numbers of cows, but the results of the analysis of individual udder quarters ( chapter six ) suggests that this decrease in NAGase activity would be seen in uninfected cows.

A carry over from the high NAGase activity during the colostral period may explain the decrease in NAGase activity within herd B, since some cows were closer to parturition at the time of the first sampling than were others. The number of cows for which composite data was available was smaller for herd B than for the other two herds, and the mean may have been affected by a small number of extreme values. In general, lactation patterns for the composite samples resemble those for the quarter foremilk data, in each herd.

### (c) EFFECTS OF THE AGE OF THE COW

The mean NAGase activity for cows within each age group, in each of the three herds, is given in chart 8.7ii.

The effect of the age of the cow on the mean NAGase activity of composite milk samples was significant within herd A (  $p < 0.05$  ), but did not reach significance in herds B or C.

Within herd A, the difference between the mean NAGase activities of the age groups is likely to be due to the high value which was obtained for the two year cows in this herd.

There were significant differences between NAGase activities at the quarter level within herd A ( chapter six ), with the two year old cows showing a significantly higher NAGase activity than either the 3 or the 4 year old cows. This same difference between the two year old cows and the older animals was not observed within herd B, while no two year old cows from herd C were included in the present trial.

It is not clear whether the high NAGase activity in the two year old cows was due to physiological factors, or to the severity of infection in terms of tissue damage in these younger cows. The level of infection in the two year old cows within herd A was comparatively low ( see chapter five ), and does not explain the high NAGase activity of the composite milk samples.

While the effect of age on the NAGase activity of composite milk samples did not reach statistical significance, there was a trend towards increasing NAGase activity as the age of the cow increased, within herds B and C. The interactions between the effects of the bacteriological status, the stage of lactation and the age of the cow on the NAGase activity of composite milk samples did not reach significance at the 5 % level within any of the three herds.

## (8) ANTITRYPSIN CONCENTRATION

### (a) EFFECTS OF THE BACTERIOLOGICAL STATUS

The mean antitrypsin concentration of the composite milk from cows in each bacteriological status is given in chart 8.8i.

The effect of the bacteriological status on the antitrypsin concentration of composite milk samples reached significance at the 5 % level in herd C only ( table 8.1 ).

Within herd C, milk from the status 3 cows showed a significantly higher, ie less negative, mean antitrypsin concentration than did that from status 1 or status 2 (  $p < 0.01$  or less ).

While the effect of bacteriological status on the antitrypsin concentration within herd B did not reach significance, there was a trend towards a higher antitrypsin concentration in milk from cows within status 3 than within that from status 1 or status 2 cows. The failure to reach significance may be attributed to the smaller number of animals which were included in the analysis within herd B than within herds A or C.

The differences between the mean antitrypsin concentrations of each bacteriological status within herd A were comparatively small. The analysis of quarter foremilk data ( chapter six ), showed the effect of bacteriological status on antitrypsin concentration to be highly significant in all three herds.

It is likely that the degree of elevation of the antitrypsin concentration in the infected quarters was insufficient to overcome the dilution effects of milk from uninfected quarters in two of the three herds.

#### (b) EFFECTS OF THE STAGE OF LACTATION

The mean antitrypsin concentration for each herd during the lactation is given in graph 8.8iii.

The effect of the stage of the lactation on the antitrypsin concentration of the composite milk samples was significant within herds B and C (  $p < 0.05$  or less ), but just failed to reach significance within herd A.

Within herd A, there was no significant change in the antitrypsin concentration before stage 6 of the lactation. The antitrypsin concentration increased significantly between stages 6 to 7 (  $p < 0.05$  ), but decreased during the final month of lactation (  $p < 0.05$  ).

Within herd B, a decrease in the mean antitrypsin concentration of the composite samples occurred between stages 1 to 2 of lactation (  $p < 0.01$  ). Thereafter, the antitrypsin concentration fluctuated, without changing significantly until the increase which occurred during the final stage of the lactation (  $p < 0.05$  ).

Within herd C there was no significant monthly change in the mean antitrypsin concentration of the composite samples during the first three stages of lactation. Between stages 3 to 4 the antitrypsin concentration decreased (  $p < 0.05$  ). A sharp

increase in the antitrypsin concentration occurred between stages 4 to 5 (  $p < 0.01$  ) with no further change occurring during the final two months of lactation.

The changes in antitrypsin concentration which occurred during the lactation in composite samples, generally mirrored those of the individual quarter foremilk samples ( chapter six ).

Other researchers have investigated the concentration of antitrypsin in composite milk. The antitrypsin concentration is high following parturition, decreases to a low point at 2 to 3 months of lactation, and then increases gradually until the end of the lactation period ( Sandholm *et al* 1984 ).

During the present trial, the antitrypsin concentration decreased during the first 2 to 3 months of lactation, ie stages 1 to 2, in 2 of the 3 herds, although the decrease was significant within herd B only.

Composite samples were not obtained for herd A during this period. From mid to late-lactation, an increase in antitrypsin concentration occurred in all three herds, although the timing varied between herds.

#### (c) EFFECTS OF THE AGE OF THE COW

The mean antitrypsin concentration for cows within each age group, in each herd, is given in chart 8.8ii. The effect of the age of the cow on the antitrypsin concentration in composite milk samples did not reach significance at the 5 % level in any of the herds ( table 8.1 ). Although significance was not reached, some trends were seen, and these were similar to those trends which were observed with quarter foremilk samples.

The differences between age groups with respect to the antitrypsin concentration were small within herd A. Within herd B, the antitrypsin concentration decreased slightly as the age of the cow increased, although the opposite was true within herd C. The reason for the apparent decrease in the milk antitrypsin concentration with increasing age within herd B is unclear, but since statistical significance was not reached, the differences between age groups may have been due to sampling variation.

### SUMMARY AND CONCLUSIONS

- (1) The bacteriological status of the cow exerted a significant effect on the somatic cell count and on the sodium concentration of composite milk samples. The effect of the bacteriological status on the NAGase activity, the lactose concentration and the antitrypsin concentration of the composite milk sample was statistically significant only in herds with a high prevalence of mammary infection. The effect of the bacteriological status of the cow on the potassium concentration, the pH or the electrical conductivity of the composite milk samples was not significant.

For a significant change in the level of a parameter to occur in the composite milk sample, the change due to infection, of the level of that parameter in the individual quarter foremilk samples must be great enough to overcome the dilution effects of the milk from the uninfected quarters within the udder. The results of the present chapter suggest that of the eight parameters which have been included in the trial, the somatic cell count and the sodium concentration are likely have the greatest ability to distinguish between infected and uninfected cows.

- (2) The stage of lactation at which the sample is taken can affect the concentration of each parameter in the composite milk sample. In general, the pattern of change in the concentration of a parameter in the composite milk samples during the lactation, reflected the changes which occurred in the individual quarters. The effects of the stage of lactation on the concentration of each parameter in the composite milk sample were not analysed separately for cows within each bacteriological status during the present trial. However the results of the analyses of individual quarter foremilk samples suggest that the changes occurred regardless of the infection status of the cow.

If the mean concentration of a parameter changes during the lactation for both infected and for uninfected cows, then the change has implications for the establishment of a threshold value for the discrimination between infected and uninfected cows. The effects of the stage of lactation and those of the season are confounded with respect to seasonal supply dairy farms in New Zealand. Nevertheless, the pattern of change in the concentration of each parameter during the lactation showed similarities to the changes which have been reported in studies which have been carried out in other dairying countries.

It is likely that a correction for the changing yield of milk during the season would go some way towards reducing the stage of lactation effects. No such correction was made during the present trial.

- (3) The effect of age on the concentration of a parameter in the composite milk sample was generally not statistically significant. The only exception to this rule was the effect of age on the NAGase activity of the composite milk samples within herd A.

Although statistical significance was generally not reached with respect to the effect of age on the concentration of each parameter during the present trial, in most cases a trend towards either an increase or a decrease in the concentration of a parameter with age was noted, the trend reflecting that observed within individual quarter foremilk samples ( chapter six ). The lack of statistical significance during the present trial may have been due to the comparatively small number of cows which were sampled. It is not clear whether the small changes in the concentration of each parameter which occurred as the age of the cow increases were due to physiological ageing effects, or to the increase in the infection rate with age.

For practical reasons, the number of cows which could be sampled during the present trial was small, and this limited the types of analyses which could be carried out on the data set. The work of Natzke *et al* ( 1972 ) suggests that with respect to the somatic cell count at least, the increase with age may be attributed largely to the effects of either current or past udder infection.

**TABLE 8.1**  
**EFFECTS OF BACTERIOLOGICAL STATUS, STAGE OF LACTATION,**  
**AND AGE OF THE COW ON LEVELS OF THE SELECTED PARAMETERS**  
**IN COMPOSITE MILK SAMPLES**

| PARAMETER    | HERD | STATUS          | AGE  | STAGE | STATUS<br>BY AGE | STATUS<br>BY STAGE | AGE BY<br>STAGE |
|--------------|------|-----------------|------|-------|------------------|--------------------|-----------------|
| $Pr > F^1$   |      |                 |      |       |                  |                    |                 |
| S.C.C.       | A    | 0.01            | 0.71 | 0.02  | 0.32             | 0.10               | 0.27            |
|              | B    | 0.01            | 0.26 | 0.01  | 0.11             | 0.08               | 0.01            |
|              | C    | 0.01            | 0.24 | 0.07  | 0.98             | 0.83               | 0.79            |
| SODIUM       | A    | 0.05            | 0.08 | 0.02  | 0.16             | 0.70               | 0.98            |
|              | B    | 0.01            | 0.52 | 0.01  | 0.52             | 0.26               | 0.39            |
|              | C    | 0.01            | 0.40 | 0.01  | 0.76             | 0.30               | 0.85            |
| POTASSIUM    | A    | 0.90            | 0.44 | 0.01  | 0.70             | 0.14               | 0.08            |
|              | B    | 0.91            | 0.93 | 0.02  | 0.57             | 0.70               | 0.89            |
|              | C    | 0.65            | 0.38 | 0.07  | 0.86             | 0.69               | 0.54            |
| CONDUCTIVITY | A    | 0.32            | 0.91 | 0.01  | 0.59             | 0.48               | 0.77            |
|              | B    | 0.88            | 0.84 | 0.05  | 0.08             | 0.39               | 0.36            |
|              | C    | ND <sup>2</sup> | ND   | ND    | ND               | ND                 | ND              |
| pH           | A    | 0.53            | 0.65 | 0.14  | 0.83             | 0.35               | 0.64            |
|              | B    | 0.44            | 0.24 | 0.02  | 0.14             | 0.21               | 0.02            |
|              | C    | ND              | ND   | ND    | ND               | ND                 | ND              |
| LACTOSE      | A    | 0.71            | 0.98 | 0.01  | 0.20             | 0.90               | 0.99            |
|              | B    | 0.22            | 0.76 | 0.01  | 0.70             | 0.45               | 0.60            |
|              | C    | 0.05            | 0.06 | 0.01  | 0.61             | 0.62               | 0.31            |
| NAGase       | A    | 0.29            | 0.05 | 0.01  | 0.40             | 0.57               | 0.49            |
|              | B    | 0.01            | 0.16 | 0.05  | 0.09             | 0.26               | 0.52            |
|              | C    | 0.01            | 0.29 | 0.04  | 0.91             | 0.90               | 0.61            |
| ANTITRYPSIN  | A    | 0.66            | 0.40 | 0.07  | 0.98             | 0.73               | 0.88            |
|              | B    | 0.11            | 0.07 | 0.01  | 0.91             | 0.08               | 0.02            |
|              | C    | 0.02            | 0.88 | 0.04  | 0.61             | 0.42               | 0.53            |

1  $Pr > F$  indicates the probability that sampling variation alone was responsible for the differences between means.

2 ND = No data

TABLE 8.2RESULTS OF CONTRAST ANALYSIS BETWEEN EACH BACTERIOLOGICALSTATUS FOR COMPOSITE MILK SAMPLES

| PARAMETER           | HERD | STATUS<br>1 vs 2 | STATUS<br>1 vs 3 | STATUS<br>2 vs 3 |
|---------------------|------|------------------|------------------|------------------|
| Pr > F <sup>1</sup> |      |                  |                  |                  |
| S.C.C.              | A    | 0.90             | 0.01             | 0.01             |
|                     | B    | 0.18             | 0.01             | 0.01             |
|                     | C    | 0.02             | 0.01             | 0.01             |
| SODIUM              | A    | 0.40             | 0.03             | 0.52             |
|                     | B    | 0.04             | 0.01             | 0.13             |
|                     | C    | 0.11             | 0.01             | 0.02             |
| LACTOSE             | C    | 0.51             | 0.02             | 0.06             |
| NAGase              | B    | 0.08             | 0.01             | 0.06             |
|                     | C    | 0.14             | 0.01             | 0.01             |
| ANTITRYPSIN         | C    | 0.85             | 0.01             | 0.01             |

1 Pr > F indicates the probability that sampling variation alone was responsible for the difference between the means.

TABLE 8.3aRESULTS OF CONTRAST ANALYSIS BETWEEN STAGES OF LACTATIONFOR COMPOSITE MILK SAMPLES - HERD A

| PARAMETER    | STAGE OF LACTATION  |        |        |        |        |        |
|--------------|---------------------|--------|--------|--------|--------|--------|
|              | 2 vs 3              | 3 vs 4 | 4 vs 5 | 5 vs 6 | 6 vs 7 | 7 vs 8 |
|              | Pr > F <sup>1</sup> |        |        |        |        |        |
| S.C.C.       | 0.32                | 0.52   | 0.60   | 0.08   | 0.75   | 0.88   |
| SODIUM       | 0.21                | 0.56   | 0.02   | 0.01   | 0.01   | 0.06   |
| POTASSIUM    | 0.19                | 0.01   | 0.01   | 0.03   | 0.18   | 0.01   |
| CONDUCTIVITY | 0.75                | 0.01   | 0.08   | 0.06   | 0.11   | 0.01   |
| pH           | 0.01                | 0.10   | 0.01   | 0.01   | 0.01   | 0.01   |
| LACTOSE      | 0.51                | 0.01   | 0.01   | 0.01   | 0.01   | 0.73   |
| NAGase       | 0.01                | 0.01   | 0.01   | 0.01   | 0.07   | 0.07   |
| ANTITRYPSIN  | 0.10                | 0.18   | 0.21   | 0.90   | 0.03   | 0.03   |

1 Pr > F indicates the probability that sampling variation alone was responsible for the difference between means.

TABLE 8.3b

RESULTS OF CONTRAST ANALYSIS BETWEEN STAGES OF LACTATION

FOR COMPOSITE MILK SAMPLES - HERD B

| PARAMETER    | STAGE OF LACTATION  |        |        |        |        |        |
|--------------|---------------------|--------|--------|--------|--------|--------|
|              | 1 vs 2              | 2 vs 3 | 3 vs 4 | 4 vs 6 | 6 vs 7 | 7 vs 8 |
|              | Pr > F <sup>1</sup> |        |        |        |        |        |
| S.C.C.       | 0.14                | 0.17   | 0.93   | 0.01   | 0.01   | 0.98   |
| SODIUM       | 0.54                | 0.08   | 0.98   | 0.01   | 0.44   | 0.01   |
| POTASSIUM    | 0.01                | 0.08   | 0.19   | 0.01   | 0.87   | 0.01   |
| CONDUCTIVITY | 0.13                | 0.75   | 0.92   | 0.01   | 0.01   | 0.01   |
| pH           | 0.01                | 0.06   | 0.01   | 0.01   | 0.03   | 0.02   |
| LACTOSE      | 0.01                | 0.08   | 0.01   | 0.01   | 0.27   | 0.75   |
| NAGase       | 0.01                | 0.01   | 0.01   | 0.01   | 0.03   | 0.15   |
| ANTITRYPSIN  | 0.01                | 0.26   | 0.62   | 0.50   | 0.96   | 0.02   |

1 Pr > F indicates the probability that sampling variation alone was responsible for the difference between means.

TABLE 8.3cRESULTS OF CONTRAST ANALYSIS BETWEEN STAGES OF LACTATIONFOR COMPOSITE MILK SAMPLES - HERD C

| PARAMETER    | STAGE OF LACTATION  |        |        |        |        |        |
|--------------|---------------------|--------|--------|--------|--------|--------|
|              | 1 vs 2              | 2 vs 3 | 3 vs 4 | 4 vs 5 | 5 vs 6 | 6 vs 7 |
|              | Pr > F <sup>1</sup> |        |        |        |        |        |
| S.C.C.       | 0.22                | 0.05   | 0.01   | 0.09   | 0.72   | 0.42   |
| SODIUM       | 0.15                | 0.59   | 0.01   | 0.01   | 0.02   | 0.01   |
| POTASSIUM    | 0.01                | 0.58   | 0.22   | 0.01   | 0.64   | 0.01   |
| CONDUCTIVITY | ND <sup>2</sup>     | ND     | ND     | ND     | ND     | ND     |
| pH           | ND                  | ND     | ND     | ND     | ND     | ND     |
| LACTOSE      | 0.27                | 0.09   | 0.01   | 0.01   | 0.02   | 0.64   |
| NAGase       | 0.99                | 0.02   | 0.23   | 0.24   | 0.85   | 0.05   |
| ANTITRYPSIN  | 0.11                | 0.21   | 0.02   | 0.01   | 0.96   | 0.62   |

1 Pr > F indicates the probability that sampling variation alone was responsible for the difference between means.

2 ND = No data

TABLE 8.4

NUMBER OF INFECTED QUARTERS PER INFECTED COW

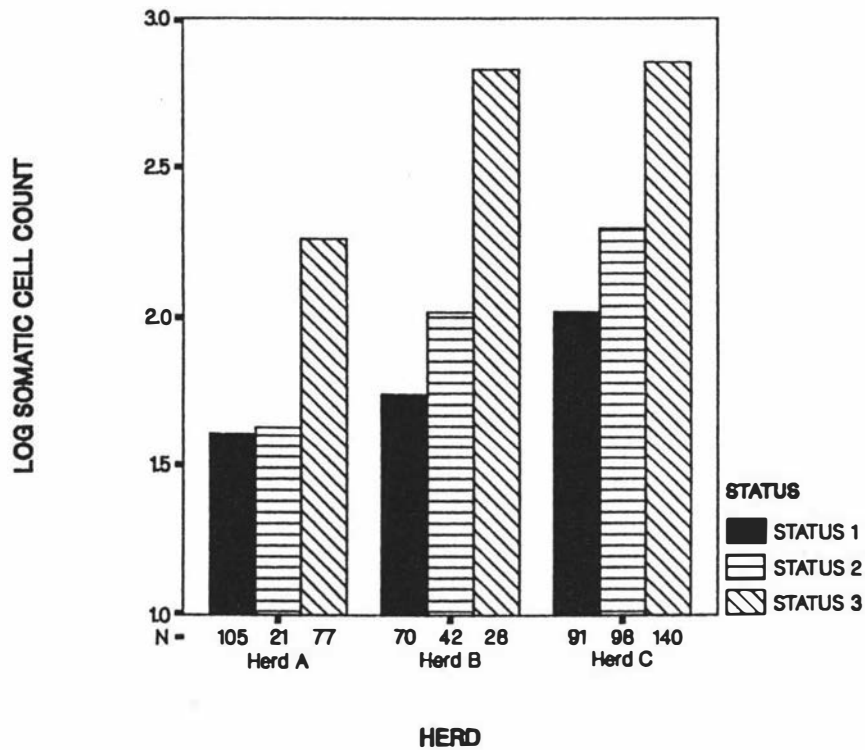
MAJOR PATHOGENS ONLY

| HERD    | 1 QUARTER | 2 QUARTERS | 3 QUARTERS | 4 QUARTERS |
|---------|-----------|------------|------------|------------|
| A       | 85.2 %    | 11.1 %     | 3.7 %      | 0.0 %      |
| B       | 57.9 %    | 15.8 %     | 5.3 %      | 21.0 %     |
| C       | 49.4 %    | 32.1 %     | 16.1 %     | 2.4 %      |
| OVERALL | 57.7 %    | 25.5 %     | 12.0 %     | 4.5 %      |

# SOMATIC CELL COUNT - HERDS A, B AND C

**CHART 8.1i**  
**LOGSCC BY STATUS WITHIN HERDS**

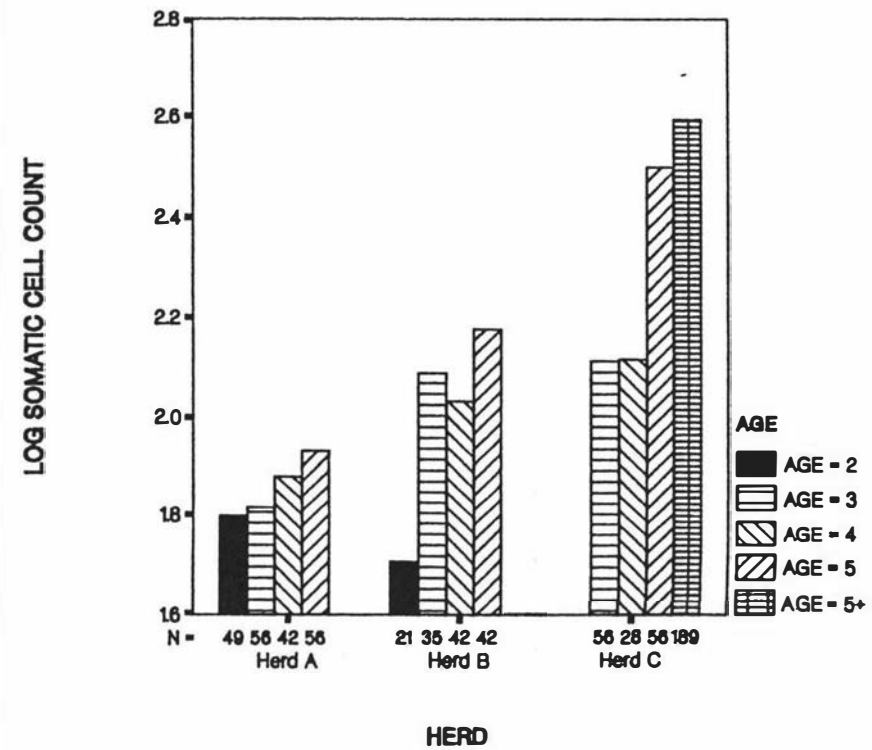
( DATA FOR COMPOSITE SAMPLES )



LOGSCC = LOG (10) SOMATIC CELL COUNT X 1000  
N = NUMBER OF OBSERVATIONS CONTRIBUTING TO THE MEAN

**CHART 8.1ii**  
**LOGSCC BY AGE WITHIN HERDS**

( DATA FOR COMPOSITE SAMPLES )

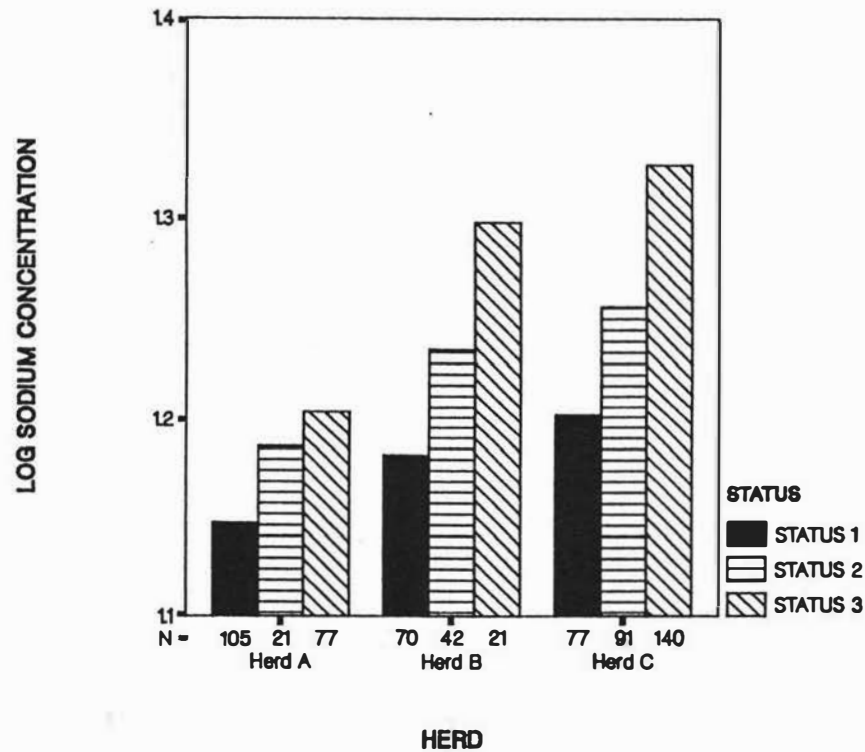


LOGSCC = LOG (10) SOMATIC CELL COUNT X 1000  
N = NUMBER OF OBSERVATIONS CONTRIBUTING TO THE MEAN

# SODIUM CONCENTRATION - HERDS A, B AND C

**CHART 8.2i**  
**LOGSOD BY STATUS WITHIN HERDS**

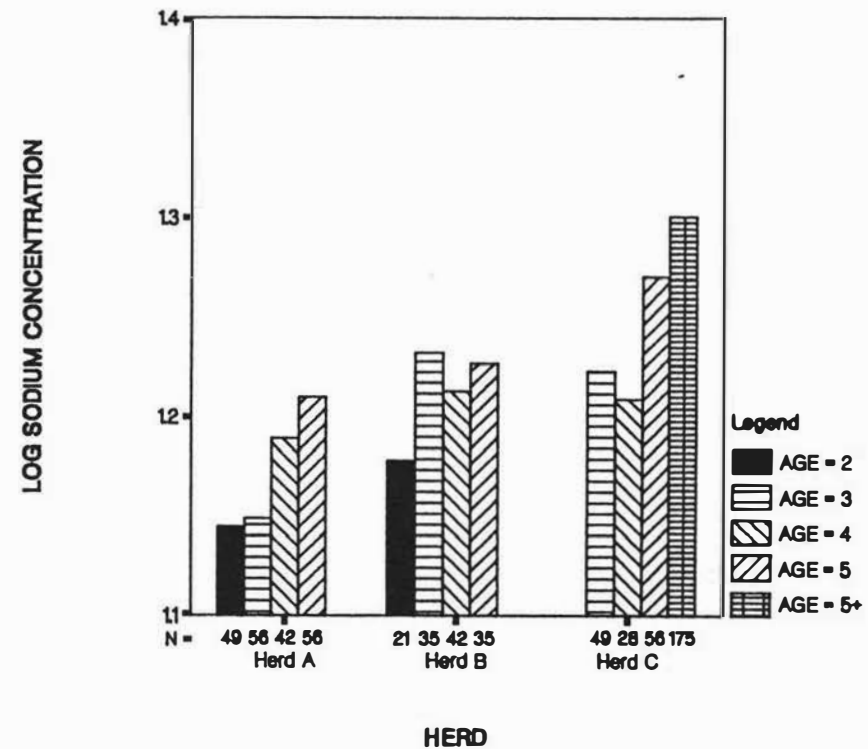
( DATA FOR COMPOSITE SAMPLES )



LOGSOD = LOG (10) SODIUM CONCENTRATION IN MILLIMOLES PER LITRE  
N = NUMBER OF OBSERVATIONS CONTRIBUTING TO THE MEAN

**CHART 8.2ii**  
**LOGSOD BY AGE WITHIN HERDS**

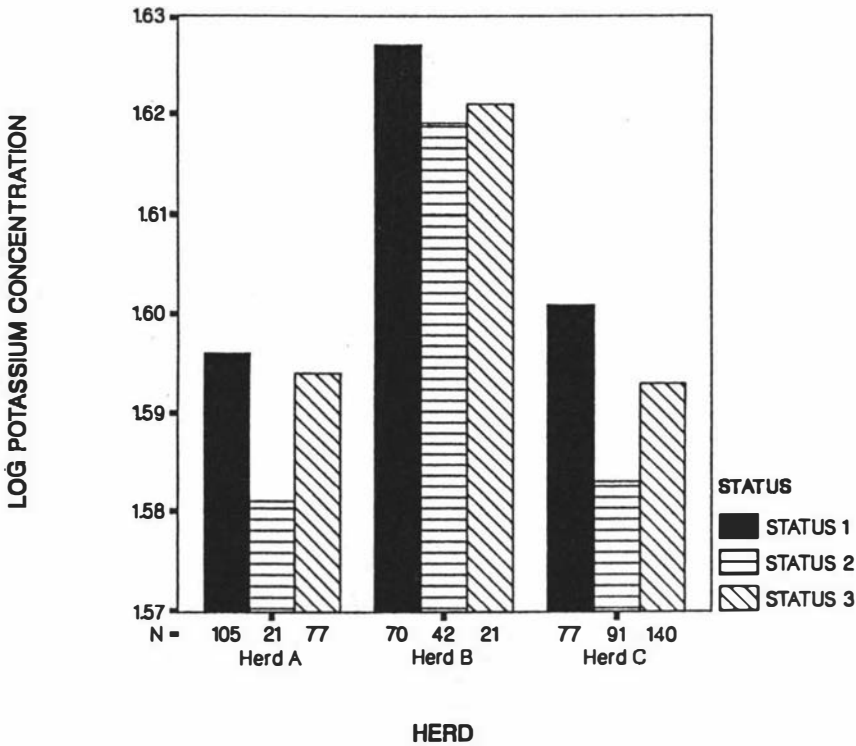
( DATA FOR COMPOSITE SAMPLES )



LOGSOD = LOG (10) SODIUM CONCENTRATION IN MILLIMOLES PER LITRE  
N = NUMBER OF OBSERVATIONS CONTRIBUTING TO THE MEAN

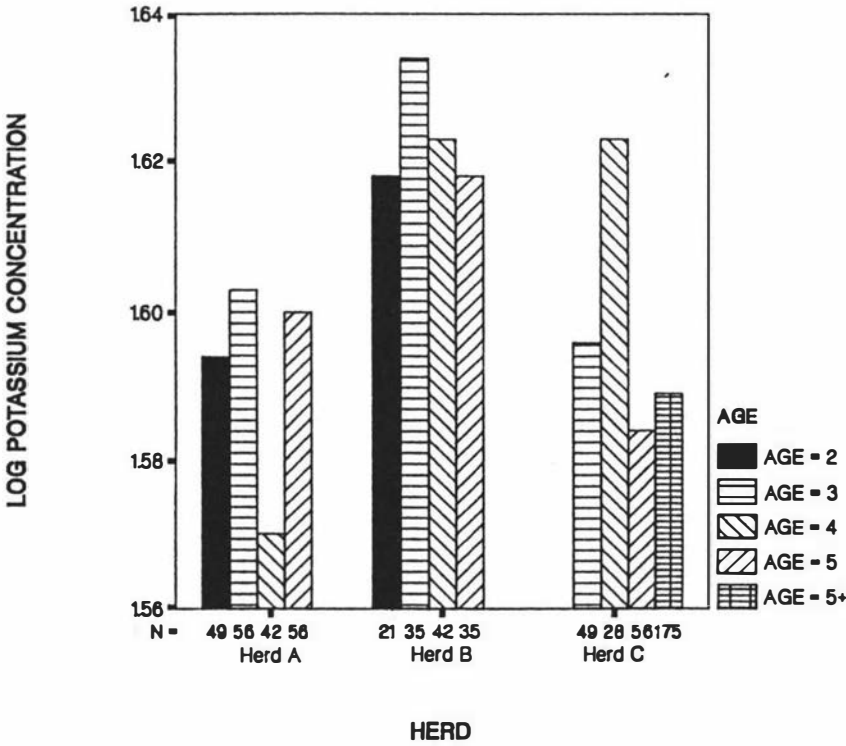
# POTASSIUM CONCENTRATION - HERDS A, B AND C

**CHART 8.3i**  
**LOGPOT BY STATUS WITHIN HERDS**  
( DATA FOR COMPOSITE SAMPLES )



LOGPOT = LOG (10) POTASSIUM CONCENTRATION IN MILLIMOLES PER LITRE  
N = NUMBER OF OBSERVATIONS CONTRIBUTING TO THE MEAN

**CHART 8.3ii**  
**LOGPOT BY AGE WITHIN HERDS**  
( DATA FOR COMPOSITE SAMPLES )

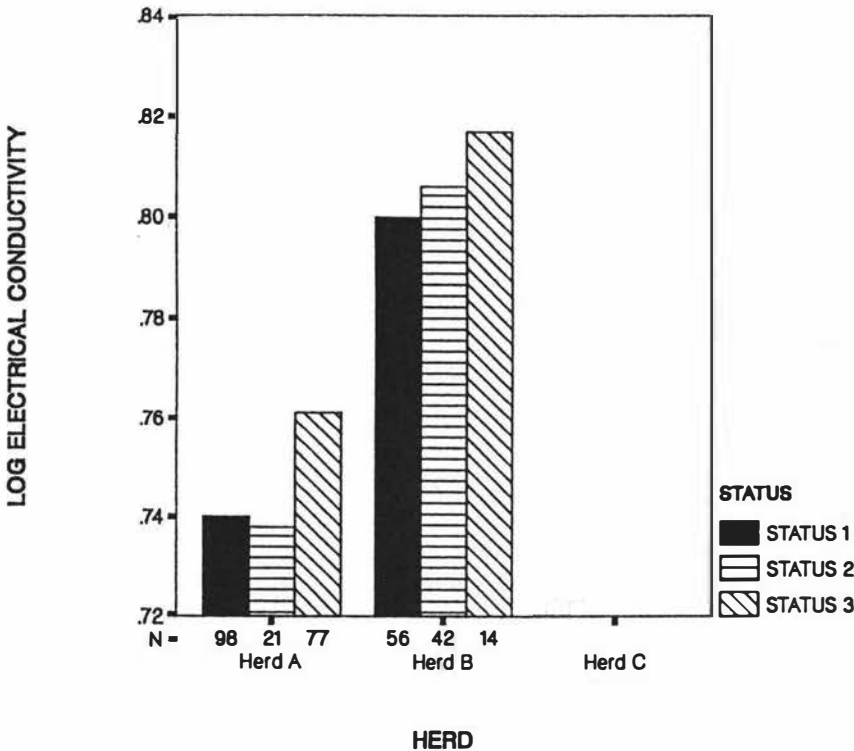


LOGPOT = LOG (10) POTASSIUM CONCENTRATION IN MILLIMOLES PER LITRE  
N = NUMBER OF OBSERVATIONS CONTRIBUTING TO THE MEAN

# ELECTRICAL CONDUCTIVITY - HERDS A, B AND C

**CHART 8.4i**  
**LOGCOND BY STATUS WITHIN HERDS**

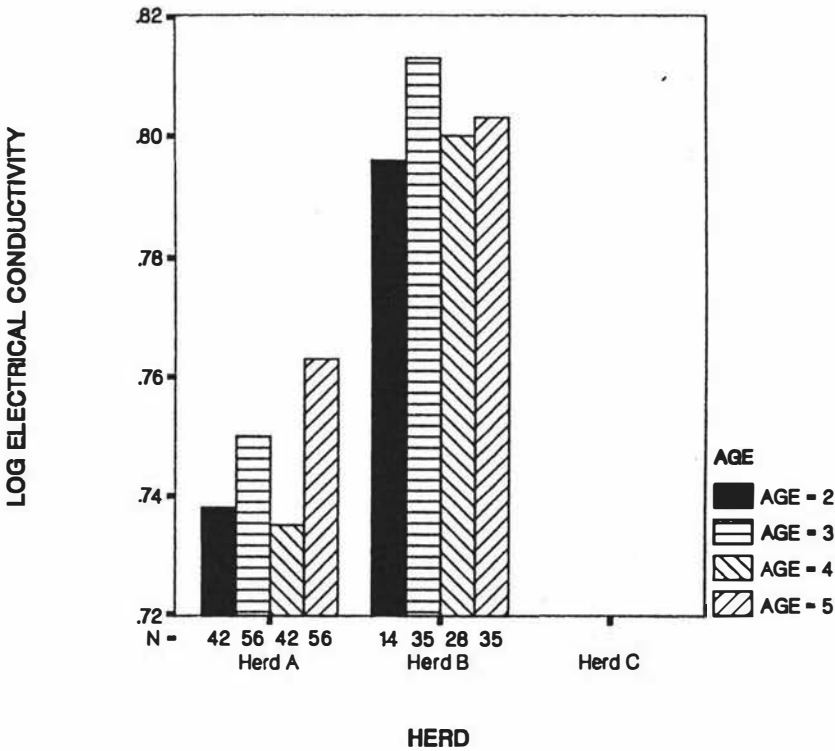
( DATA FOR COMPOSITE SAMPLES )



LOGCOND = LOG (10) ELECTRICAL CONDUCTIVITY IN MILLISIEMENS  
N = NUMBER OF OBSERVATIONS CONTRIBUTING TO THE MEAN

**CHART 8.4ii**  
**LOGCOND BY AGE WITHIN HERDS**

( DATA FOR COMPOSITE SAMPLES )

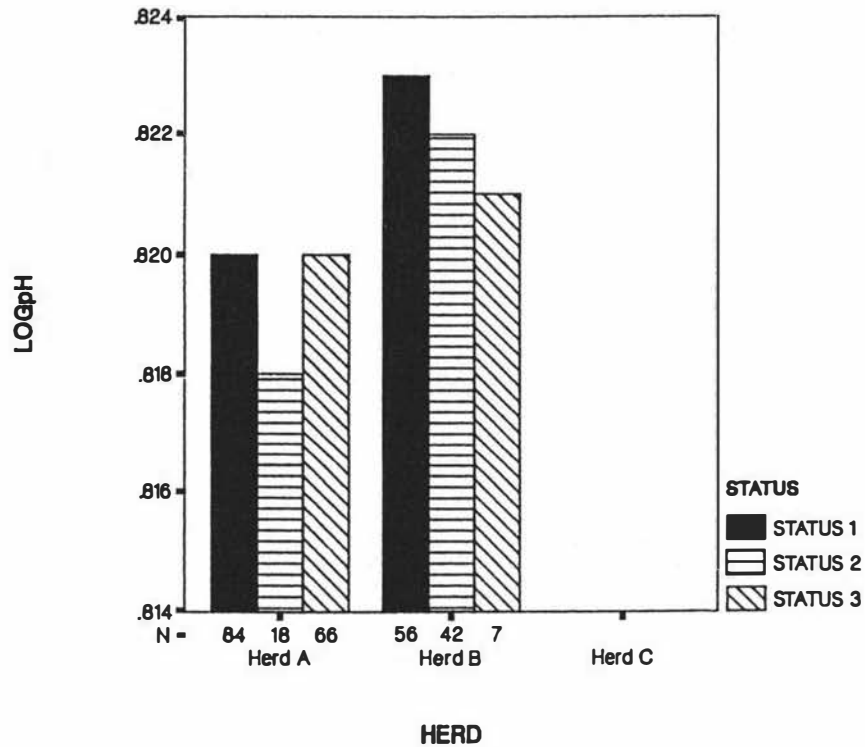


LOGCOND = LOG (10) ELECTRICAL CONDUCTIVITY IN MILLISIEMENS  
N = NUMBER OF OBSERVATIONS CONTRIBUTING TO THE MEAN

# pH OF MILK - HERDS A, B AND C

## CHART 8.5i LOGpH BY STATUS WITHIN HERDS

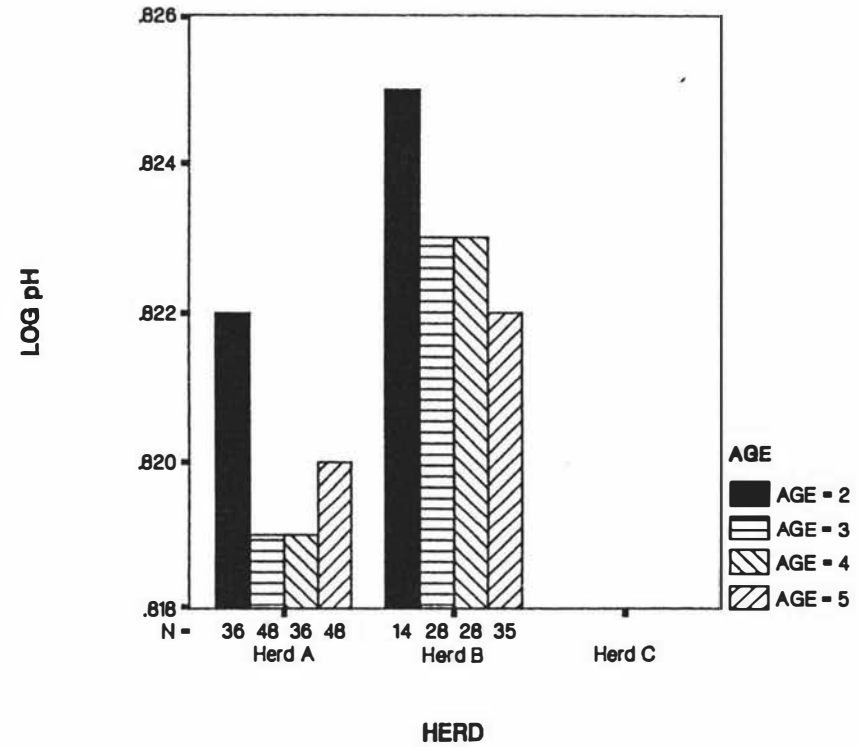
( DATA FOR COMPOSITE SAMPLES )



LOGpH = LOG (10) pH  
N = NUMBER OF OBSERVATIONS CONTRIBUTING TO THE MEAN

## CHART 8.5ii LOGpH BY AGE WITHIN HERDS

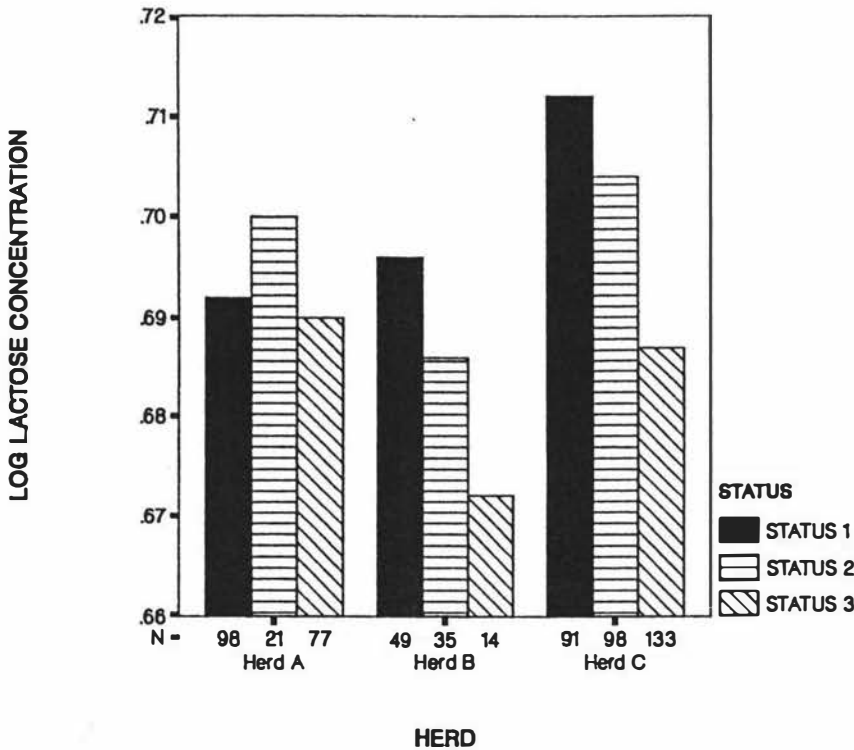
( DATA FOR COMPOSITE SAMPLES )



LOGpH = LOG (10) pH  
N = NUMBER OF OBSERVATIONS CONTRIBUTING TO THE MEAN

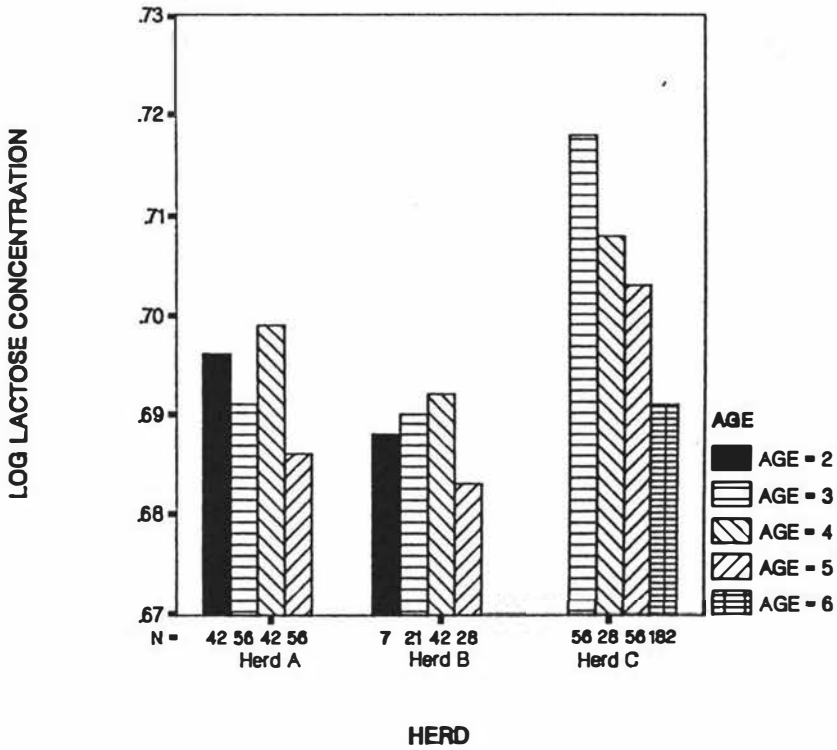
# LACTOSE CONCENTRATION - HERDS A, B AND C

**CHART 8.6i**  
**LOGLACT BY STATUS WITHIN HERDS**  
( DATA FOR COMPOSITE SAMPLES )



LOGLACT = LOG (10) LACTOSE CONCENTRATION ( % )  
N = NUMBER OF OBSERVATIONS CONTRIBUTING TO THE MEAN

**CHART 8.6ii**  
**LOGLACT BY AGE WITHIN HERDS**  
( DATA FOR COMPOSITE SAMPLES )

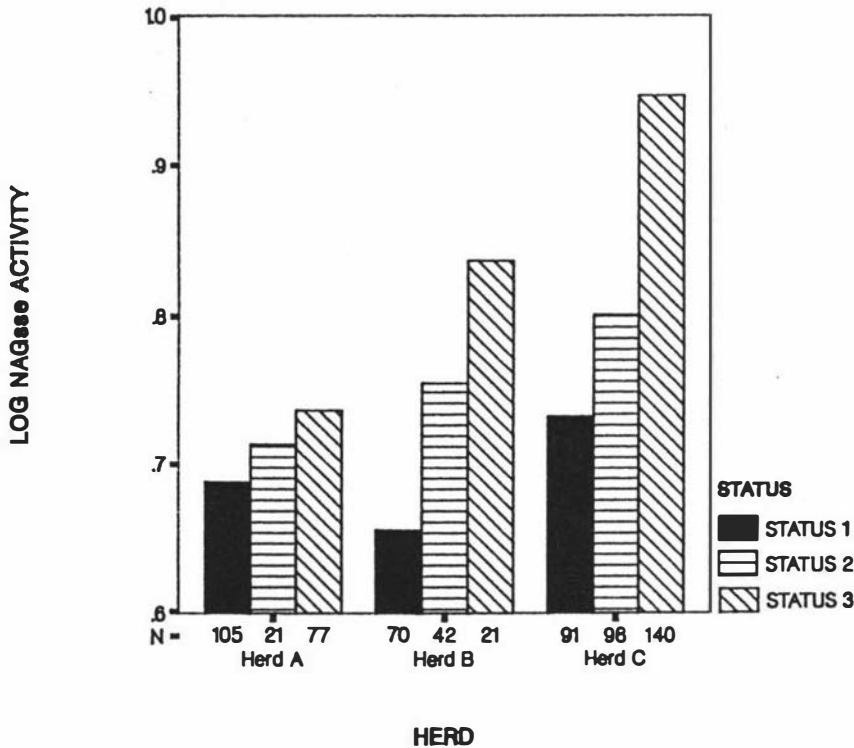


LOGLACT = LOG (10) LACTOSE CONCENTRATION ( % )  
N = NUMBER OF OBSERVATIONS CONTRIBUTING TO THE MEAN

# NAGase ACTIVITY - HERDS A, B AND C

**CHART 8.7i**  
**LOGNAG BY STATUS WITHIN HERDS**

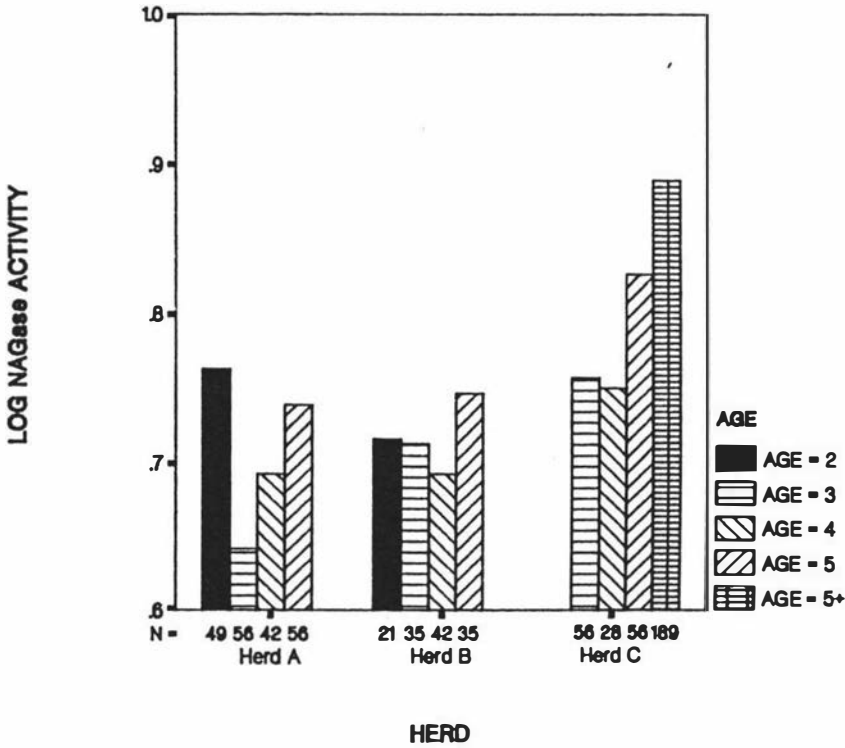
( DATA FOR COMPOSITE SAMPLES )



LOGNAG = LOG (10) NAGase ACTIVITY ( NANOMOL/ML/MIN )  
N = NUMBER OF OBSERVATIONS CONTRIBUTING TO THE MEAN

**CHART 8.7ii**  
**LOGNAG BY AGE WITHIN HERDS**

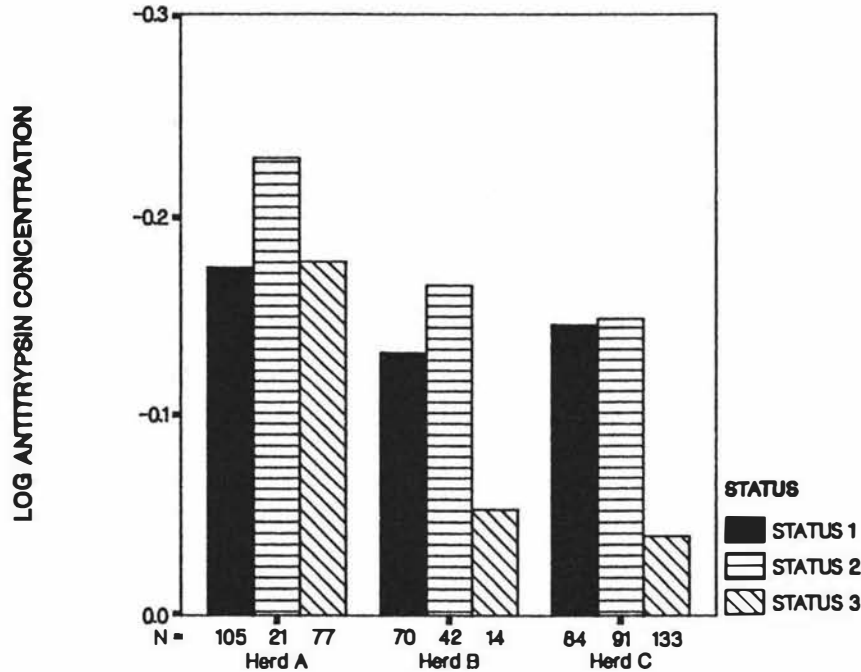
( DATA FOR COMPOSITE SAMPLES )



LOGNAG = LOG (10) NAGase ACTIVITY ( NANOMOL/ML/MIN )  
N = NUMBER OF OBSERVATIONS CONTRIBUTING TO THE MEAN

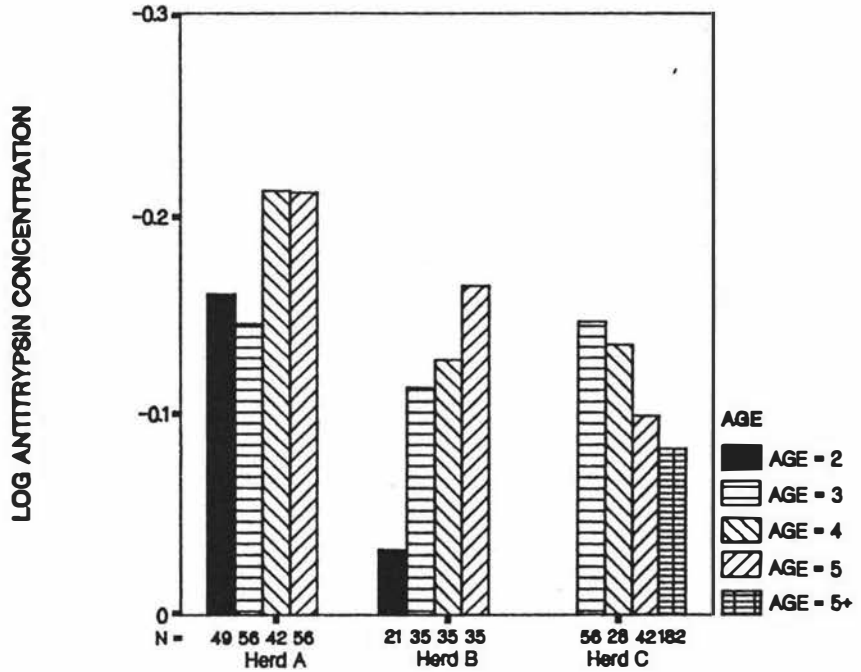
# ANTITRYPSIN CONCENTRATION - HERDS A, B AND C

**CHART 8.8i**  
**LOGANT BY STATUS WITHIN HERDS**  
( DATA FOR COMPOSITE SAMPLES )



LOGANT = LOG (10) ANTITRYPSIN CONCENTRATION ( RELATIVE UNITS )  
N = NUMBER OF OBSERVATIONS CONTRIBUTING TO THE MEAN

**CHART 8.8ii**  
**LOGANT BY AGE WITHIN HERDS**  
( DATA FOR COMPOSITE SAMPLES )

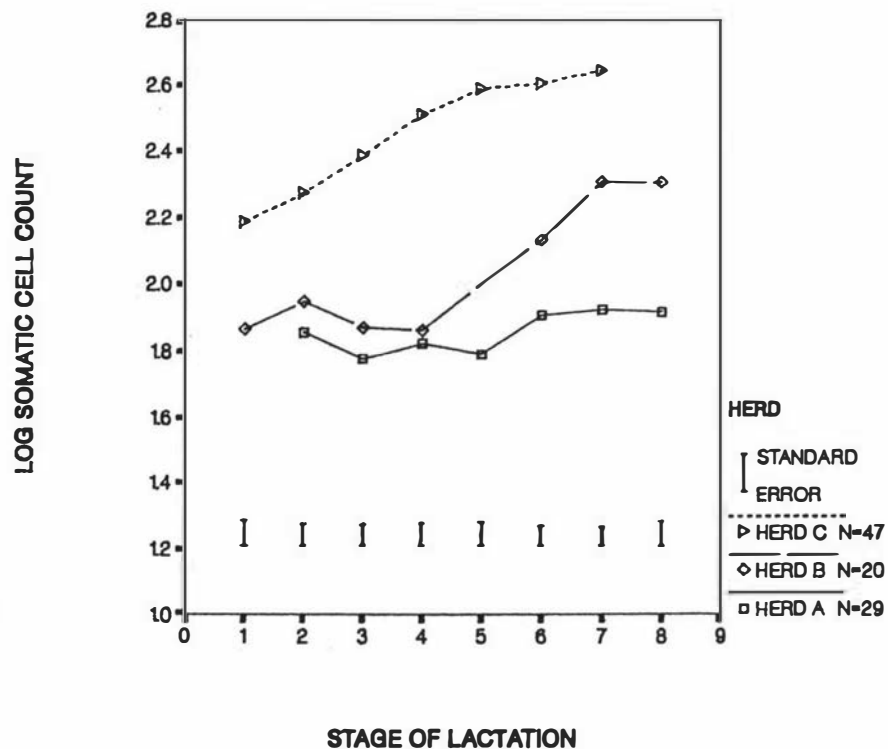


LOGANT = LOG (10) ANTITRYPSIN CONCENTRATION ( RELATIVE UNITS )  
N = NUMBER OF OBSERVATIONS CONTRIBUTING TO THE MEAN

# SOMATIC CELL COUNT & SODIUM CONCENTRATION

**GRAPH 8.1iii**  
**LOGSCC VS STAGE OF LACTATION**

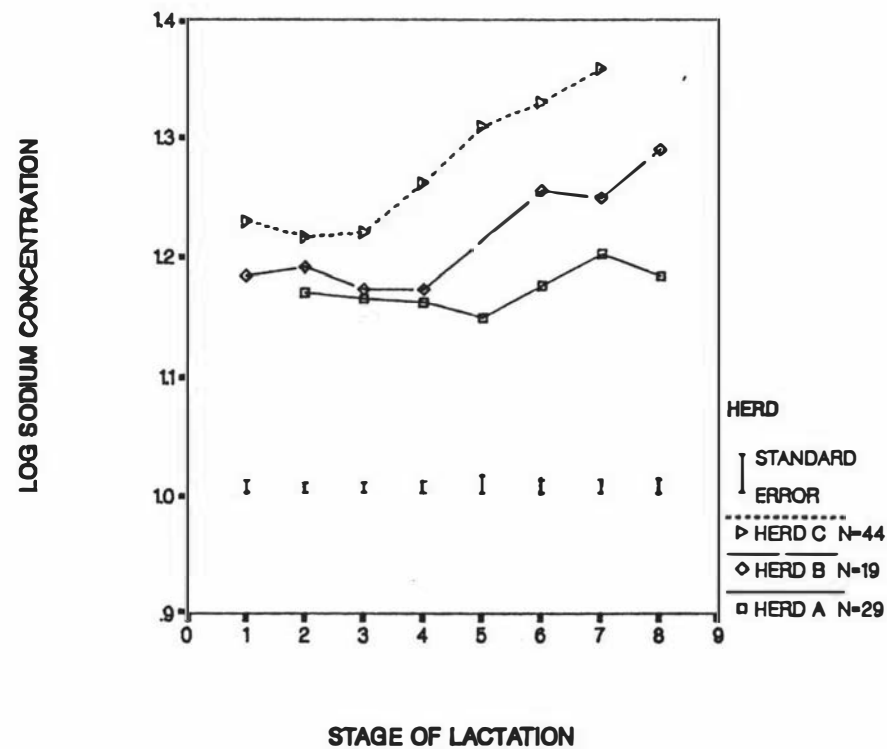
BY HERD ( DATA FOR COMPOSITE SAMPLES )



LOGSCC = LOG (10) SOMATIC CELL COUNT ( x 1000 )  
STANDARD ERROR = POOLED MONTHLY STANDARD ERROR

**GRAPH 8.2iii**  
**LOGSOD VS STAGE OF LACTATION**

BY HERD ( DATA FOR COMPOSITE SAMPLES )

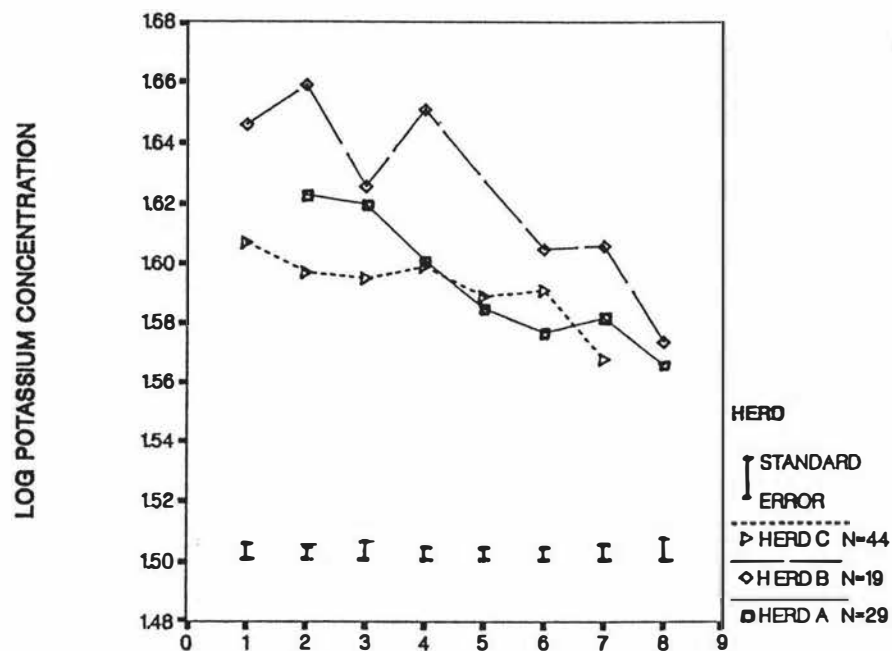


LOGSOD = LOG (10) SODIUM CONCENTRATION IN MILLIMOLES PER LITRE  
STANDARD ERROR = POOLED MONTHLY STANDARD ERROR

# POTASSIUM CONCENTRATION & ELECTRICAL CONDUCTIVITY

**GRAPH 8.3iii**  
**LOGPOT VS STAGE OF LACTATION**

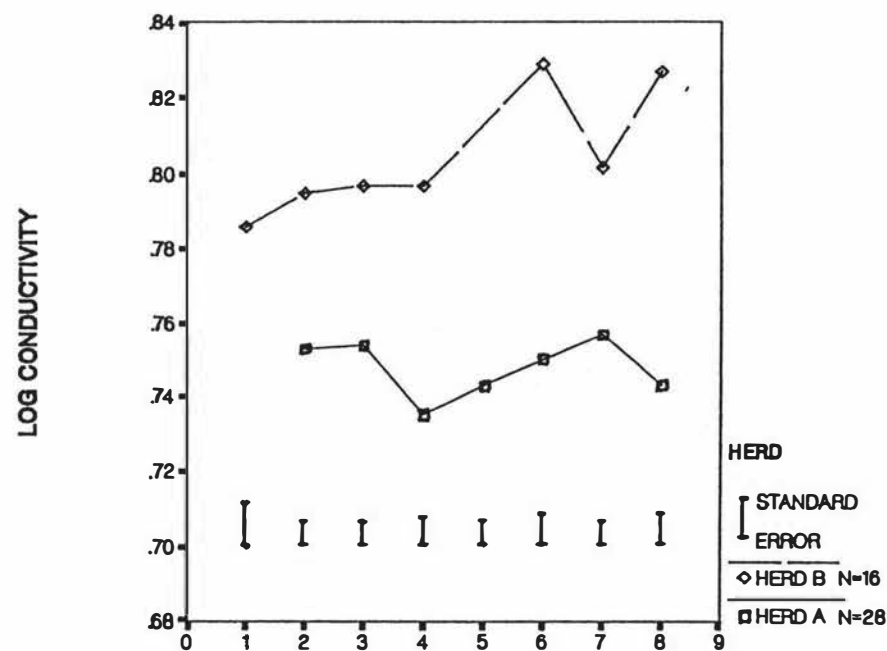
BY HERD ( DATA FOR COMPOSITE SAMPLES )



LOGPOT = LOG (10) POTASSIUM CONCENTRATION IN MILLIMOLES PER LITRE  
STANDARD ERROR = POOLED MONTHLY STANDARD ERROR

**GRAPH 8.4iii**  
**LOGCOND VS STAGE OF LACTATION**

BY HERD ( DATA FOR COMPOSITE SAMPLES )

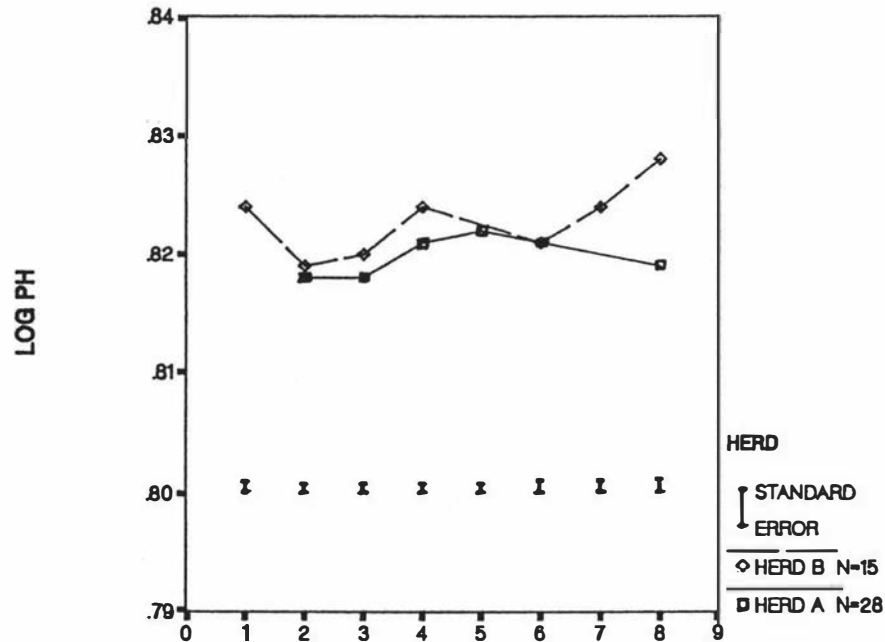


LOGCOND = LOG (10) ELECTRICAL CONDUCTIVITY IN MILLISEMENS  
STANDARD ERROR = POOLED MONTHLY STANDARD ERROR

# pH OF MILK & LACTOSE CONCENTRATION

**GRAPH 8.5iii**  
**LOGPH VS STAGE OF LACTATION**

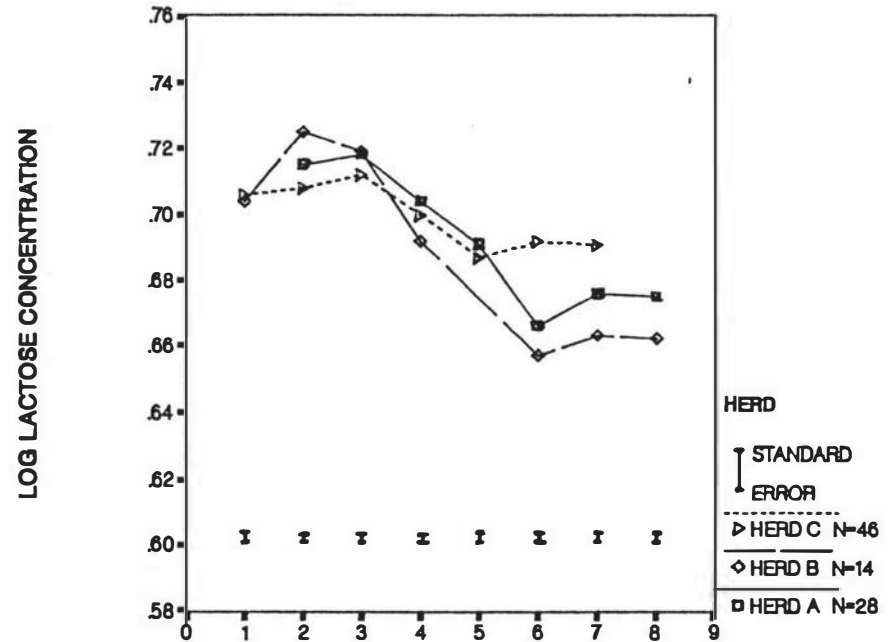
BY HERD ( DATA FOR COMPOSITE SAMPLES )



LOGPH = LOG (10) PH  
STANDARD ERROR = POOLED MONTHLY STANDARD ERROR

**GRAPH 8.6iii**  
**LOGLACT VS STAGE OF LACTATION**

BY HERD ( DATA FOR COMPOSITE SAMPLES )

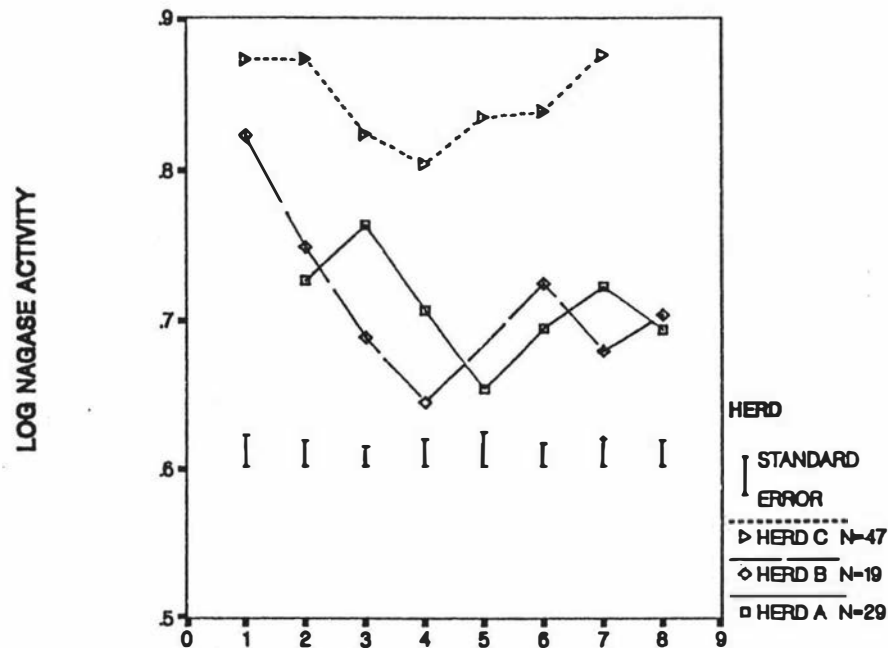


LOGLACT = LOG (10) LACTOSE CONCENTRATION ( % )  
STANDARD ERROR = POOLED MONTHLY STANDARD ERROR

# NAGase ACTIVITY & ANTITRYPSIN CONCENTRATION

**GRAPH 8.7iii**  
**LOGNAG VS STAGE OF LACTATION**

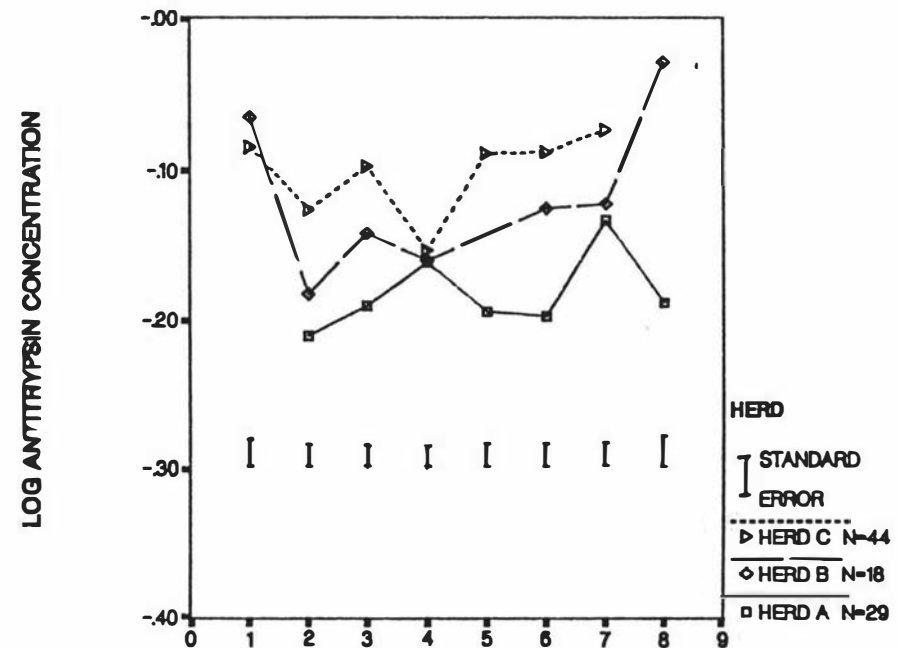
BY HERD ( DATA FOR COMPOSITE SAMPLES )



LOGNAG = LOG (10) NAGASE ACTIVITY ( NANOMOL / ML / MIN )  
STANDARD ERROR = POOLED MONTHLY STANDARD ERROR

**GRAPH 8.8iii**  
**LOGANT VS STAGE OF LACTATION**

BY HERD ( DATA FOR COMPOSITE SAMPLES )



LOGANT = LOG ANTITRYPSIN CONCENTRATION ( RELATIVE UNITS )  
STANDARD ERROR = POOLED MONTHLY STANDARD ERROR

## CHAPTER NINE

### THE DIAGNOSIS OF INFECTED UDDERS USING COMPOSITE MILK SAMPLES

#### INTRODUCTION

At present in New Zealand, the Livestock Improvement Corporation offers the dairy farmer the option of individual cow somatic cell counts which may be carried out on one or more occasions during the dairy season. These figures, when interpreted appropriately, provide information about the mastitis status of each cow during the lactation, and this information can contribute to the decision making process about the selection of cows for dry cow therapy, or for culling from the herd.

The interpretation of individual cow somatic cell counts is often difficult, due in part to a lack of systematic information about the relationship between the somatic cell count and the infection status within New Zealand herds.

The present chapter evaluates the performance of the somatic cell count as well as that of each of the other seven selected parameters, ie the concentration of sodium, potassium, lactose and antitrypsin, the pH, the electrical conductivity and the NAGase activity, in the discrimination between infected and uninfected cows.

Infection was measured on samples from individual quarters, and this was related to the parameters measured in the composite milk samples.

The use of a combination of the somatic cell count with each of the alternative parameters is also studied to determine whether the accuracy with which the cows are identified as being infected or uninfected is improved, when compared with use of the somatic cell count alone.

Threshold values are determined for each parameter, both for the overall data set, and within individual herds.

## MATERIALS AND METHODS

Composite milk samples were taken at monthly intervals from the selected cows within each of the three herds. A description of the herds, and of the sampling procedure is given in chapter three of this thesis.

### (1) DEFINITION OF INFECTION

The cows were classified according to two different systems, on the basis of the isolation of pathogenic bacteria from the individual quarter foremilk samples during the lactation:

#### (i) BACTERIOLOGICAL CLASSIFICATION 1

A cow was deemed to be infected if one or more quarter foremilk samples which were taken at the same milking as the respective composite sample, yielded a major pathogen. Cows which yielded only a minor pathogen, or those from which no mammary pathogens were isolated from any quarter, were considered to be uninfected.

#### (ii) BACTERIOLOGICAL CLASSIFICATION 2

A cow was deemed to be infected if one or more quarter foremilk samples yielded either a major or a minor pathogen. Only those cows which yielded no recognized mammary pathogen, from any of the four udder quarters, was considered to be uninfected.

In both classification schemes, the individual quarters were considered to be infected with a pathogen if that organism was isolated on the test day only. No attempt was made to confirm the infection status of the quarter by repeated sampling ( see chapter seven for discussion regarding this point ).

The assay methods which were used for each of the eight parameters have been described ( chapter three ).

## (2) PREDICTION OF THE INFECTION STATUS OF THE UDDER

Two methods were used to predict the presence or absence of infection in the udder:

### (i) ESTIMATION OF THE PROBABILITY OF MISCLASSIFICATION AND THE CRITICAL THRESHOLD VALUES FOR EACH PARAMETER

The critical threshold of each parameter, both for the total data set, and within individual herds, and the probability of misclassification of the infection status of the cows were estimated using a program written in BASIC. A brief description of the program has been given ( chapter seven ), and the program is listed in the appendix.

Terms such as the " critical threshold ", and the " probability of misclassification " have been defined previously ( chapter seven ).

### (ii) DISCRIMINANT FUNCTION ANALYSIS

The ability of each parameter to distinguish between infected and uninfected cows was also determined using discriminant function analysis ( Statistical Analytical Systems, 1985 ). This procedure computes a linear or quadratic discriminant function for classifying observations into two or more groups on the basis of one or more numeric variables, by determination of the generalised squared distance between groups. This procedure allows for the application of the discriminant function to a second data set.

The discriminative power of the somatic cell count in combination with each of the alternative parameters was determined using discriminant function analysis.

All discriminant analyses were carried out on log (10) transformed data, as the untransformed data were markedly skewed.

## (3) THE STAGE OF LACTATION

For the purposes of the analysis, the lactation was divided into four stages ( see chapter seven for details ).

## RESULTS

### (1) THRESHOLD VALUES AND PROBABILITIES OF MISCLASSIFICATION FOR ALL HERDS, THROUGHOUT THE LACTATION PERIOD

Table 9.1i gives the threshold values and the probabilities of misclassification for each parameter, within the total data set, throughout the trial period.

#### (i) BACTERIOLOGICAL CLASSIFICATION 1

At the critical threshold of 185,000 cells per ml of milk, the somatic cell count misclassified 24.9 % of samples when bacteriological classification 1 ( BC1 ) was used.

The remaining parameters showed a higher probability of misclassification, ie a lower ability to correctly classify the cows as being infected or uninfected. The NAGase activity misclassified 32 % of samples, while 33 % of composite samples were misclassified when the sodium concentration of the milk was used to distinguish between infected and uninfected cows. The potassium concentration, the electrical conductivity, the pH, the lactose concentration and the antitrypsin concentration of the milk each misclassified more than 40 % of samples, the parameter with the highest probability of misclassification being the pH of the milk.

The percentage of cows which were infected, was lower for the electrical conductivity and for the pH of the milk than for the remaining parameters, since the pH and conductivity were not measured on samples from cows within herd C, and the prevalence of infection with major pathogens was higher within herd C, than within herds A or B.

#### (ii) BACTERIOLOGICAL CLASSIFICATION 2

When bacteriological classification 2 ( BC2 ) was used, the parameter with the lowest probability of misclassification was once again the somatic cell count. At a critical threshold of 80,000 cells per ml of milk, the somatic cell count misclassified just under 23 % of samples, a figure similar to that achieved using BC1.

The sodium concentration of the milk misclassified 29 % of samples, a slight improvement in diagnostic accuracy over the result when BC1 was used. However, the NAGase activity misclassified 40 % of samples, this figure being higher than that obtained when BC1 was used.

The parameter with the highest probability of misclassification under BC2 was the potassium concentration of the milk, which along with the pH, electrical conductivity, lactose concentration and the antitrypsin concentration, misclassified more than 40 % of composite milk samples.

The critical threshold for the somatic cell count was lower under BC2, than under BC1. Similarly, the respective threshold values for the sodium concentration, the electrical conductivity, the NAGase activity and the antitrypsin concentration were lower under BC2 than under BC1, while the opposite was true with regard to the lactose concentration. The critical threshold values for the potassium concentration and for the pH of the milk differed little between the bacteriological classification schemes.

## (2) THRESHOLD VALUES AND PROBABILITIES OF MISCLASSIFICATION WITHIN INDIVIDUAL HERDS, THROUGHOUT THE TRIAL PERIOD

### (i) BACTERIOLOGICAL CLASSIFICATION 1

Table 9.1ii gives the threshold values and the probabilities of misclassification for each parameter, within individual herds, throughout the lactation.

Within each herd, the parameter with the lowest probability of misclassification was the somatic cell count.

The probability of misclassification for the somatic cell count was similar in each of the three herds, ranging from approximately 20 % within herd A, to 24 % of samples within herd C.

The critical threshold value for the somatic cell count varied markedly between herds, ranging from 73,000 cells per ml within herd A, to 344,000 cells per ml within herd C.

The critical thresholds for the sodium concentration and for the NAGase activity were also highest within herd C, and lowest within herd A. The critical threshold for the antitrypsin concentration was similar within herds B and C, but was lower within herd A, while that for the lactose concentration was similar within herds A and B, but slightly higher within herd C. The critical threshold values for the pH and the electrical conductivity were slightly higher within herd B, than within herd A.

### (ii) BACTERIOLOGICAL CLASSIFICATION 2

The somatic cell count was the parameter with the lowest probability of misclassification in each of the three herds when BC2 was applied to the data. The

probabilities of misclassification for the somatic cell count within the three herds were similar, ranging from 21 % within herd A, to 25 % of samples within herd C.

Slightly higher probabilities of misclassification were recorded for the sodium concentration and for the electrical conductivity of the milk, although neither parameter showed large differences in the probability of misclassification between herds.

The abilities of the remaining parameters to distinguish between infected and uninfected cows were lower, with the probabilities of misclassification exceeding 30 % of samples.

The variation between herds with respect to the probability of misclassification was larger for certain parameters eg the lactose concentration and the antitrypsin concentration, than for the somatic cell count or the sodium concentration of the milk.

The probability of misclassification was lowest within herd A for some parameters, whilst for other parameters, lower figures were recorded within herds B or C.

### (3) THRESHOLD VALUES AND PROBABILITIES OF MISCLASSIFICATION FOR ALL HERDS, AT EACH STAGE OF THE LACTATION

Table 9.1iii gives the threshold values and the probabilities of misclassification for each parameter, within the total data set, at each of the four stages of lactation.

The somatic cell count showed a lower probability of misclassification than did any of the other parameters, at each stage of lactation. The probability of misclassification for the somatic cell count was similar at each stage of lactation, ranging from 21 % of samples at stage 2, to 27 % of samples at stage 3.

Stage 2 of the lactation was the period during which the sodium concentration, the potassium concentration, the NAGase activity, and the antitrypsin concentration showed the lowest probabilities of misclassification although the differences between the stages of the lactation with respect to the NAGase activity and the potassium concentration were relatively small.

The lowest probability of misclassification for the electrical conductivity and for the pH of the milk occurred during the first stage of lactation.

The critical threshold for the somatic cell count increased markedly during the first three stages of lactation, from 105,000 cells per ml, to 255,000 cells per ml of milk.

The other parameters showed smaller changes in critical threshold values during the course of the lactation, than did the somatic cell count. For those parameters which tend to increase in concentration as a result of infection, there was generally an increase in the critical threshold as the lactation progressed, while the opposite was true of the lactose concentration and the potassium concentration of the milk.

#### (4) DISCRIMINANT FUNCTION ANALYSIS IN THE TOTAL DATA SET THROUGHOUT THE LACTATION, USING A SINGLE PARAMETER

Table 9.2i gives the results of the discriminant function analysis for each parameter, in the total data set, throughout the lactation period, using bacteriological classification 1. The statistical significance of the differences between the results of the various parameters were not measured since each parameter was tested separately.

##### (i) PREDICTIVE ABILITY FOR ALL COWS

The parameter with the greatest predictive ability, ie the parameter which could correctly classify the greatest percentage of samples as coming from either an infected or an uninfected cow, was the somatic cell count of the milk. The somatic cell count, when used alone, correctly classified 76 % of composite milk samples.

The next most accurate tests were the NAGase activity and the sodium concentration of the milk, which correctly classified 73 % and 69 % of the composite milk samples respectively. The predictive abilities of the remaining parameters were lower, the parameter with the lowest predictive ability being the pH of the milk.

##### (ii) PREDICTIVE ABILITY FOR INFECTED COWS

The parameters differed markedly with respect to their abilities to correctly classify samples which had been taken from infected cows. The somatic cell count correctly classified 72 % of such samples, while the other parameters correctly classified between 42 % and 56 % of samples from infected cows, the lower figure being that recorded for the antitrypsin concentration of the milk.

### (iii) PREDICTIVE ABILITY FOR UNINFECTED COWS

The predictive abilities of the parameters, with respect to samples from uninfected cows, were generally higher than were those for samples from infected cows, the only exception being that of the pH of the milk.

The parameter with the greatest predictive ability for uninfected cows was the NAGase activity, which correctly classified nearly 85 % of such samples. The somatic cell count and the sodium concentration each correctly classified over 75 % of samples from uninfected cows, while the antitrypsin concentration, the electrical conductivity, the pH, the potassium concentration and the lactose concentration each classified less than 75 % of such samples.

## (5) DISCRIMINANT FUNCTION ANALYSIS WITHIN INDIVIDUAL HERDS THROUGHOUT THE LACTATION, USING A SINGLE PARAMETER

Table 9.2ii gives the results of the discriminant function analysis for each parameter, in each of the three herds, throughout the lactation period, using bacteriological classification 1. The statistical significance of the differences in predictive abilities of each parameter between the three herds were not measured.

### (i) PREDICTIVE ABILITY FOR ALL COWS

Within each herd, the somatic cell count showed a higher predictive ability than did any other parameter, when the data from all the cows, ie those which were infected and those which were uninfected, were combined.

The predictive ability of the somatic cell count exceeded 75 % of samples within each of the three herds, ranging from 77 % of samples within herd C, to 85 % of samples within herd B.

The predictive abilities of the sodium concentration and of the NAGase activity, were greatest within herd B, whereas the predictive abilities of the potassium concentration, the lactose concentration, and the antitrypsin concentration were greatest within herd C, although in the case of some parameters, the differences between the herds with respect to the predictive ability were small.

### (ii) PREDICTIVE ABILITY FOR INFECTED COWS

The somatic cell count correctly classified over 70 % of samples taken from infected cows, within each of the three herds.

The somatic cell count showed the greatest predictive ability of all the parameters within herds A and C, but was slightly inferior to the potassium concentration and to the electrical conductivity of the milk, with respect to the samples from infected cows within herd B.

While little variation occurred between herds with respect to the ability of the somatic cell count to correctly classify samples from infected cows, the abilities of each of the remaining parameters varied widely between herds. The greatest between-herd variation was recorded for the potassium concentration, which correctly classified 31.7 % of samples taken from infected cows within herd C, but 78.4 % of such samples within herd B, a difference between herds of nearly 47 percentage points.

Although certain of the parameters showed relatively high predictive abilities with respect to the samples which had been taken from infected cows within one or other of the three herds, only the somatic cell count showed a consistently high predictive ability in this respect.

### (iii) PREDICTIVE ABILITY FOR UNINFECTED COWS

The accuracy with which the samples from uninfected cows were classified varied between the herds, although this variation was greater for some parameters than for others.

The somatic cell count correctly classified over 78 % of samples which had been taken from uninfected cows, in each of the three herds, the greatest predictive ability occurring within herd B, in which 89 % of such samples were correctly classified as having come from an uninfected cow.

The somatic cell count showed a higher predictive ability for samples from uninfected cows than did any other parameter within herds A and B. Within herd C, the somatic cell count showed a slightly lower predictive ability with respect to samples from uninfected cows, than did the NAGase activity, although the difference between the two parameters was only 3 percentage points.

(6) DISCRIMINANT FUNCTION ANALYSIS IN THE TOTAL DATA SET,  
THROUGHOUT THE LACTATION, FOR THE SOMATIC CELL COUNT  
PLUS ONE OTHER PARAMETER

Table 9.2iii gives the result of the discriminant function analysis for the total data set, throughout the lactation, when each of the alternative parameters was combined, in turn, with the somatic cell count. Bacteriological classification 1 was used in each analysis. The statistical significance of the differences between the predictive abilities of the various parameters were not measured.

(i) PREDICTIVE ABILITY FOR ALL COWS

The somatic cell count alone correctly classified over 76 % of the composite milk samples as having come from an infected or an uninfected cow.

The addition of any one of several of the parameters to the model containing the somatic cell count alone, appeared to cause small increases in the predictive ability of the system. However, the apparent increase in the predictive ability which occurred when the somatic cell count and either the electrical conductivity or the pH were combined results from the fact that no electrical conductivity or pH measurements were made on samples from herd C. The somatic cell count alone, was better able to classify samples from herds A and B, than those from herd C.

From table 9.2ii, the somatic cell count alone correctly classified 82.8 % and 85.4 % of samples within herds A and B respectively. Thus if a weighted average figure of 83.9 % is used for herds A and B, then the combination of the somatic cell count with the electrical conductivity or the pH did not result in a real improvement in the predictive ability compared with that achieved using the somatic cell count alone.

The greatest real improvement in the predictive ability resulted from the combination of the somatic cell count with the NAGase activity, the predictive ability being 1.1 percentage points higher than that of the somatic cell count alone.

(ii) PREDICTIVE ABILITY FOR INFECTED COWS

The somatic cell count alone correctly classified approximately 72 % of samples which had been taken from infected cows. The greatest improvement in predictive ability compared with that which was achieved using the model containing the somatic cell count alone, resulted from the combination of the somatic cell count plus the antitrypsin concentration, an improvement of 1.1 percentage points.

With respect to the somatic cell count alone, the weighted average predictive ability for samples from infected cows in herds A and B was 75.2 % of samples. The predictive abilities of the models which contained the somatic cell count plus the electrical conductivity, or the somatic cell count plus the pH, were lower than that of the model which contained the somatic cell count alone, by 4.8 percentage points and 3.4 percentage points respectively.

### (iii) PREDICTIVE ABILITY FOR UNINFECTED COWS

The somatic cell count alone correctly classified 78.5 % of the samples which had been taken from uninfected cows. The greatest real improvement in the predictive ability with respect to the samples from uninfected cows resulted from the combination of the somatic cell count with the NAGase activity, the improvement amounting to 3.2 percentage points over that obtained using the somatic cell count alone.

The weighted average predictive ability for the somatic cell count alone within herds A and B, was 87.4 % of samples. The combination of the somatic cell count plus the electrical conductivity, or the somatic cell count plus the pH, resulted in decreases in predictive ability of 3.0 percentage points and 4.1 percentage points respectively, compared with that achieved using the somatic cell count alone.

## (7) DISCRIMINANT FUNCTION ANALYSIS WITHIN INDIVIDUAL HERDS, THROUGHOUT THE LACTATION, FOR THE SOMATIC CELL COUNT PLUS ONE OTHER PARAMETER

Table 9.2iv gives the results of the discriminant function analysis within individual herds, when each of the alternative parameters was combined, in turn, with the somatic cell count, using bacteriological classification 1.

### (i) PREDICTIVE ABILITY FOR ALL COWS

The somatic cell count alone correctly classified 82.8 %, 85.4 % and 76.5 % of composite milk samples, from herds A, B, and C respectively, when the data for all the cows were combined.

Within herd A, the addition of either the NAGase activity or the antitrypsin concentration to the model resulted in marginal improvements in the predictive ability, compared with that obtained using the somatic cell count alone. The use of a model

containing the somatic cell count plus the electrical conductivity resulted in an improvement in the predictive ability of 2.8 percentage points.

Within herd B, the only parameter which improved the predictive ability of the model was the NAGase activity, although the improvement was only marginal. The addition of several of the parameters to the model containing the somatic cell count alone resulted in a decrease in the predictive ability, the largest decrease occurring when the somatic cell count and the antitrypsin concentration were combined.

Within herd C, the combination of the sodium concentration, the potassium concentration, the lactose concentration, the NAGase activity or the antitrypsin concentration with the somatic cell count each resulted in a small improvement in the predictive ability, in comparison with that of the somatic cell count alone. The greatest increase in predictive ability resulted from the use of the somatic cell count plus the potassium concentration of the milk.

#### (ii) PREDICTIVE ABILITY FOR INFECTED COWS

The somatic cell count alone correctly classified 76.4 %, 73.7 % and 74.6 % of samples which had been taken from infected cows in herds A, B and C respectively.

Within herd A, the predictive ability was improved by the addition of either the sodium concentration, the electrical conductivity or the NAGase activity to the model. The use of a model containing the somatic cell count plus the electrical conductivity, gave a predictive ability which was 6.9 percentage points higher than the model which included the somatic cell count alone.

Within herd B, the combination of the somatic cell count with the potassium concentration resulted in an increase in the predictive ability of 4.7 percentage points over that obtained using the somatic cell count alone. Smaller increases in the predictive ability resulted from the use of the somatic cell count plus either the electrical conductivity, the pH, the lactose concentration, or the antitrypsin concentration of the milk.

Within herd C, marginal improvements in the predictive ability resulted from the combination of the somatic cell count with the sodium concentration, the potassium concentration or the lactose concentration. However, decreases in the predictive ability occurred when either the NAGase activity or the antitrypsin concentration were used in combination with the somatic cell count.

### (iii) PREDICTIVE ABILITY FOR UNINFECTED COWS

The somatic cell count alone correctly classified 86.1 %, 89.1 % and 78.1 % of samples which had been taken from uninfected cows within herds A, B and C respectively.

Within herd A, a small increase in the predictive ability resulted from the combination of the somatic cell count with either the electrical conductivity or the antitrypsin concentration, while the combination of the somatic cell count with the remaining parameters caused a slightly lower predictive ability than was obtained using the somatic cell count alone.

Within herd B, the addition of a second parameter to the model generally caused a decrease in the predictive ability. The largest decrease in the predictive ability resulted from the combination of the somatic cell count with either the potassium concentration, or the antitrypsin concentration.

Within herd C, the addition of a second parameter to the model generally resulted in a slight increase in the predictive ability with respect to composite milk samples from uninfected cows.

### (8) CORRELATION BETWEEN THE SOMATIC CELL COUNT AND THE ALTERNATIVE PARAMETERS IN COMPOSITE MILK SAMPLES

Table 9.3 gives the correlation coefficients between the somatic cell count and each of the alternative parameters, both within the overall data set and within individual herds.

Both the sodium concentration and the NAGase activity were highly correlated with the somatic cell count in composite milk samples. A weaker relationship existed between the somatic cell count and each of the remaining parameters, particularly the pH of the milk.

In general, the correlation between the somatic cell count and each of the alternative parameters was higher within samples from infected cows, than in samples from uninfected cows. An exception to this finding was the relationship between the somatic cell count and the electrical conductivity, for which a higher correlation coefficient was recorded for samples from uninfected than from infected cows.

The correlation coefficients between the somatic cell count and the alternative parameters were generally higher within herds B and C than within herd A.

## DISCUSSION

### (1) THE PROBABILITY OF MISCLASSIFICATION OF SAMPLES

When the data from the three herds were combined, the somatic cell count showed a lower probability of misclassification than did any of the other parameters which were studied. This finding was not unexpected, following the results which have been presented with respect to the diagnosis of infection within individual udder quarters ( chapter seven ).

A number of researchers have studied the association between bacterial infection of the udder and the somatic cell count of the composite milk samples. The mean somatic cell counts for milk from cows which were uninfected, infected with minor pathogens, or infected with major pathogens were 170,000, 227,000 and 998,000 cells per ml of milk respectively ( Schultz *et al*, 1977b ).

Only 6 % of composite milk samples from cows which were harbouring major pathogens fell into the somatic cell count range of 0 to 200,000 cells per ml, while 60 % of milk samples from uninfected cows fell within this range ( Duirs and MacMillan, 1979 ). Similarly, McDermott *et al*, ( 1982 ) reported that 5 % of cows with a composite somatic cell count of between 0 to 99,000 cells per ml were infected, whereas 61 % of cows with somatic cell counts of over 600,000 cells per ml were infected.

The results of several studies regarding the ability of the somatic cell count to distinguish between infected and uninfected cows are compared in table 9.4. While arbitrary threshold values were used in the studies which are cited, the critical threshold, ie the threshold value at which the proportion of false negative and false positive results are equal, was used during the present trial.

Differences between trials with respect to definitions of infection, the bacterial flora of the cows, and sampling techniques make comparisons between separate studies hazardous.

The results of the present trial confirm the findings of the cited trials, that use of the somatic cell count of composite milk samples provides a reasonably accurate prediction of the infection status of the cow.

The sodium concentration and the NAGase activity were ranked second and third, after the somatic cell count, in terms of their ability to distinguish between infected and uninfected cows. Once again, this finding bears out the results of chapter seven, in which the sodium concentration and the NAGase activity were better able to predict the infection status of individual udder quarters than were the remaining parameters, with the exception of the somatic cell count. Other workers have shown the sodium

concentration ( Fernando et al, 1985 ) and the NAGase activity ( Kitchen et al, 1984a ) of the composite milk to be capable of predicting the presence or absence of infection with a degree of accuracy similar to that observed during the present trial.

The remaining parameters showed higher probabilities of misclassification than did the somatic cell count, the sodium concentration, or the NAGase activity. Previous reports have indicated similar results to those of the present study with respect to the inability of the potassium concentration ( Fernando et al, 1985 ), the lactose concentration or the pH of the composite milk samples ( Mijnen et al, 1982 ) to accurately predict the infection status of the cow.

During the present trial, the electrical conductivity of the composite milk sample was found to have a relatively high probability of misclassification, ie a low ability to distinguish between infected and uninfected cows. Similar results have been reported by other researchers ( Batra and McAllister, 1984 ). Fernando et al ( 1985 ) reported the electrical conductivity of the milk to be superior to the somatic cell count for predicting the infection status of the cow, although those authors used somatic cell count results which had been transformed to log values, while the electrical conductivity readings were not transformed.

## (2) THE EFFECT OF THE MILK FRACTION ON THE PROBABILITY OF MISCLASSIFICATION OF MILK SAMPLES

For a parameter to be able to predict the infection status of a cow, the deflection due to infection, ie the amount by which the concentration of the parameter changes as a result of the infection, must be great enough to overcome the dilution effects of the uninfected quarters within the infected udder. The deflection due to infection, within the individual udder quarters, was greater for the somatic cell count, the sodium concentration and the NAGase activity than for the remaining parameters ( chapter six ). As a result of the lower deflection due to infection, the electrical conductivity, the pH, the lactose concentration, the potassium concentration and the antitrypsin concentration showed high probabilities of misclassification.

In composite milk samples, the milk from infected quarters will be diluted by milk from uninfected quarters. Therefore, it might have been expected that the probability of misclassification by each parameter would be higher with respect to composite milk samples than with respect to individual quarter samples. That this was not the case during the present trial, reflects the nature of the two types of sample. The quarter foremilk samples were taken prior to machine milking, but following the removal of 10 to 15 ml of strict foremilk. In contrast, the composite sample was representative of

the milk removed throughout the milking process, after removal of the first 20 to 30 ml of foremilk.

It was shown in chapter four, that the concentration of the parameters tends to change during the course of the milking process, and that in general, the difference in the concentration of a parameter between infected and uninfected quarters is greater towards the end of the milking than at the start of the milking process. Thus the ability of the concentration of each parameter in the composite milk samples to classify the sample as having come from an infected or an uninfected cow, was better than might have been predicted from the results of the quarter samples.

### (3) THE EFFECT OF THE BACTERIOLOGICAL CLASSIFICATION SYSTEM ON THE PROBABILITY OF MISCLASSIFICATION AND ON THE CRITICAL THRESHOLD

The somatic cell count showed a similar probability of misclassification for both bacteriological classification systems 1 and 2 although the critical threshold was lower when bacteriological classification 2 ( BC2 ) was used.

The difference between the critical threshold values for the two bacteriological classification systems demonstrates the effect of the minor pathogens on the somatic cell count. As was the case with respect to the diagnosis of infection within individual udder quarters, a different threshold is required if the aim of the exercise is to identify those cows which are infected with major pathogens only, from that required to identify cows which are infected by both major and/or minor pathogens.

A number of the alternative parameters also showed a lower critical threshold when BC2 was used, than when BC1 was used, suggesting that the minor pathogens exert an effect on the concentration of these parameters in the composite milk samples.

The fact that the NAGase activity showed a higher probability of misclassification with BC2 than with BC1 suggests that the NAGase activity is affected to a lesser extent than is the somatic cell count, by the presence of minor pathogens.

#### (4) THE VARIATION BETWEEN HERDS WITH RESPECT TO THE PROBABILITY OF MISCLASSIFICATION AND TO THE CRITICAL THRESHOLD VALUES

The probability of misclassification of the somatic cell count was remarkably consistent between herds, regardless of the bacteriological classification system used. Within each herd, more than 75 % of the composite milk samples were correctly classified by the somatic cell count.

The other parameters generally showed larger differences in probability of misclassification than did the somatic cell count, particularly when BC1 was used.

For a diagnostic test to be of practical use, it must be able to consistently identify infected cows, within a wide variety of herds. In this respect, the somatic cell count was superior to all of the other parameters which were tested.

The variation in the critical threshold values between herds was more marked for the somatic cell count than for the other parameters and this may be a disadvantage if the somatic cell count is used for screening milk samples from large numbers of herds. The differences between herds with respect to the critical threshold values reflect the effects of minor pathogens and of previous infections on the somatic cell count of the individual udder quarters ( chapter seven ). The fact that the critical threshold values for each herd varied less for the parameters other than the somatic cell count, suggests that the effects of minor pathogens and previous infections on the levels of the alternative parameters were not as great as were the effects on the somatic cell count of the composite milk samples. Bacteria, damaged tissue, and leukocytes themselves may all release chemotaxins ( Murphy, 1976 ). Thus the neutrophils which have migrated into the udder tissue and then into the milk may recruit still more neutrophils from the blood, the system being very sensitive.

#### (5) THE EFFECT OF THE STAGE OF THE LACTATION ON THE PROBABILITIES OF MISCLASSIFICATION, AND ON THE CRITICAL THRESHOLD VALUES

The probability of misclassification remained relatively constant at all stages of lactation, for all parameters ( table 9.1iii ). For example, the values for the somatic cell count ranged from 21 % during stage 2, to 25 % during stage 4.

However, the values for the critical thresholds changed during lactation, particularly for the somatic cell count. In general, parameters which were increased by infection showed increases in threshold value later in the lactation, whereas parameters which were decreased by infection showed decreases in threshold value during lactation. The critical

threshold for the NAGase activity showed little variation during the course of the season. These changes may reflect the increasing infection rate for minor pathogens ( chapter five ). However, the results of chapter six show that the concentration of the parameters change during the lactation, within both infected and uninfected udder quarters. These changes in concentration are probably due to the variation in the milk yield and to involutionary effects within the secretory tissue of the udder. Thus the changing critical threshold values are probably due mainly to normal physiological processes.

(6) THE EFFECT OF USING A FIXED THRESHOLD LEVEL  
RATHER THAN THE CRITICAL THRESHOLD ON THE DIAGNOSIS  
OF UDDER INFECTION

The results of the present trial have implications for the somatic cell counting scheme which is offered by the Livestock Improvement Corporation. At present, a single threshold value of 250,000 cells per ml is recommended as the cut-off point between normal and mastitic cows, this figure being used throughout the lactation. The effects of using such a fixed 250,000 cells per ml threshold may be seen in table 9.5.

Within herd A, 19.6 % of the samples from infected cows were misclassified when the critical threshold for that herd was used ( see table 9.1ii ). Using the fixed threshold, nearly 63 % of the samples which were taken from infected cows would have been classed as negative, ie false negative results. By the same token, use of the critical threshold resulted in 19.6 % of the samples from uninfected cows being misclassified, while only 3 % of such samples would have been misclassified had the fixed 250,000 cell per ml threshold been applied.

Within herd B, 29 % of the samples from infected cows would have been misclassified, had the fixed threshold been used, compared with 21 % of such samples when the critical threshold of 142,000 cells per ml was used. At the same time, approximately 5 % of the samples from the uninfected cows would have been misclassified by the fixed threshold, compared to 21 % of such samples when the critical threshold was used.

Within herd C, use of the fixed threshold would have caused a lower percentage of the samples from the infected cows to be misclassified than would have use of the critical threshold, while the percentage of samples from uninfected cows which would be misclassified would be higher if the fixed threshold was applied than was the case when the critical threshold was used.

The actual threshold which is selected, will obviously depend upon the use to which the information is to be put. It will also depend upon the economic consequences of not identifying, and therefore failing to treat infected cows, against the consequences of misdiagnosing and therefore treating or culling uninfected cows unnecessarily ( see chapter seven ).

During the present trial, the herds with a greater incidence of udder infection, and a higher bulk milk somatic cell count, showed a higher critical threshold than did herd A, in which both the incidence of infection and the bulk milk somatic cell count were low. It might therefore be possible to use the bulk milk somatic cell count to select a suitable threshold level for a particular herd. This is clearly an area in which further research is required.

If the somatic cell count threshold were to be increased during the season, then the results of the present trial suggest that a consistent distinction between infected and uninfected cows could be achieved.

#### (7) DISCRIMINANT FUNCTION ANALYSIS USING EACH PARAMETER ALONE

For all parameters, the values for the percentage of correct diagnoses determined by discriminant function analyses were very similar to those determined by the critical threshold program ( tables 9.1i and 9.2i ). For the somatic cell count, both forms of analysis correctly classified more than 75 % of all samples, either in the whole data set, or within each herd.

In chapter seven, it was shown that the somatic cell count was superior to the other parameters with respect to the classification of samples from infected quarters, ie the somatic cell count has a high sensitivity. The results of the present chapter show that the same is true of the somatic cell count with respect to the classification of infected cows.

If one of the alternative parameters was to replace the somatic cell count as a diagnostic test of udder health, then a substantial number of infected cows would be missed.

Of the eight parameters which were tested, the NAGase activity showed the highest specificity, ie the greatest ability to correctly classify samples from uninfected cows. However, the difference between the specificity of the NAGase activity and that of the somatic cell count was relatively small.

In general, the specificity of each parameter was higher than was the corresponding sensitivity. A similar finding has been reported for individual udder quarters ( chapter seven ). The somatic cell count was the only parameter which showed high values for both sensitivity and for specificity.

The use of a diagnostic test which has both a high sensitivity and a high specificity, will maximise the number of infected cows which can be identified and subsequently treated or culled from the herd, while minimising the number of uninfected cows which are treated or culled unnecessarily.

#### (8) DISCRIMINANT FUNCTION ANALYSIS WITHIN INDIVIDUAL HERDS

While the predictive ability of the somatic cell count with respect to the total data set differed between herds, being highest within herd B, and lowest within herd C, no parameter showed a greater predictive ability than did the somatic cell count in any of the three herds, a finding which confirms the results of the threshold program.

Although the sensitivity of a number of the parameters was high within one of the three herds, that of the somatic cell count was consistently high, suggesting that only the somatic cell count can consistently identify infected cows in herds with varying levels of infection.

The difference between herds, with respect to the sensitivity of the parameters reflects the differences in the bacterial flora, and the effects of the various pathogens on the concentration of the parameter in question.

A more consistent change in the somatic cell count, than in the concentration of the other parameters, was caused by the major pathogens within each herd.

The somatic cell count showed a higher specificity than did any other parameter within herds A and B. Within herd C, the NAGase activity showed the highest specificity of all the parameters, although the difference between the specificity of the NAGase activity and that of the somatic cell count was relatively small.

The somatic cell count showed a consistently high specificity within each of the three herds, while a number of the other parameters showed large differences between herds with respect to specificity. The specificity of the sodium concentration was consistently high, between herds, but was lower than that of the somatic cell count within each herd.

(9) THE EFFECT OF USING TWO PARAMETERS  
FOR THE DIAGNOSIS OF INFECTION

(i) TOTAL DATA SET

In chapter seven, it was shown that the addition of a second parameter to the model containing the somatic cell count, resulted in little improvement in the accuracy with which the quarter foremilk samples could be classified as having been taken from either an infected or from an uninfected quarter.

The results of the present chapter show a similar situation with respect to the use of composite milk samples for the diagnosis of infection within individual cows.

Clearly, the parameters other than the somatic cell count gave little additional useful information as to the presence or the absence of infection within the udder.

The NAGase activity of the milk, when combined with the somatic cell count, resulted in a marginal increase in the predictive ability of the model with respect to the the total data set, reflecting the slightly higher specificity of the NAGase activity than of the somatic cell count of the milk.

When the infected cows were examined, either the potassium concentration, or the antitrypsin concentration, in combination with the somatic cell count gave marginally higher predictive abilities than were associated with the use of the somatic cell count alone, suggesting that in a small number of cows, the somatic cell count was affected to a lesser extent than was the concentration of the antitrypsin or of the potassium ions. However, the improvements in the predictive ability which were associated with the use of the two parameter model were generally small and may have been due to sampling variation.

In the case of several of the parameters, the use of the two parameter model resulted in a decrease in the predictive ability in comparison with that achieved using the somatic cell count alone.

The improvement in the classification of uninfected cows by the addition of the NAGase activity to the somatic cell count is not surprising in view of the greater specificity of the NAGase activity, once again suggesting that the NAGase activity of the uninfected cows under BC1, was less affected by minor pathogens, or by the previous infection of the gland than was the somatic cell count.

## (ii) WITHIN INDIVIDUAL HERDS

The addition of a second parameter to the model containing the somatic cell count generally resulted in very little improvement in the predictive ability of the system in any of the three herds, when data from both the infected and uninfected cows were combined.

While several parameters, when combined with the somatic cell count, resulted in a small improvement in the predictive ability compared with that of the somatic cell count alone, within 1 or 2 of the herds, a decrease in the predictive ability of the model usually occurred in the remaining herd or herds. Only the combination of the somatic cell count plus the NAGase activity resulted in an increase in the predictive ability of the model within each of the three herds, although the increase in 2 of the 3 herds amounted to less than 1 percentage point.

To introduce a second parameter for the diagnosis of udder health, on a commercial scale, the extra expenditure would need to be balanced by a substantial increase in the predictive ability of the two parameter system, in comparison with that of the single parameter which is currently in use. Whether the increase in the predictive ability which might result from the measuring of both the somatic cell count and the NAGase activity of the milk would justify the extra expenditure will depend upon costs associated with misdiagnosing the infected cows as being uninfected, and misdiagnosing the uninfected cows as being infected.

A detailed analysis of the cost-benefit ratio is beyond the scope of this thesis, but it appears unlikely that the adoption of a two parameter system would be economically viable.

## (10) CORRELATION BETWEEN THE SOMATIC CELL COUNT AND THE OTHER PARAMETERS

The correlation coefficients between the somatic cell count and each of the other parameters within the composite milk samples, show similar trends to the corresponding correlation coefficients in quarter foremilk samples ( chapter seven ).

The NAGase activity and the somatic cell count were highly correlated. Similar findings have been reported by other researchers ( Kitchen, 1976; Kitchen and Middleton, 1976a; Obara et al, 1983; Miller and Paape, 1988 ).

It is perhaps surprising, therefore, that of all the parameters, the NAGase activity should show the greatest improvement in the predictive ability when combined with

the somatic cell count, since two parameters which are highly correlated will be likely to classify a sample in a similar fashion.

The higher correlation between parameters within the milk of infected cows than in that of uninfected cows has also been found in a previous study ( Emanuelson et al, 1987 ) and may be attributed to effects of factors other than the presence of bacteria on the levels of the parameters, these effects being greater within uninfected than in infected glands.

The higher correlation coefficients within herd C than within herd A, probably reflect the higher rate of infection with major pathogens which occurred within herd C ( chapter five ).

### SUMMARY AND CONCLUSIONS

The results presented in chapter nine in relation to the diagnosis of udder infection by the use of composite milk samples lead to conclusions which are similar in many respects to those drawn in chapter seven in relation to the diagnosis of infection within individual udder quarters using quarter foremilk samples.

- (1) The somatic cell count proved to have a greater ability to distinguish between infected and uninfected cows than did the sodium concentration, the potassium concentration, the pH, the electrical conductivity, the lactose concentration, the NAGase activity or the antitrypsin concentration of the composite milk samples.
- (2) The ability of the somatic cell count to distinguish between infected and uninfected cows was consistently high in the three herds. While several of the other parameters were able to distinguish between infected and uninfected cows with a similar degree of accuracy within individual herds, no parameter other than the somatic cell count was so consistently accurate in this respect.
- (3) The ability of the somatic cell count to distinguish between infected and uninfected cows was consistently high throughout the lactation period, provided that the critical threshold value was used.
- (4) The critical threshold values for the parameters differed between the three herds, the difference being particularly marked with respect to the critical threshold of the somatic cell count. This between-herd variation in the critical threshold may cause problems with regard to the use of the somatic cell count as a screening test for udder infection. The difference in the critical threshold values between herds reflects the effects of the rate of infection with minor pathogens and possibly the effects of previous infection on the concentration of the parameter.
- (5) A higher threshold value will be required for the identification of cows which are infected with major pathogens only, than for the identification of cows which are infected with either major or minor pathogens.
- (6) The critical threshold values for the parameters changed during the course of the lactation. The critical threshold values for the somatic cell count, and for other parameters which tend to increase in concentration as a result of infection, generally increased during lactation, while the reverse was true of those parameters, the concentration of which tend to decrease as a result of infection. An exception to this trend occurred with respect to the NAGase

activity, the critical threshold of which showed a slight decrease as the season progressed.

The variation in the critical threshold values during the lactation are probably due to physiological changes within the udder, although some of the variation may be due to the changes in the infection rate with minor pathogens.

- (7) The use of a set threshold for the determination of udder health within all herds, will result in a high proportion of false negative results within herds with a low incidence of infection, and a high proportion of false positive results in herds with a high incidence of udder infection, regardless of the parameter used.
- (8) The threshold which is chosen for a particular herd should be appropriate for that herd.
- (9) There is little to be gained from using a two parameter system for predicting the infection status of the udder. It is unlikely that the increase in diagnostic accuracy which might be achieved would compensate for the extra cost which would be involved.
- (10) The diagnosis of udder infection within cows, using composite milk samples was only slightly less accurate than was the diagnosis of infection within individual udder quarters, using individual quarter samples, when the somatic cell count was used as the diagnostic test.

The somatic cell count of the composite milk sample is an accurate and a reliable indicator of the infection status of the cow, being able to predict the presence or absence of major pathogens in over 75 % of samples from herds with differing levels of infection, provided that the critical threshold value for each herd is used. By sampling on more than one occasion during the course of the dairy season, it is likely that the accuracy will be further increased. During the present trial, the accuracy of diagnosis was assessed on the basis of the bacteriological status of the cow at the time of sampling only.

TABLE 9.11RESULTS OF THRESHOLD PROGRAM WITHIN THE TOTAL DATA SETCOMPOSITE MILK SAMPLES

| NAME OF<br>PARAMETER                               | <u>BACTERIOLOGICAL<br/>CLASSIFICATION 1</u> |                                     | <u>BACTERIOLOGICAL<br/>CLASSIFICATION 2</u> |                                     |
|----------------------------------------------------|---------------------------------------------|-------------------------------------|---------------------------------------------|-------------------------------------|
|                                                    | CRITICAL<br>THRESHOLD                       | PROBABILITY OF<br>MISCLASSIFICATION | CRITICAL<br>THRESHOLD                       | PROBABILITY OF<br>MISCLASSIFICATION |
| SOMATIC<br>CELL<br>COUNT<br>( cells/ml )           | 185,000                                     | 24.9 %                              | 80,000                                      | 22.8 %                              |
| SODIUM<br>CONCENTRATION<br>( mMoles/litre )        | 16.8                                        | 33.0 %                              | 15.4                                        | 28.7 %                              |
| POTASSIUM<br>CONCENTRATION<br>( mMoles/litre )     | 40.1                                        | 46.3 %                              | 40.1                                        | 49.7 %                              |
| ELECTRICAL<br>CONDUCTIVITY<br>( mSiemens/cm )      | 5.85                                        | 44.2 %                              | 5.70                                        | 29.3 %                              |
| pH                                                 | 6.62                                        | 52.4 %                              | 6.62                                        | 46.7 %                              |
| LACTOSE<br>CONCENTRATION<br>( % )                  | 4.98                                        | 40.7 %                              | 5.03                                        | 43.7 %                              |
| NAGase<br>ACTIVITY<br>( nmoles/ml/min )            | 5.85                                        | 32.1 %                              | 5.50                                        | 40.2 %                              |
| ANTITRYPSIN<br>CONCENTRATION<br>( relative units ) | 0.760                                       | 41.8 %                              | 0.720                                       | 37.8 %                              |

TABLE 9.111RESULTS OF THRESHOLD PROGRAM WITHIN INDIVIDUAL HERDSCOMPOSITE MILK SAMPLES

| PARAMETER NAME                                  | HERD | <u>BACTERIOLOGICAL CLASSIFICATION 1</u> |                                  | <u>BACTERIOLOGICAL CLASSIFICATION 2</u> |                                  |
|-------------------------------------------------|------|-----------------------------------------|----------------------------------|-----------------------------------------|----------------------------------|
|                                                 |      | CRITICAL THRESHOLD                      | PROBABILITY OF MISCLASSIFICATION | CRITICAL THRESHOLD                      | PROBABILITY OF MISCLASSIFICATION |
| SOMATIC CELL COUNT<br>( cells/ml )              | A    | 73,000                                  | 19.6 %                           | 60,000                                  | 21.1 %                           |
|                                                 | B    | 142,000                                 | 20.6 %                           | 50,000                                  | 21.2 %                           |
|                                                 | C    | 344,000                                 | 23.5 %                           | 160,000                                 | 24.5 %                           |
| SODIUM CONCENTRATION<br>( mMoles/L )            | A    | 14.9                                    | 30.6 %                           | 14.6                                    | 28.8 %                           |
|                                                 | B    | 17.5                                    | 24.8 %                           | 15.2                                    | 31.4 %                           |
|                                                 | C    | 18.5                                    | 35.0 %                           | 17.2                                    | 33.5 %                           |
| POTASSIUM CONCENTRATION<br>( mMoles/L )         | A    | 39.4                                    | 43.4 %                           | 39.6                                    | 47.4 %                           |
|                                                 | B    | 42.2                                    | 40.9 %                           | 42.9                                    | 43.3 %                           |
|                                                 | C    | 39.5                                    | 49.6 %                           | 39.5                                    | 50.8 %                           |
| ELECTRICAL CONDUCTIVITY<br>( mS/cm )            | A    | 5.55                                    | 43.4 %                           | 5.55                                    | 28.3 %                           |
|                                                 | B    | 6.30                                    | 41.5 %                           | 6.15                                    | 30.6 %                           |
|                                                 | C    | ND <sup>1</sup>                         | ND                               | ND                                      | ND                               |
| pH                                              | A    | 6.61                                    | 47.5 %                           | 6.61                                    | 49.8 %                           |
|                                                 | B    | 6.64                                    | 53.3 %                           | 6.65                                    | 44.3 %                           |
|                                                 | C    | ND                                      | ND                               | ND                                      | ND                               |
| LACTOSE CONCENTRATION<br>( % )                  | A    | 4.92                                    | 48.2 %                           | 4.93                                    | 47.1 %                           |
|                                                 | B    | 4.91                                    | 47.0 %                           | 5.12                                    | 32.3 %                           |
|                                                 | C    | 5.04                                    | 35.4 %                           | 5.13                                    | 35.3 %                           |
| NAGase ACTIVITY<br>( nM/ml/min )                | A    | 5.15                                    | 39.9 %                           | 5.10                                    | 39.3 %                           |
|                                                 | B    | 5.40                                    | 31.8 %                           | 5.10                                    | 44.9 %                           |
|                                                 | C    | 6.90                                    | 26.2 %                           | 6.20                                    | 39.1 %                           |
| ANTITRYPSIN CONCENTRATION<br>( relative units ) | A    | 0.660                                   | 49.1 %                           | 0.660                                   | 44.9 %                           |
|                                                 | B    | 0.800                                   | 38.2 %                           | 0.780                                   | 49.8 %                           |
|                                                 | C    | 0.800                                   | 35.6 %                           | 0.740                                   | 35.5 %                           |

1 ND = No data

TABLE 9.1111RESULTS OF THRESHOLD PROGRAM BY STAGE OF LACTATION,WITHIN THE TOTAL DATA SET - BACTERIOLOGICAL CLASSIFICATION 1COMPOSITE MILK SAMPLES

| PARAMETER<br>NAME                                  | STAGE | CRITICAL<br>THRESHOLD | PROBABILITY OF<br>MISCLASSIFICATION |
|----------------------------------------------------|-------|-----------------------|-------------------------------------|
| SOMATIC CELL<br>COUNT<br>( cells/ml )              | 1     | 105,000               | 26.2 %                              |
|                                                    | 2     | 195,000               | 21.0 %                              |
|                                                    | 3     | 255,000               | 27.3 %                              |
|                                                    | 4     | 200,000               | 24.9 %                              |
| SODIUM<br>CONCENTRATION<br>( mMoles/litre )        | 1     | 15.8                  | 34.7 %                              |
|                                                    | 2     | 15.8                  | 26.3 %                              |
|                                                    | 3     | 18.4                  | 35.9 %                              |
|                                                    | 4     | 18.8                  | 35.0 %                              |
| POTASSIUM<br>CONCENTRATION<br>( mMoles/litre )     | 1     | 41.2                  | 46.8 %                              |
|                                                    | 2     | 40.9                  | 46.2 %                              |
|                                                    | 3     | 39.1                  | 47.2 %                              |
|                                                    | 4     | 38.2                  | 46.2 %                              |
| ELECTRICAL<br>CONDUCTIVITY<br>( mSiemens/cm )      | 1     | 5.85                  | 40.5 %                              |
|                                                    | 2     | 5.80                  | 45.7 %                              |
|                                                    | 3     | 5.75                  | 44.1 %                              |
|                                                    | 4     | 6.00                  | 43.9 %                              |
| pH                                                 | 1     | 6.61                  | 45.0 %                              |
|                                                    | 2     | 6.61                  | 52.9 %                              |
|                                                    | 3     | 6.63                  | 51.5 %                              |
|                                                    | 4     | 6.66                  | 54.9 %                              |
| LACTOSE<br>CONCENTRATION<br>( % )                  | 1     | 5.17                  | 36.7 %                              |
|                                                    | 2     | 5.14                  | 41.6 %                              |
|                                                    | 3     | 4.78                  | 38.4 %                              |
|                                                    | 4     | 4.74                  | 44.3 %                              |
| NAGase<br>ACTIVITY<br>( nMoles/ml/min )            | 1     | 6.40                  | 34.2 %                              |
|                                                    | 2     | 5.80                  | 30.2 %                              |
|                                                    | 3     | 5.70                  | 31.4 %                              |
|                                                    | 4     | 5.60                  | 34.3 %                              |
| ANTITRYPSIN<br>CONCENTRATION<br>( relative units ) | 1     | 0.750                 | 43.1 %                              |
|                                                    | 2     | 0.750                 | 31.9 %                              |
|                                                    | 3     | 0.770                 | 45.4 %                              |
|                                                    | 4     | 0.780                 | 46.4 %                              |

TABLE 9.21RESULTS OF DISCRIMINANT FUNCTION ANALYSIS FOR THE TOTAL DATA SETUSING EACH PARAMETER ALONE - BACTERIOLOGICAL CLASSIFICATION 1COMPOSITE MILK SAMPLES


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| PERCENTAGE OF CORRECT DIAGNOSES                    |                  |                                        |                                          |                           |
|----------------------------------------------------|------------------|----------------------------------------|------------------------------------------|---------------------------|
| PARAMETER                                          | TOTAL<br>SAMPLES | INFECTED<br>SAMPLES<br>( SENSITIVITY ) | UNINFECTED<br>SAMPLES<br>( SPECIFICITY ) | NUMBER OF<br>OBSERVATIONS |
| <hr/>                                              |                  |                                        |                                          |                           |
| SOMATIC CELL<br>COUNT<br>( cells/ml )              | 76.1 %           | 72.0 %                                 | 78.5 %                                   | 736                       |
| SODIUM<br>CONCENTRATION<br>( mMoles/litre )        | 69.4 %           | 53.1 %                                 | 79.1 %                                   | 732                       |
| POTASSIUM<br>CONCENTRATION<br>( mMoles/litre )     | 55.5 %           | 49.5 %                                 | 59.0 %                                   | 732                       |
| ELECTRICAL<br>CONDUCTIVITY<br>( mSiemens/cm )      | 57.8 %           | 55.6 %                                 | 58.7 %                                   | 360                       |
| pH                                                 | 50.3 %           | 55.3 %                                 | 48.1 %                                   | 344                       |
| LACTOSE<br>CONCENTRATION<br>( % )                  | 60.4 %           | 55.2 %                                 | 63.5 %                                   | 724                       |
| NAGase<br>ACTIVITY<br>( nMoles/ml/min )            | 72.8 %           | 52.9 %                                 | 84.6 %                                   | 735                       |
| ANTITRYPSIN<br>CONCENTRATION<br>( relative units ) | 62.5 %           | 41.7 %                                 | 74.8 %                                   | 731                       |

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TABLE 9.211RESULTS OF DISCRIMINANT FUNCTION ANALYSIS WITHIN INDIVIDUAL HERDS,USING EACH PARAMETER ALONE - BACTERIOLOGICAL CLASSIFICATION 1COMPOSITE MILK SAMPLES

| PERCENTAGE OF CORRECT DIAGNOSES                       |      |                  |                                        |                                          |                           |
|-------------------------------------------------------|------|------------------|----------------------------------------|------------------------------------------|---------------------------|
| PARAMETER                                             | HERD | TOTAL<br>SAMPLES | INFECTED<br>SAMPLES<br>( SENSITIVITY ) | UNINFECTED<br>SAMPLES<br>( SPECIFICITY ) | NUMBER OF<br>OBSERVATIONS |
| SOMATIC CELL<br>COUNT<br>( cells/ml )                 | A    | 82.8 %           | 76.4 %                                 | 86.1 %                                   | 209                       |
|                                                       | B    | 85.4 %           | 73.7 %                                 | 89.1 %                                   | 157                       |
|                                                       | C    | 76.5 %           | 74.6 %                                 | 78.1 %                                   | 370                       |
| SODIUM<br>CONCENTRATION<br>( mMoles/litre )           | A    | 67.9 %           | 54.2 %                                 | 75.2 %                                   | 209                       |
|                                                       | B    | 78.2 %           | 73.0 %                                 | 79.8 %                                   | 156                       |
|                                                       | C    | 67.6 %           | 57.3 %                                 | 75.9 %                                   | 367                       |
| POTASSIUM<br>CONCENTRATION<br>( mMoles/litre )        | A    | 56.5 %           | 52.8 %                                 | 58.4 %                                   | 209                       |
|                                                       | B    | 55.1 %           | 78.4 %                                 | 47.9 %                                   | 156                       |
|                                                       | C    | 55.6 %           | 31.7 %                                 | 74.9 %                                   | 367                       |
| ELECTRICAL<br>CONDUCTIVITY<br>( mSiemens/cm )         | A    | 66.3 %           | 37.5 %                                 | 81.6 %                                   | 208                       |
|                                                       | B    | 52.6 %           | 77.8 %                                 | 44.8 %                                   | 152                       |
|                                                       | C    | ND <sup>1</sup>  | ND                                     | ND                                       |                           |
| pH                                                    | A    | 53.4 %           | 44.1 %                                 | 58.4 %                                   | 193                       |
|                                                       | B    | 51.7 %           | 60.0 %                                 | 49.1 %                                   | 151                       |
|                                                       | C    | ND               | ND                                     | ND                                       |                           |
| LACTOSE<br>CONCENTRATION<br>( % )                     | A    | 58.7 %           | 36.1 %                                 | 70.6 %                                   | 208                       |
|                                                       | B    | 55.1 %           | 52.8 %                                 | 55.9 %                                   | 147                       |
|                                                       | C    | 68.0 %           | 55.5 %                                 | 78.1 %                                   | 369                       |
| NAGase<br>ACTIVITY<br>( nM/ml/min )                   | A    | 60.8 %           | 59.7 %                                 | 61.3 %                                   | 209                       |
|                                                       | B    | 76.3 %           | 46.0 %                                 | 85.7 %                                   | 156                       |
|                                                       | C    | 74.6 %           | 66.7 %                                 | 81.0 %                                   | 370                       |
| ANTITRYPSIN<br>CONCENTRATION<br>( relative<br>units ) | A    | 50.2 %           | 76.4 %                                 | 36.5 %                                   | 209                       |
|                                                       | B    | 63.9 %           | 55.6 %                                 | 66.4 %                                   | 155                       |
|                                                       | C    | 65.9 %           | 54.0 %                                 | 75.5 %                                   | 367                       |

1 ND = No data

TABLE 9.2111RESULTS OF DISCRIMINANT FUNCTION ANALYSISWITHIN THE TOTAL DATA SET USING THE SOMATIC CELL COUNTPLUS ONE OTHER PARAMETER - BACTERIOLOGICAL CLASSIFICATION 1COMPOSITE MILK SAMPLES

| PARAMETER                             | PERCENTAGE OF CORRECT DIAGNOSES |                                     |                                       | NUMBER OF OBSERVATIONS |
|---------------------------------------|---------------------------------|-------------------------------------|---------------------------------------|------------------------|
|                                       | TOTAL SAMPLES                   | INFECTED SAMPLES<br>( SENSITIVITY ) | UNINFECTED SAMPLES<br>( SPECIFICITY ) |                        |
| SCC ALONE<br>HERDS A+B+C              | 76.1 %                          | 72.0 %                              | 78.5 %                                | 736                    |
| SCC ALONE<br>HERDS A+B ONLY           | 83.9 %                          | 75.2 %                              | 87.4 %                                | 366                    |
| SCC +<br>SODIUM<br>CONCENTRATION      | 75.4 % ( -0.7 ) <sup>1</sup>    | 71.8 % ( -0.2 )                     | 77.6 % ( -0.9 )                       | 729                    |
| SCC +<br>POTASSIUM<br>CONCENTRATION   | 76.0 % ( -0.1 )                 | 72.5 % ( +0.5 )                     | 78.1 % ( -0.4 )                       | 729                    |
| SCC +<br>ELECTRICAL<br>CONDUCTIVITY   | 80.2 % ( -3.7 ) <sup>2</sup>    | 70.4 % ( -4.8 )                     | 84.4 % ( -3.0 )                       | 358                    |
| SCC +<br>pH                           | 79.8 % ( -4.1 ) <sup>2</sup>    | 71.8 % ( -3.4 )                     | 83.3 % ( -4.1 )                       | 342                    |
| SCC +<br>LACTOSE<br>CONCENTRATION     | 75.9 % ( -0.2 )                 | 71.3 % ( -0.7 )                     | 78.7 % ( +0.2 )                       | 722                    |
| SCC +<br>NAGase<br>ACTIVITY           | 77.2 % ( +1.1 )                 | 69.7 % ( -2.3 )                     | 81.7 % ( +3.2 )                       | 732                    |
| SCC +<br>ANTITRYPSIN<br>CONCENTRATION | 75.7 % ( -0.4 )                 | 73.1 % ( +1.1 )                     | 77.2 % ( -1.3 )                       | 728                    |

1 Figures in parentheses represent the difference in the predictive ability between the somatic cell count alone, and the somatic cell count plus the parameter concerned ( the significance of the difference was not tested ).

2 Electrical conductivity and pH readings were not taken on samples from herd C - see text for explanation.

TABLE 9.21v

RESULTS OF DISCRIMINANT FUNCTION ANALYSISWITHIN INDIVIDUAL HERDS USING THE SOMATIC CELL COUNTPLUS ONE OTHER PARAMETER - BACTERIOLOGICAL CLASSIFICATION 1COMPOSITE MILK SAMPLES

| PARAMETER                       | HERD | PERCENTAGE OF CORRECT DIAGNOSES |                                     |                                       | NUMBER OF OBSERVATIONS |
|---------------------------------|------|---------------------------------|-------------------------------------|---------------------------------------|------------------------|
|                                 |      | TOTAL SAMPLES                   | INFECTED SAMPLES<br>( SENSITIVITY ) | UNINFECTED SAMPLES<br>( SPECIFICITY ) |                        |
| SOMATIC CELL COUNT ALONE        | A    | 82.8 %                          | 76.4 %                              | 86.1 %                                | 209                    |
|                                 | B    | 85.4 %                          | 73.7 %                              | 89.1 %                                | 157                    |
|                                 | C    | 76.5 %                          | 74.6 %                              | 78.1 %                                | 370                    |
| SCC + SODIUM CONCENTRATION      | A    | 82.3 % ( -0.5 ) <sup>1</sup>    | 79.2 % ( +2.8 )                     | 83.9 % ( -2.2 )                       | 209                    |
|                                 | B    | 82.5 % ( -2.9 )                 | 73.0 % ( -0.7 )                     | 85.5 % ( -3.6 )                       | 154                    |
|                                 | C    | 77.0 % ( +0.5 )                 | 75.0 % ( +0.4 )                     | 78.7 % ( +0.6 )                       | 366                    |
| SCC + POTASSIUM CONCENTRATION   | A    | 81.8 % ( -1.0 )                 | 76.4 % ( +0.0 )                     | 84.7 % ( -1.4 )                       | 209                    |
|                                 | B    | 81.8 % ( -3.6 )                 | 78.4 % ( +4.7 )                     | 82.9 % ( -6.2 )                       | 154                    |
|                                 | C    | 79.0 % ( +2.5 )                 | 75.6 % ( +1.0 )                     | 81.7 % ( +2.6 )                       | 366                    |
| SCC + ELECTRICAL CONDUCTIVITY   | A    | 85.6 % ( +2.8 )                 | 83.3 % ( +6.9 )                     | 86.8 % ( +0.7 )                       | 208                    |
|                                 | B    | 82.0 % ( -3.4 )                 | 75.0 % ( +1.3 )                     | 84.2 % ( -4.9 )                       | 150                    |
|                                 | C    | ND <sup>2</sup>                 | ND                                  | ND                                    |                        |
| SCC + pH                        | A    | 81.3 % ( -1.5 )                 | 75.0 % ( -1.4 )                     | 84.8 % ( -1.3 )                       | 193                    |
|                                 | B    | 85.2 % ( -0.2 )                 | 77.1 % ( +3.4 )                     | 87.7 % ( -1.4 )                       | 149                    |
|                                 | C    | ND                              | ND                                  | ND                                    |                        |
| SCC + LACTOSE CONCENTRATION     | A    | 82.7 % ( -0.1 )                 | 76.4 % ( +0.0 )                     | 86.0 % ( -0.1 )                       | 208                    |
|                                 | B    | 84.2 % ( -1.2 )                 | 77.8 % ( +4.1 )                     | 86.4 % ( -2.7 )                       | 146                    |
|                                 | C    | 78.5 % ( +2.0 )                 | 75.0 % ( +0.4 )                     | 81.4 % ( +3.3 )                       | 368                    |
| SCC + NAGase ACTIVITY           | A    | 83.3 % ( +0.5 )                 | 77.8 % ( +1.4 )                     | 86.1 % ( +0.0 )                       | 209                    |
|                                 | B    | 85.7 % ( +0.3 )                 | 73.0 % ( -0.7 )                     | 89.7 % ( +0.6 )                       | 154                    |
|                                 | C    | 78.3 % ( +1.8 )                 | 73.9 % ( -0.7 )                     | 81.9 % ( +3.8 )                       | 369                    |
| SCC + ANTITRYPSIN CONCENTRATION | A    | 83.7 % ( +0.9 )                 | 76.4 % ( +0.0 )                     | 87.6 % ( +1.6 )                       | 209                    |
|                                 | B    | 81.7 % ( -3.7 )                 | 77.8 % ( +4.1 )                     | 82.9 % ( -6.2 )                       | 153                    |
|                                 | C    | 77.3 % ( +0.8 )                 | 69.9 % ( -4.7 )                     | 83.3 % ( +4.2 )                       | 366                    |

1 Figures in parentheses represent the difference in the predictive ability between the somatic cell count alone, and the somatic cell count plus the parameter concerned ( the significance of the difference was not tested ).

2 ND = No data

TABLE 9.3

VALUES FOR CORRELATION COEFFICIENTS FOR RELATIONS  
BETWEEN THE SOMATIC CELL COUNT AND THE OTHER PARAMETERS

COMPOSITE MILK SAMPLES

| HERD   | DATA       | SOD  | POT   | CON             | pH   | LAC   | NAG  | ANT  |
|--------|------------|------|-------|-----------------|------|-------|------|------|
| TOTAL  | UNINFECTED | 0.67 | -0.20 | 0.28            | 0.14 | -0.18 | 0.40 | 0.23 |
|        | INFECTED   | 0.70 | -0.23 | 0.17            | 0.20 | -0.35 | 0.71 | 0.64 |
|        | OVERALL    | 0.74 | -0.24 | 0.23            | 0.13 | -0.33 | 0.65 | 0.46 |
| HERD A | UNINFECTED | 0.52 | -0.07 | 0.26            | 0.04 | -0.17 | 0.29 | 0.22 |
|        | INFECTED   | 0.12 | -0.11 | -0.12           | 0.11 | -0.03 | 0.25 | 0.31 |
|        | OVERALL    | 0.49 | -0.16 | 0.19            | 0.10 | -0.14 | 0.32 | 0.18 |
| HERD B | UNINFECTED | 0.61 | -0.24 | 0.17            | 0.16 | -0.32 | 0.09 | 0.17 |
|        | INFECTED   | 0.70 | -0.53 | 0.04            | 0.27 | -0.34 | 0.77 | 0.69 |
|        | OVERALL    | 0.76 | -0.30 | 0.19            | 0.10 | -0.32 | 0.55 | 0.38 |
| HERD C | UNINFECTED | 0.61 | -0.14 | ND <sup>1</sup> | ND   | -0.57 | 0.40 | 0.28 |
|        | INFECTED   | 0.72 | -0.28 | ND              | ND   | -0.51 | 0.67 | 0.58 |
|        | OVERALL    | 0.72 | -0.20 | ND              | ND   | -0.62 | 0.65 | 0.54 |

1 ND = No data

TABLE 9.4

SUMMARY OF PREVIOUS STUDIES INTO THE ABILITY  
OF THE SOMATIC CELL COUNT TO DISTINGUISH  
BETWEEN INFECTED AND UNINFECTED COWS

| AUTHORS                                                  | THRESHOLD<br>VALUE | FALSE<br>NEGATIVE<br>RESULTS | FALSE<br>POSITIVE<br>RESULTS |
|----------------------------------------------------------|--------------------|------------------------------|------------------------------|
| Renner<br>( 1975 )                                       | 400,000            | 21 %                         | 15 %                         |
| Lindstrom <u>et al</u><br>( 1981 )                       | 250,000            | 50 %                         | 11 %                         |
| Andrews <u>et al</u><br>( 1983 )                         | 250,000            | 2 %                          | 21 %                         |
| Batra and<br>McAllister<br>( 1984 )                      | 250,000            | 35 %                         | 6 %                          |
| Present trial<br>( Bacteriological<br>Classification 1 ) | 185,000            | 25 %                         | 25 %                         |

TABLE 9.5

THE EFFECT OF USING A FIXED THRESHOLD OF 250,000 CELLS PER ML  
OF MILK ON THE DIAGNOSTIC ACCURACY OF THE SOMATIC CELL COUNT

|                                 | HERD         |              |              |
|---------------------------------|--------------|--------------|--------------|
|                                 | A            | B            | C            |
| TOTAL<br>DIAGNOSES              | 209          | 157          | 370          |
| CORRECT<br>DIAGNOSES            | 160 ( 77 % ) | 140 ( 89 % ) | 267 ( 72 % ) |
| FALSE<br>NEGATIVES              | 45 ( 22 % )  | 11 ( 7 % )   | 30 ( 8 % )   |
| FALSE<br>POSITIVES              | 4 ( 2 % )    | 6 ( 4 % )    | 73 ( 20 % )  |
| TYPE 1<br>MISCLASS <sup>1</sup> | 63 %         | 29 %         | 18 %         |
| TYPE 2<br>MISCLASS <sup>2</sup> | 3 %          | 5 %          | 36 %         |
| TOTAL<br>INFECTED               | 72           | 38           | 165          |
| TOTAL<br>UNINFECTED             | 137          | 119          | 205          |

1 TYPE 1 MISCLASS = FALSE NEGATIVES AS A PROPORTION OF TOTAL INFECTED.

2 TYPE 2 MISCLASS = FALSE POSITIVES AS A PROPORTION OF TOTAL UNINFECTED.

## CHAPTER TEN

### SUMMARY AND GENERAL DISCUSSION

#### INTRODUCTION

Despite the time and effort which have been invested in researching the causes, effects, diagnosis and control of bovine mastitis, this disease complex remains a major problem for the dairy industry.

Part of the problem is that only a small fraction of the mastitis cases is evident to the farmer, the remainder exist in the subclinical form, making them more difficult to detect.

While obligate pathogens such as Streptococcus agalactiae may be eliminated from a herd ( Newbould 1975 ), other pathogens exist in the environment, forming a reservoir of infection which cannot be eliminated. Thus, efforts are made to control the disease by:

- (1) Regular checking of the milking machine.
- (2) Dipping or spraying the teats with an antiseptic solution following milk removal.
- (3) Treating clinically infected cows during lactation.
- (4) Culling chronically infected cows.
- (5) Treating subclinically infected cows, or those cows which have shown an increased susceptibility to infection, at the end of the lactation period, ie using dry cow antibiotic therapy.

The first two procedures are preventative, while the third procedure is aimed at curing those infections which are evident to the farmer. Implementation of the fourth and fifth procedures requires that both clinically and subclinically infected cows are identified. It is the identification of the subclinically infected cows which has been the subject of this thesis.

The present study has provided a wealth of information regarding the ability of a number of laboratory tests to discriminate between infected and uninfected cows, or between infected and uninfected udder quarters, under New Zealand conditions of dairy farming.

In the current chapter, these findings are summarised and discussed with respect to practical applications, and the need for further research.

## SUMMARY OF FINDINGS

### (1) CHANGES IN THE CONCENTRATION OF THE SELECTED PARAMETERS IN MILK, DURING THE PROCESS OF MILK REMOVAL

The parameters whose concentration in the milk is increased during infection of the mammary gland, viz the somatic cell count, sodium concentration, and NAGase activity, tended to increase in concentration during the process of milk removal, both within infected and within uninfected glands. The change in concentration during the milking process tended to be greater within infected quarters than within uninfected quarters. Those parameters which tend to decrease in concentration as a result of infection, eg lactose, generally decreased in concentration during the milking process.

These findings suggest that should quarter sampling be undertaken, then to maximise the discrimination between infected and uninfected quarters, the samples should be taken towards the end of the milking process. However, a number of practical problems are associated with the taking of milk samples following cessation of machine milking, ( see chapter four ).

Of greater relevance to the current scheme for mastitis diagnosis, ie the counting of somatic cells in composite milk samples which is offered by the Livestock Improvement Corporation, is the effect of the point in time during the milking process at which the cups are removed from the udder, on the somatic cell count of the milk. The more complete the removal of the milk, the higher will be the somatic cell count in the resulting composite milk sample. While this means a greater potential to distinguish between infected and uninfected cows, problems will arise in selecting a reliable threshold value. The greater adoption of automatic cup-removers might make a standardized milking endpoint easier to achieve, thereby decreasing the effect of the stage of milking on the somatic cell count of the milk.

### (2) DIFFERENCES BETWEEN HERDS WITH RESPECT TO THE MICROBIAL FLORA OF THE MAMMARY GLAND

The three herds which were sampled during the present trial differed with respect to the incidence, average duration of infection, and therefore the average level of infection with major and minor pathogens. In each of the three herds, coagulase positive staphylococci were the most commonly isolated major pathogens. Streptococcus agalactiae was isolated from a single cow within one of the three herds, while coliforms were isolated from one cow in a different herd. Corynebacterium bovis was present within each of the three herds, although the prevalence differed between herds.

The incidence of infection tended to increase with lactation number or age of the cow.

The prevalence of infection with certain pathogens changed during the course of the dairy season, this change being particularly marked with respect to the minor pathogen Corynebacterium bovis.

The percentage of cows and of individual udder quarters which were uninfected decreased during the course of the dairy season.

The average duration of infection for a particular pathogen varied between herds.

The between-herd variability with respect to the prevalence and the average duration of infection with a particular pathogen may reflect differences in virulence between strains of the pathogen, as well as differences in the genetic make-up of the cows within each herd. Another contributory factor may have been differences in the application of mastitis control procedures between the herds, for example differences in the teat spraying of cows following milking.

The results of the present trial show that it is possible to achieve a comparatively low rate of infection within an average sized New Zealand dairy herd, and to maintain that low infection rate during the course of the dairy season ( herd A ).

The increase in the incidence of infection with the age of the cow has been found in previous studies ( see chapter five ), and probably reflects a cumulative effect, the rate of new infection being greater than that of elimination of existing infections, rather than an increasing susceptibility to infection with increasing age. The lower rate of infection within young cows than in their older herdmates suggests that care should be taken to prevent the spread of infection from the latter group. This end might be achieved by milking the younger animals first whenever possible.

The increase in the prevalence of infection during the course of the season probably reflects both the increased exposure to mammary pathogens during the season, and the efficacy of dry cow therapy as a mastitis control technique during the dry and early post-partum periods. Selective dry cow antibiotic therapy was used in each of the three herds prior to the commencement of the present trial. The increase in the prevalence of infection was particularly marked with respect to Corynebacterium bovis during the present trial.

Corynebacterium bovis is susceptible to dry cow therapy, with the cure rate for existing infections exceeding 80 % ( Crist *et al*, 1982 ) However, this minor pathogen has a very high infectivity and quickly recolonizes the gland in the absence of effective teat dipping or spraying ( Honkanen-Buzalski and Bramley, 1984 ).

While the importance of Corynebacterium bovis with respect to decreases in milk yield and changes in milk composition may be debated, infection does cause an increase in the somatic cell count, and cases of clinical mastitis due to this organism have been recorded (Counter, 1981). It may be concluded that in 2 of the 3 herds which were studied during the present trial, the teat spraying was ineffective in controlling the rate of new infections with Corynebacterium bovis, the prevalence of which exceeded 50 % of quarters within these two herds. Thus while the changes in the milk composition which are caused by this organism are smaller in scale than are those changes caused by the major pathogens, the very high prevalence of infection makes Corynebacterium bovis an important cause of reduced milk quality in terms of the bulk milk.

Other organisms, notably some of the major pathogens showed little change in prevalence during the season, with the new infection rate approximately balancing the rate of elimination.

### (3) THE EFFECT OF BACTERIOLOGICAL STATUS ON THE LEVELS OF THE SELECTED PARAMETERS IN MILK

The bacteriological status of the individual udder quarter exerted a statistically significant effect on the level of each of the parameters in the milk sample taken from that quarter, although the effects on changes in certain parameters, notably those of the pH and the potassium concentration were smaller in degree than were the changes in the remaining parameters.

The effect of the bacteriological status of the whole udder on the levels of the parameters within composite milk samples did not reach statistical significance in every instance. The effects of the bacteriological status of the udder on the somatic cell count and on the sodium concentration of the milk were significant within each of the three herds, while the effect on the NAGase activity was statistically significant within two of the three herds. The effects of the bacteriological status of the udder on the concentrations of lactose and of antitrypsin in composite milk samples were significant within one herd only, while the presence of infection did not exert a statistically significant effect on the potassium concentration, the pH or the electrical conductivity of composite milk samples in any of the herds.

The stronger the effect of the bacteriological status on a given parameter, the greater is the likelihood that the parameter will be effective in the discrimination between infected and uninfected quarters, or between infected and uninfected cows.

The levels of a number of parameters, including the somatic cell count, were increased by the presence of minor pathogens, when compared with quarters which were free of bacterial infection. This indicates that the minor pathogens are capable of causing a mild degree of damage to the mammary gland. It has been argued that minor pathogens might serve to protect the mammary gland from infection with major pathogens ( Bramley, 1975; Bramley, 1978 ), although this has been disputed by other authors ( Honkanen-Buzalski *et al.*, 1984 ).

The pertinent question is whether the protective effect is quantitatively more important than the damage which is caused by the minor pathogens. Although the degree of damage which is caused by minor pathogens is less than that typically seen in quarters which are infected with major pathogens, during the present trial as many as 60 % of the udder quarters were shown to harbour minor pathogens, predominantly Corynebacterium bovis. Thus the level of infection which may be attained by minor pathogens can make them quantitatively as important, with respect to their effect on the bulk herd milk, as are the major pathogens.

Another problem created by the minor pathogens is the effect on the discrimination between infected and uninfected quarters or cows. If only those quarters which are infected with major pathogens are actively sought, then the quarters which harbour minor pathogens will be included in the "uninfected quarters", thereby affecting the mean level of the selected parameter for these "uninfected quarters" and decreasing the distinction between the infected and uninfected quarters. If quarters which are infected with either major or minor pathogens are sought, then the effect of the minor pathogens will be to lower the mean level of the parameter within the "infected" quarters, once again decreasing the discrimination between infected and uninfected quarters. Thus the threshold value which is required to distinguish between infected and uninfected quarters or cows will vary with the particular definition of the word "infection".

From the foregoing discussion, it would seem prudent to aim for the control of both major and minor pathogens within the dairy herd, rather than to tolerate or actively promote the colonization of the gland with minor pathogens in the expectation that this will decrease the rate of infection with the major pathogens.

#### (4) THE EFFECT OF THE STAGE OF LACTATION ON THE LEVELS OF THE SELECTED PARAMETERS IN MILK

During the present trial, the effect of the stage of lactation on the level of each of the eight selected parameters in quarter foremilk samples was significant within each of the three herds. The effect of the stage of lactation on the levels of the parameters in composite milk samples also reached significance in many instances.

The level of those parameters which increase as a result of mastitis, eg the somatic cell count, sodium concentration and the NAGase activity tended to increase as the season progressed, while those parameters which decrease in concentration as a result of mastitis, tended to decrease in concentration as the lactation progressed. These changes were evident both within infected and within uninfected quarters, leading to the conclusion that much of the variation may be attributed to physiological factors such as involution of individual lobules within the lactating gland, and the decrease in milk yield which occurs towards the end of the lactation period.

The fact that in the case of a number of parameters, the changes were quantitatively greater within infected than within uninfected glands suggests that infection may exacerbate the natural changes which are taking place within the mammary gland.

As was noted earlier in the thesis, within New Zealand seasonal supply dairy herds, the stage of lactation effects are confounded with those of the season. Since seasonal effects are likely to vary from district to district, and from year to year, as a result of changing climatic influences, the effects on the milk yield and composition are also likely to vary between years, and between geographical areas within any given year.

The increase in the somatic cell count which occurs towards the end of the season is associated with a decrease in the volume of milk which is produced ( Brolund, 1985 ). Thus some of the increase in the somatic cell count, or of the other parameters such as the NAGase activity will be due to simple concentration effects in a smaller volume of milk.

By adjusting the somatic cell count for the milk yield, the effect of the stage of lactation on the somatic cell count might be decreased, making interpretation of the somatic cell count results more reliable. The somatic cell counting scheme which is currently offered by the Livestock Improvement Corporation is usually carried out in conjunction with production testing, thus the relevant milk yield figures will generally be available.

The effect of adjusting for milk yield on the accuracy of diagnosis of subclinical mastitis was not studied during the present trial, but the topic will be addressed during a subsequent research project.

#### (5) THE EFFECT OF AGE OF THE COW ON THE LEVELS OF THE SELECTED PARAMETERS IN THE MILK

The effects of the age of the cow on the levels of a number of selected parameters in the milk from individual quarters were statistically significant during the present trial.

The effects of the age of the cow on the levels of the parameters in composite milk samples failed to reach statistical significance in any instance.

As was noted earlier in the current chapter, the infection rate increased with the age of the cow. Thus the change in the level of a parameter as the cow's age increases is probably due to the increased prevalence of infection, rather than to the effect of age per se.

The results of a previous study in which the cows were followed for a number of years ( Natzke *et al.*, 1972a ) suggest that for uninfected cows, there is little increase in the somatic cell count with age.

Consequently there is no need to adjust the somatic cell count results for the effects of age of the cow.

#### (6) THE DIAGNOSIS OF UDDER INFECTION

##### (i) PREDICTIVE ABILITY OF THE SELECTED PARAMETERS

Of the eight parameters studied, the somatic cell count showed the greatest ability to discriminate between infected and uninfected quarters, and also between infected and uninfected cows, regardless of whether those quarters or cows which were infected with minor pathogens were classed as infected or uninfected.

The specificity of each parameter, ie the ability of the parameter to correctly classify uninfected cows or quarters as being truly uninfected was generally high, with comparatively few false positive results being obtained. However, the sensitivity of the parameters, ie the ability of the parameter to correctly classify infected cows or quarters as being truly infected was generally lower than was the corresponding specificity, the only exception being the somatic cell count, which showed both high sensitivity and high specificity.

The consequences of misdiagnosing the infection status of the cow can be costly. Misdiagnosing an infected cow as being uninfected may result in severe damage to the gland, with a reduction in milk yield, and undesirable changes in the composition of

the milk which is produced. Failure to detect udder infection also results in a continuing reservoir of pathogenic bacteria which may infect other animals within the herd.

Misdiagnosing an uninfected cow as being infected may result in unnecessary expenditure on antibiotics, and ultimately culling of valuable animals from the dairy herd.

For a diagnostic indicator of bovine subclinical mastitis to be of practical value therefore, it must be both sensitive and specific. During the present trial, this ideal was achieved only by the somatic cell count of the milk.

The reason for the greater sensitivity of the somatic cell count than of the other selected parameters probably lies in the nature of the test. The somatic cell count represents an estimate of the total number of cells in the milk. This includes epithelial cells from the walls of the gland, as well as leukocytes which are attracted to the infected gland by the release of chemotaxins during the growth of the bacterial cells. Once initiated, the process of chemotaxis is autocatalytic, with leukocytes themselves releasing chemotaxins. Thus the process of chemotaxis is extremely sensitive.

The other seven selected parameters all reflect damage to the mammary gland, either through demonstration of altered permeability between the blood and the milk, or altered biosynthetic capacity of the mammary gland. The results of the present trial suggest that at some stages of the infection cycle, the multiplying bacteria may cause an influx of leukocytes from the blood, at a point in time when the degree of damage to the gland is insufficient to be detected by the measurement of the levels of the alternative parameters.

The predictive ability of the somatic cell count was high within all three of the herds which were sampled during the present trial. This consistency between herds was not a feature of all of the selected parameters, some being better able to discriminate between infected and uninfected cows or udder quarters within one herd than within the other two herds.

From a practical point of view, a diagnostic indicator of mastitis must be able to distinguish between infected and uninfected cows within the wide range of herds which are found within New Zealand. A diagnostic test which is known to perform well only within certain herds is likely to cause a lack of confidence amongst users of the test. The three herds which were studied during the present trial differed with respect to herd size, milking shed design, use of teat spraying, and the incidence of mastitis, past and present.

During the present trial, the parameters showed little variation in their ability to discriminate between infected and uninfected quarters or cows during the course of the dairy season, providing that stage of lactation effects were taken into account in the establishment of the threshold level for the parameter. This was true of the somatic cell count of the milk, although it must be remembered that samples were not taken within one month of calving, a time at which the somatic cell count has been shown to elevated during previous trials ( Cullen, 1968; Natzke et al, 1972a ).

The consistency in predictive ability during the course of the dairy season reinforces the value of the recommendation by the Livestock Improvement Corporation that individual cow somatic cell counts be carried out at monthly or two-monthly intervals throughout the dairy season. By following the health of the udder during the season, rather than at one point in time only, the chance of detecting a chronic infection is increased.

In addition, those cows which have been infected during part of the season, and in which the infection has been spontaneously eliminated, or cured by therapy, are more likely to be detected than would be the case if sampling on one occasion was carried out. The identification of such cows allows the farmer the option of using dry cow therapy, to reduce the new infection rate over the dry period.

#### (ii) THRESHOLD VALUES OF THE SELECTED PARAMETERS

During the present trial, the critical threshold, ie the cut-off point at which the proportion of false positive and false negative results are equal, was used to discriminate between infected and uninfected cows or quarters.

The critical threshold values for the selected parameters varied between herds, the variation being particularly marked in the case of the somatic cell count of the milk. In general, those parameters which increase in concentration as a result of infection, eg the somatic cell count, the sodium concentration and the NAGase activity tended to show a higher critical threshold within herds B and C, than within herd A, while the opposite was true of the parameters which decrease in concentration as a result of infection.

The effects of previous infections, and the effects of infection with minor pathogens probably contribute to differences between herds with respect to critical threshold values.

The fact that individual dairy herds require different thresholds to discriminate between infected and uninfected cows has implications for the current somatic cell counting service which is provided by the Livestock Improvement Corporation. At present, this

service recommends the use of a threshold value of 250,000 cells per ml for all herds, irrespective of the level of infection within the herd.

The effects of using a fixed threshold for all dairy herds have been addressed within chapters seven and nine.

To provide a consistent sensitivity and specificity within each New Zealand dairy herd, the level of infection within the herds should be taken into account. It is impractical to sample each herd using aseptic techniques, therefore a method of estimating the level of infection within a herd would need to be found.

The bulk milk somatic cell count has been shown to be related to the level of infection within the dairy herd ( Westgarth, 1971 ). Thus the bulk milk somatic cell count could be used to split the herds into several groups, with a common somatic cell count threshold being used on herds within each group.

Further work is required to ascertain the relationship between the bulk milk somatic cell count and the level of infection within New Zealand dairy herds. The bulk milk somatic cell count could be measured in a sample of milk from the vat, or estimated using individual cow somatic cell count figures weighted according to the milk yield of the cow. Naturally, the group to which a particular herd is assigned would vary over time, as a result of changing levels of infection within the herd.

The ability of a given parameter to discriminate between infected and uninfected cows within a particular herd tended to show little variation during the course of the dairy season during the present trial, but the same was not true of the critical threshold. For those parameters which increase in concentration as a result of infection, the critical threshold tended to increase during the lactation period, while for those parameters which decrease in concentration as a result of infection, the critical threshold tended to decrease.

Once again, these findings have implications for the current somatic cell counting scheme, in that the effects of the stage of lactation on the level of the selected parameter must be taken into account when discriminating between infected and uninfected cows.

The use of a fixed threshold within a herd, during the course of the lactation, will not give consistent results with respect to the diagnosis of udder infection. As was suggested earlier, milk yield data could be used to reduce the effects of the stage of lactation on the somatic cell count of the milk.

### (iii) DIAGNOSIS OF UDDER INFECTION USING A COMBINATION OF TESTS

During the present trial, the somatic cell count showed a greater ability to correctly classify udders or individual udder quarters as being infected or uninfected than did the sodium concentration, the potassium concentration, the electrical conductivity, the pH, the lactose concentration, the NAGase activity or the antitrypsin concentration of the milk. Since the parameters measure different facets of the infectious or inflammatory processes, which do not necessarily coincide, the use of a combination of tests has been suggested as having the potential to improve the diagnosis of udder infection ( Kitchen *et al*, 1984b ).

This was not found to be the case during the present trial. The addition of a second test to a model containing the somatic cell count provided little if any increase in the proportion of quarters or of cows which were correctly classified.

For the addition of a second test to be justified economically, the financial benefits attributable to the increased proportion of cows which are correctly classified would have to outweigh the increased costs associated with setting up and running a two-test system. It is highly unlikely that the small increases in diagnostic ability which occurred during the present trial would justify the use of a second test under current circumstances.

### FURTHER POINTS OF INTEREST

#### (1) METHODS OF ESTIMATING THE SOMATIC CELL COUNT

Over the last 100 years, a number of techniques for the estimation of the somatic cell count of milk have been developed. These range from the manual staining and counting of cells using a light microscope to electronic counting methods such as the Coulter counter, or the Fossomatic fluoro-optical counter.

For the past 10 to 12 years, the Livestock Improvement Corporation of the New Zealand Dairy Board has used the Fossomatic method to count the somatic cells in individual cow milk samples. The Fossomatic counter was also used during the current project.

However, several alternative methods for estimating the somatic cell count of milk have been described. These include:

- (a) Flow cytometry ( Lutz et al, 1975; Breer et al, 1976 ).
- (b) Adenosine triphosphate ( ATP ) analysis ( Bossuyt, 1978; Nelson et al, 1985 ).

A major drawback of the Fossomatic fluoro-optical counter is the capital cost of the equipment. It is possible that one of the alternative methods of counting the cells might prove to be less expensive. Emanuelson et al ( 1987 ) have shown the ATP content of the milk to be a reliable indicator of the infection status of the udder quarter. However, problems relating to sample storage may rule out the ATP assay as a practical means of diagnosing udder infection on a commercial scale.

Clearly, developments in the field of somatic cell counting technology should be noted, and further research initiated to ensure that the service which is offered to the dairy farmer is both reliable and cost effective.

## (2) THE DISTINCTION BETWEEN MASTITIS AND UDDER INFECTION

Mastitis is by definition, an inflammatory reaction of the mammary gland. According to the International Dairy Federation ( 1987 ), mastitis may be classified as infectious, traumatic or toxic, with infectious mastitis being the most common form.

Guidelines for the diagnosis of mastitis published by the International Dairy Federation ( 1971 ) take into account both the bacteriological status of the udder, and the somatic cell count of the milk, as shown in table 10.1.

Latent infection occurs when bacteria are isolated from the udder quarter, but the somatic cell count of the milk falls below the threshold value. However, during the course of a chronic infection, the somatic cell count tends to fluctuate, and may fall below the selected threshold on some occasions. Thus a latent infection may be merely a function of the time during the course of the infection cycle at which the sample is taken.

It is also possible that a latent infection represents an infection of the teat canal, rather than of the secretory or ductal tissue within the gland itself. This point raises the question of the practical significance of teat canal infections.

Giesecke and Viljoen ( 1974 ) have classified teat canal infections as being "relevant" if the infection is accompanied by an increase in the somatic cell count, or "irrelevant" if

no increase in the somatic cell count of the milk is evident. In a trial by Du Preez ( 1987 ), 6 % of the quarters were truly mastitic ( ie were infected, showed an elevated somatic cell count and an increased BSA concentration in the milk ) with a similar percentage of the quarters being classified as having a relevant teat canal infection, in which the BSA content of the milk was not elevated. A further 14 % of quarters were deemed to have irrelevant teat canal infections, with bacteria being isolated, but no elevation of the somatic cell count or BSA content of the milk. Disinfectant teat dipping was routinely used following milking.

During an earlier trial by Giesecke and Viljoen ( 1974 ), out of a total of 126 quarters, four showed evidence of irrelevant teat canal infections, while 19 quarters were classified as having relevant teat canal infections.

In a separate trial, Giesecke and Viljoen ( 1974 ) took milk samples over a period of three days, from 23 udder quarters. The mean somatic cell counts of the quarters which were classified as normal, irrelevant teat canal infections, relevant teat canal infections, or truly mastitic are given in table 10.2.

The 14 quarters which were classified as having teat canal infections showed a mean somatic cell count of 1,435,000 cells per ml of milk. Even the quarters which were classified as having irrelevant teat canal infections showed a mean somatic cell count of over 250,000 cells per ml, compared with the normal quarters, in which the milk contained fewer than 100,000 cells per ml. These figures demonstrate that teat canal infections do have a marked effect on the somatic cell count of the milk. Giesecke and Viljoen ( 1974 ) showed that the increase in the somatic cell count of the milk during teat canal infection is probably due to the elaboration and diffusion of toxins during the growth of pathogens within the teat canal.

Akers and Thompson ( 1987 ) stimulated a leukocytosis into the udder using sterile oyster glycogen. These authors concluded that the migration of leukocytes into the udder, regardless of the presence or absence of micro-organisms, disrupts the secretory epithelium and may be responsible for altered composition of milk, and decreased milk production. Thus the leukocytosis which is caused by teat canal infections is also likely to have deleterious effects on the volume and composition of the milk within the gland.

In addition to the effects of the teat canal infections on the composition and the volume of milk which is produced, these localized infections may lead to the establishment of infection within the deeper regions of the mammary gland. Forbes and Herbert ( 1968 ) showed that the majority of intramammary infections caused by staphylococci which developed during the season, were preceded by teat canal infections. Furthermore, it was suggested that intramammary infections may be maintained by teat canal infections.

Similarly, Du Preez ( 1987 ) concluded that teat canal infections serve as potential sources of infection for the udder parenchyma, and that teat canal infections and infections of the parenchyma may exist simultaneously.

The time taken for bacteria in the teat canal to penetrate the defences of the cow, and to establish infection within the udder may vary. Murphy and Stuart ( 1953 ) inoculated the streak canals of 17 udder quarters with Streptococcus agalactiae. Infection of the mammary gland occurred within seven quarters, taking between 2 and 18 milkings for the infections to become established. Beech and Forbes ( 1965 ) reported that teat canal infections caused by Staphylococcus aureus may exist for up to seven weeks before appearing in milk taken from the teat sinus.

Teat canal infections may also exist for long periods of time before being spontaneously eliminated, without infection of the udder being established ( Beech and Forbes, 1965 ). While this might suggest that such teat canal infections are of doubtful importance, it must be remembered that the infected teat canals represent a source of pathogenic bacteria for other quarters within the infected cows, as well as for other cows within the dairy herd. In addition, both the volume and the composition of the milk may be affected during the period of teat canal infection ( see above ).

The diagnosis of a latent infection, using the International Dairy Federation guidelines might also result from contamination of the sample as a result of inadequate aseptic techniques being applied at the time of sampling. Staphylococcus aureus may cause lesions on the exterior surface of the udder, ( McDonald, 1977 ) and these lesions could contaminate the milk sample. However, the close proximity of these lesions to the teat orifice make them a potential source of infection for the interior of the gland.

It is clear, therefore, that both latent udder infection, and infection of the teat canal are potentially important states of udder health.

Non-specific mastitis is defined as the presence of an elevated somatic cell count in quarters from which pathogenic bacteria are not isolated ( International Dairy Federation, 1971 ). This state may result from the failure to demonstrate bacteria which nevertheless are present within the affected quarter. Failure to isolate bacteria may be due to low numbers of viable organisms. Such a situation might be expected to occur when the organisms are present within a lesion which has been walled-off to form an abscess. Abscess formation is commonly a feature of staphylococcal mastitis ( Stabenfeldt and Spencer, 1965 ).

During a chronic infection, the numbers of bacteria per ml of milk tend to vary ( Schalm et al, 1971 ). As the numbers of bacteria increase, the metabolites which are produced stimulate an increase in the number of leukocytes within the milk. The numbers of bacteria then decrease as a result of the increase in phagocytosis. Thus the

failure to isolate pathogenic bacteria, and the consequent classification of the gland as having non-specific mastitis may, like the classification of latent infection, be a function of the time at which the sample is taken.

It is likely that certain cases of non-specific mastitis may be due to either trauma to the mammary gland, or to toxic damage caused by contact of the tissue with chemical irritants. Trauma, or mechanical injury is well recognized as a cause of inflammation ( Curran and Harnden, 1972 ) and in the context of mastitis might result from the udder being trodden on or kicked by other cows. Such damage is likely to be less common in New Zealand than in other dairying countries in which the cows are housed indoors. The effect of physical damage to the udder on the milk yield and composition has not been widely reported in the literature.

A number of substances, when placed within the mammary gland, are capable of causing inflammation. These substances include antibiotics ( Giesecke, 1978 ), oyster glycogen ( Fox *et al*, 1986 ) and some anti-inflammatory agents ( Pyörälä, 1988 ). However, for toxic damage to be a cause of inflammation, the chemical must first pass through the teat canal, which under normal circumstances, is filled by a keratin plug ( Nickerson, 1985 ). The teat canal may remain patent for a period following milk removal, ( Nickerson, 1985 ), hence it is conceivable that teat spraying or dipping with antiseptic solutions might cause some degree of inflammation. However, the low somatic cell counts which are a feature of uninfected udder quarters within herds in which teat spraying or dipping is regularly used suggests that this potential source of toxic damage is not important in practice.

The deliberate infusion of antibiotics into the teat sinus is likely to cause a rise in the somatic cell count ( Giesecke, 1978 ). However, the treatment of cows during the lactation is followed by a withholding period, during which time the milk is discarded. Dry cow therapy is usually given following the final milking of the season. The inflammation which follows infusion of antibiotics into the udder during normal lactation is transient ( Holdaway, unpublished ). Thus the mastitis caused by such treatment is of little practical importance.

Neither traumatic nor toxic factors are likely to cause a lasting inflammation within the mammary gland unless exposure to the particular factor is prolonged or repeated. Infectious mastitis, on the other hand, is usually present for a considerable period of time ( see chapter five ).

While infection of the mammary gland is not tantamount to mastitis, it is clear from the foregoing discussion, that the state of udder health must be considered over a period of time, rather than on the day of sampling alone. In this sense, the isolation of pathogenic bacteria from the milk of a cow will always be significant, since even if no

inflammation is present at the time of sampling, it may well occur at a later date, while in the meantime, the infected udder acts as a source of infection for other cows.

From a practical point of view, the control measures which are available to the dairy farmer, generally rely on either the prevention or treatment of infection, rather than of inflammation. Thus any parameter which is selected for the determination of udder health, should be capable of detecting not only inflammation of the gland, but also the presence or absence of infection. The somatic cell count of the milk is such an indicator, since chemotaxis is stimulated by bacterial metabolic by-products, as well as by damaged tissue. The remaining parameters which were studied during the present trial, ie the concentrations of sodium ions, potassium ions, lactose and antitrypsin, and the pH, electrical conductivity and NAGase activity, measure either the damage to the gland, or the resulting inflammatory response, but not the presence or absence of infection.

By using any of the selected parameters, a large proportion of the established infections which have already caused significant damage to the mammary gland will be detected. Only by using the somatic cell count as the diagnostic test, is there a chance of detecting the infection before this damage can be caused.

### (3) THE RELATIVE MERITS OF INDIVIDUAL UDDER QUARTER SAMPLES, AND OF WHOLE UDDER COMPOSITE SAMPLES FOR THE DIAGNOSIS OF UDDER INFECTION

During the present trial, both individual udder quarter samples, and whole udder composite milk samples were taken, and were used to determine the ability of a number of selected diagnostic tests to discriminate between infected and uninfected quarters, and between infected and uninfected cows respectively.

Infection of the udder may occur within individual quarters, while the other quarters within the infected udder remain uninfected ( see chapter five ). Since the composite milk sample contains milk from each of the four quarters, changes in the concentration of a particular parameter in the milk from an infected quarter will be masked by the milk of normal composition which is produced by the uninfected quarters. Consequently, the rate of detection of infection is likely to be higher when milk samples are taken from individual udder quarters than is the case when the composite samples are used.

However, in practice, the advantage of a higher detection rate for infected cows must be weighed against the increased sampling and analytical costs which are associated with the sampling of individual quarters.

An assessment of the relative economics of taking individual quarter, or whole udder composite milk samples for the diagnosis of udder infection is beyond the scope of this thesis. It must be remembered, however, that the current diagnostic scheme which is operated by the Livestock Improvement Corporation generally involves the estimation of the somatic cell count in composite milk samples which have been obtained for the primary purpose of production testing. Thus any change to the sampling scheme for the diagnosis of udder infection might necessitate the taking of an additional set of samples, thereby generating extra costs and an increase in disruption to normal milking routines.

A change to a quarter based system would also involve a large capital investment with respect to the development and production of sampling equipment.

The results of the present trial ( chapters four, seven and nine ) suggest that the increase in sensitivity which might be achieved by sampling foremilk from individual quarters, over that obtainable with the status quo, ie composite sampling, would be small. A larger increase in sensitivity could be expected if the quarter sample was to be taken at a later stage of the milking process.

An advantage associated with quarter sampling is the fact that a comparison may be made between quarters within the udder, with respect to the level of a parameter in the milk. The use of such interquarter ratios, or interquarter differences for the diagnosis of infection has been advocated by a number of researchers ( Davis, 1947; Davis, 1975; Woolford and Williamson, 1982; Sheldrake *et al*, 1983a; Oshima, 1985; Sandholm and Mattila, 1985; Kangasniemi *et al*, 1986; ).

The rationale behind the use of the interquarter comparison is that non-bacteriological causes of variation in levels of a parameter in the milk are likely to affect all four quarters of the cow similarly.

Had interquarter analyses been undertaken during the present trial, it is likely that the discrimination between infected and uninfected quarters would have been improved over that shown when absolute levels of the parameter were used, since both the stage of the lactation, and the age of the cow were shown to exert a statistically significant effect on the levels of a number of the parameters in quarter milk samples ( chapter six ).

The greatest potential benefits of interquarter comparisons lie in the application of the method to the in-line determination of milk cations or electrical conductivity. The inclusion of four ion-specific electrodes, or conductivity cells in the milking cluster, obviates the need to take milk samples for the diagnosis of udder infection. Should the use of interquarter analysis provide a high degree of sensitivity and specificity, then the

use of in-line diagnostic systems could be a major advance in the diagnosis of subclinical disease. Clearly, more research needs to be carried out in this respect.

\* \* \* \* \*

In conclusion, despite years of research, mastitis, caused principally by udder infection, continues to cause major problems for the dairy industry. While certain pathogens may be eliminated from a herd, emphasis remains on the control of mastitis, rather than on eradication.

Central to the control of mastitis, is the identification of those cows which have subclinical udder infection, or those which have shown themselves to be at risk of further attacks of the disease. Since bacteriology is time consuming, expensive, and requires an aseptically taken milk sample, other indirect tests must be used to diagnose infection.

During the present trial, a number of parameters were compared with respect to their ability to discriminate between infected and uninfected cows and between individual udder quarters. The results show that the somatic cell count of the milk had a higher predictive ability than did any of the alternative parameters, ie the concentrations of sodium, potassium, lactose, or antitrypsin, the pH, electrical conductivity or the NAGase activity of the milk.

The somatic cell count was able to discriminate between infected and uninfected cows in dairy herds with differing levels of infection.

The ability of the somatic cell count to discriminate between infected and uninfected cows was consistent during the course of the lactation, provided that the effects of the stage of lactation on the somatic cell count were taken into account.

The NAGase activity and the sodium concentration of the milk each showed the ability to correctly classify a high percentage of cows with respect to udder infection, but both parameters were less sensitive than was the somatic cell count. It was concluded that the remaining parameters would be ineffective in the diagnosis of subclinical udder infection.

The use of a second test, in addition to the somatic cell count did not generally result in a marked improvement in the predictive ability of the model, and it is unlikely that the use of a two-test system would be justified on economic grounds at the present time.

The Livestock Improvement Corporation currently offers a somatic cell counting service for individual cows, with the recommendation that the tests be carried out on a number of occasions during the season. The results of the present trial suggest that this system has the potential to provide the dairy farmer with valuable information regarding the extent of subclinical udder infection within the herd. However, the threshold levels which are used as a basis for decisions such as the application of dry cow therapy, or culling, should reflect the level of infection which exists in the particular herd.

Use of a fixed threshold value leads to inconsistent results with respect to discrimination between infected and uninfected cows in herds with a high or a low incidence of udder infection. The threshold value for the somatic cell count appears to be increased in herds with a high incidence of udder infection.

Since the stage of lactation affects the somatic cell count of both infected and of uninfected cows, correction for this factor should improve the ability of the somatic cell count to provide consistent results with respect to the diagnosis of subclinical udder infection during the course of the New Zealand dairy season.

While the somatic cell count proved to be the best laboratory test which is currently available for the diagnosis of udder infection, the detection of infection using in-line systems should remain a long term goal for mastitis research.

TABLE 10.1

INTERNATIONAL DAIRY FEDERATION GUIDELINES FOR THE  
DIAGNOSIS OF SUBCLINICAL MASTITIS

| SOMATIC CELL COUNT | PATHOGENIC MICRO-ORGANISMS |                  |
|--------------------|----------------------------|------------------|
|                    | NOT ISOLATED               | ISOLATED         |
| < 500,000          | NORMAL SECRETION           | LATENT INFECTION |
| > 500,000          | NONSPECIFIC MASTITIS       | MASTITIS         |

TABLE 10.2

EFFECT OF INFECTION OF THE UDDER AND OF THE TEAT CANAL  
ON THE SOMATIC CELL COUNT OF THE MILK - RESULTS OF A PREVIOUS TRIAL<sup>1</sup>

| CLASSIFICATION                     | MEAN SOMATIC CELL COUNT OVER<br>THREE SUCCESSIVE DAYS | NUMBER OF QUARTERS |
|------------------------------------|-------------------------------------------------------|--------------------|
| NORMAL SECRETION                   | 90,000                                                | 5                  |
| IRRELEVANT TEAT<br>CANAL INFECTION | 286,000                                               | 6                  |
| RELEVANT TEAT<br>CANAL INFECTION   | 2,296,000                                             | 8                  |
| MASTITIS                           | 5,296,000                                             | 4                  |

1 Adapted from Giesecke and Viljoen ( 1974 )

## BIBLIOGRAPHY

Abu-Lehia, I.H. ( 1987 )

A simple and rapid colourimetric method for lactose determination in milk. Food Chemistry 24 : 233-240.

Adachi, S. and Yamaji, A. ( 1978 )

The determination of lactose in milk: A comparison of gas-liquid chromatography with the Lane and Eynon method. Journal of Dairy Research 45 : 127-129.

Akers, R.M. and Thompson, W. ( 1987 )

Effect of induced leukocyte migration on mammary cell morphology and milk component biosynthesis. Journal of Dairy Science 70 : 1685-1695 .

Anderson, M. and Andrews, A.T. ( 1977 )

Progressive changes in individual milk protein concentrations associated with high somatic cell counts. Journal of Dairy Research 44 : 223-235.

Anderson, M., Brooker, B.E., Andrews, A.T. and Alichanidis, E. ( 1974 )

Membrane material isolated from milk of mastitic and normal cows. Journal of Dairy Science 57 : 1448-1458.

Anderson, M., Brooker, B.E., Andrews, A.T. and Alichanidis, E. ( 1975 )

Membrane material in bovine skim-milk from udder quarters infused with endotoxin and pathogenic organisms. Journal of Dairy Research 42 : 401-417.

Anderson, P.H.R. and Stone, D.M. ( 1955 )

Staphylococcal food poisoning associated with spray-dried milk. Journal of Hygiene , 53 : 387-397.

Andrews, A.T. ( 1983 )

Breakdown of caseins by proteinases in bovine milks with high somatic cell counts arising from mastitis or infusion with bacterial endotoxin. Journal of Dairy Research 50 : 57-66.

Andrews, A.T. and Alichanidis, E. ( 1975 )

The acid phosphatases of bovine leukocytes, plasma and milk of healthy and mastitic cows. Journal of Dairy Research 42 : 391-400.

Andrews, R.J., Kitchen, B.J., Kwee, W.S. and Duncalfe, F. ( 1983 )

Relationship between individual cow somatic cell counts and the mastitis infection status of the udder. Australian Journal of Dairy Technology 38 : 71-74.

Anon ( 1985 )

A bioluminescent screening method for mastitis. Bioluminescence Today, LKB Wallac, Application Note 515.

Ardö, Y. ( 1978 )

The antimicrobial agent bronopol used as a preservative in milk samples to be analysed for fat, protein and cell count. In Proceedings of the XXth International Dairy Congress, Volume E, p 176-177.

Ashworth, U.S., Forster, T.L. and Luedecke, L.O. ( 1967 )

Relationship between California Mastitis Test reaction and composition of milk from opposite quarters. Journal of Dairy Science 50 : 1078-1082.

Balulescu, L. ( 1985 )

An automated ion-selective electrode method for salt determination in foods. Food Technology 39 : 38-40.

Barry, J.G. and Donnelly, W.J. ( 1981 )

The effect of secretory disturbance on casein composition in freshly drawn bovine milks. Journal of Dairy Research 48 : 437-446.

Batra, T.R. and McAllister, A.J. ( 1984 )

A comparison of mastitis detection methods in dairy cattle. Canadian Journal of Animal Science 64 : 305-312.

Beech, J.A. and Forbes, D. ( 1965 )

Pathogens in the streak canal. The Veterinary Record 77 : 1085-1086.

Benson, R.J and Dettmann, E.B. ( 1977 )

The New South Wales Mastitis Control Program 3. The effect on production and economics. Australian Veterinary Journal 53 : 538-541.

Berning, L.M., Paape, M.J. and Miller, R.H. ( 1987a )

Effects of estradiol benzoate and estrus on N-acetyl- $\beta$ -D-glucosaminidase activity and the somatic cell concentration in milk. Journal of Dairy Science 70 : 1302-1306.

- Berning, L.M., Paape, M.J., Miller, R.H. and LeDane, R.A. ( 1987b )  
Variation in N-acetyl- $\beta$ -D-glucosaminidase activity and somatic cell count among various milk fractions. Journal of Dairy Science 70 : 1054-1060.
- Biggs, D.A. ( 1972 )  
Precision and accuracy of infrared milk analysis. Journal of the Association of Official Analytical Chemists 55 : 488-497.
- Black, R.T., Marshall, R.T. and Bourland, C.T. ( 1972 )  
Locus of mammary gland infections of Corynebacterium bovis. Journal of Dairy Science 55 : 413-416.
- Blackburn, P.S. ( 1968 )  
The cell count of cow's milk and the micro-organisms cultured from the milk. Journal of Dairy Research 35 : 59-65.
- Blackiston's Gould Medical Dictionary. ( 1972 )  
Edited by A. Olsen. New York : McGraw-Hill Book Company. 3rd Edition p 914.
- Bodoh, G.W., Battista, W.J., Schultz, L.H. and Johnston, R.P. ( 1976 )  
Variation in somatic cell counts in Dairy Herd Improvement samples. Journal of Dairy Science 59 : 1119-1123.
- Bogin, E. and Ziv, G. ( 1973 )  
Enzymes and minerals in normal and mastitic milks. Cornell Veterinarian 63 : 666-676.
- Bogin, E., Ziv, G., Rivetz, B., Gordon, S. and Saran, A. ( 1977 )  
Distribution of lactate dehydrogenase isoenzymes in normal and inflamed bovine udders and milk. Research in Veterinary Science 22 : 198-200.
- Booth, R.A., Morse, G.E. and Treece, J.M. ( 1965 )  
Relationship of A-esterase activity, blood serum albumin and leukocyte count during experimental exposure to Streptococcus agalactiae ( Cornell strain 48 ). Journal of Dairy Science 48 : 822.
- Bossuyt, R. ( 1978 )  
Usefulness of an ATP assay technique in evaluating the somatic cell content of milk. Milchwissenschaft 33 : 11-13.

**Bramley, A.J. ( 1975 )**

Infection of the udder with coagulase negative micrococci and Corynebacterium bovis. International Dairy Federation Bulletin, Document Number 85 : 377-381.

**Bramley, A.J. ( 1976 )**

Variations in the susceptibility of lactating and nonlactating bovine udders when infused with Escherichia coli. Journal of Dairy Research 43 : 205-211.

**Bramley, A.J. ( 1978 )**

The effect of subclinical Staphylococcus epidermidis infection of the lactating bovine udder on its susceptibility to infection with Streptococcus agalactiae or Escherichia coli. British Veterinary Journal 134 : 146-151.

**Brazis, A.R., Reyes, A.L. Donnelly, C.B., Read, R.B. and Peeler, J.T. ( 1967 )**

Comparison of results of mastitis screening tests of milk from individual and pooled cow quarters. Journal of Dairy Science 50 : 500-504.

**Breer, C. Lutz, H. and Super, B.S. ( 1976 )**

Counting somatic cells in milk with a rapid flow-through cytophotometer. Journal of Milk and Food Technology 39 : 624-627.

**Bremel R.D., Schultz, L.H., Gabler, F.R. and Peters, J. ( 1977 )**

Estimating somatic cells in milk samples by the membrane-filter-DNA procedure . Journal of Food Protection 40 : 32-38.

**Broadhurst, J. and Paley, C. ( 1939 )**

A single-dip stain for the direct examination of milk. American Veterinary Medical Association Journal 94 : 525-526.

**Brolund, L. ( 1985 )**

Cell counts in bovine milk. Causes of variation and applicability for diagnosis of subclinical mastitis. Acta Veterinaria Scandinavica Supplementum 80 : 123p

**Brookbanks, E.O. ( 1966 )**

A report on surveys of the incidence of mastitis infection in New Zealand dairy herds. New Zealand Veterinary Journal 14 : 62-70.

**Brooks, I.B., Luster, G.A. and Easterly, D.G. ( 1970 )**

A procedure for the rapid determination of the major cations in milk by atomic absorption spectrophotometry. Atomic Absorption Newsletter 9 : 93-94.

Brooks, B.W. and Barnum, D.A. ( 1984 )

The susceptibility of bovine udder quarters colonized with Corynebacterium bovis to experimental infection with Staphylococcus aureus or Streptococcus agalactiae. Canadian Journal of Comparative Medicine 48 : 146-150.

Brooks, B.W., Barnum, D.A. and Meek, A.H. ( 1982 )

A survey of mastitis in selected Ontario dairy herds. Canadian Veterinary Journal 23 : 156-159.

Brus, D.H.J. and Jaartsveld, F.H.J. ( 1971a )

Comparison of batches of spray-dried milk powder prepared from milks with a low and a high cell count. Netherlands Milk and Dairy Journal 25 : 221-223.

Brus, D.H.J. and Jaartsveld, F.H.J. ( 1971b )

Comparison of batches of Gouda cheese, made from bulk milk with a low and a high cell count. Netherlands Milk and Dairy Journal 25 : 224-228.

Brus, D.H.J., Jaartsveld, F.H.J., Van Werven, E. and Bannenberg, H.J. ( 1966 )

Comparison of batches of butter prepared from milks with a low and a high cell count. Netherlands Milk and Dairy Journal 20 : 37-47.

Bryan, C.S. ( 1932 )

Examination of milk for streptococci of mastitis. American Journal of Public Health 22 : 749-751.

Bryan, C.S. ( 1935 )

The microscopic diagnosis of infectious mastitis. Veterinary Medicine 30 : 1-7.

Bryan, C.S. and Devereux, E.D. ( 1937 )

A comparison of the microscopic test, Hotis test and blood agar plate in detecting streptococci of mastitis in milk. Cornell Veterinarian 27 : 68-74.

Buchberger, J., Graml, R. and Wolfschoon-Pombo, A.F. ( 1986 )

Untersuchungen zum gefrierpunkt und zur zusammensetzung der milch. Deutsche Molkereizeitung 107 : 1066-1068, 1070-1071.

Cagienard, B. ( 1983 )

Mastitis prevalence and the selection of dry cow therapy programmes in dairy herds. New Zealand Veterinary Journal 31 : 30-31.

Callieri, C.A., Corbellini, C.N., Kuck, A.M., Montero, H.L., Murphy, M.I. and Muset, G.B. ( 1987 )

Comparison of somatic cell count and N-acetyl- $\beta$ -D-glucosaminidase activity of quarter milk samples. Revista de Medicina Veterinaria, Argentina 68 : 69-72 and 74-75.

Capuco, A.V., Paape, M.J. and Nickerson, S.C. ( 1986 )

In vitro study of polymorphonuclear leukocyte damage to mammary tissues of lactating cows. American Journal of Veterinary Research 47 : 663-668.

Chamings, R.J., Murray, G. and Booth, J.M. ( 1984 )

Use of a conductivity meter for the detection of subclinical mastitis. The Veterinary Record 114 : 243-245.

Christensen, W.B. ( 1946 )

Urea decomposition as a means of differentiating proteus and paracolon cultures from each other and from salmonella and shigella. Journal of Bacteriology 52 : 461-466.

Christie, R., Atkins, N.E. and Munch-Petersen, E. ( 1944 )

A note on a lytic phenomenon shown by group B streptococci. Australian Journal of Experimental Biology and Medical Science 22 : 197-200.

Cobb, R.W. and Walley, J.K. ( 1962 )

Corynebacterium bovis as a probable cause of bovine mastitis. The Veterinary Record 74 : 101-102.

Conner, J.G., Eckersall, P.D., Doherty, M. and Douglas, T.A. ( 1986 )

Acute phase response and mastitis in the cow. Research in Veterinary Science 41 : 126-128.

Corbellini, C.N. ( 1984 )

The determination of sodium and potassium in milk and its relation with the concentration of somatic cells, and its use in the monitoring of subclinical mastitis. International Dairy Federation Bulletin, Document Number 177 : 161.

Corbellini, C.N., Kuck, A.M., Miquet, J., Montero, H.L., Murphy, M.I., Muset, G.B. and Vena, M.M. ( 1987 )

Relationship between N-acetyl- $\beta$ -D-glucosaminidase concentration in milk and infection in udder quarters. Revista de Medicina Veterinaria Argentina 68 : 132-140.

Counter, D.E. ( 1981 )

Outbreak of bovine mastitis associated with Corynebacterium bovis. The Veterinary Record 108 : 560-561.

Cousins, C.M. and Bramley, A.J. ( 1981 )

The microbiology of raw milk. In Dairy Microbiology - Volume 1, The microbiology of milk. Edited by Robinson, J.O., Essex, England : Applied Science Publishers. p 119-163.

Cowan, S.T. and Steel, K.J. ( 1974 )

Identification of medical bacteria. Cambridge : Cambridge University Press. Second edition. 238 p.

Crist, W.L., Harmon, R.J., Hemken R.W. and Langlois, B.E. ( 1982 )

Effect of intramammary dry treatment on prevalence of minor udder pathogens at parturition. Journal of Dairy Science 65 Suppl 1 : 161-162.

Cullen, G.A. ( 1967a )

A method of counting cells in milk using an electronic cell counter. The Veterinary Record 80 : 188-195.

Cullen, G.A. ( 1967b )

Short term variations in the cell count of cow's milk. The Veterinary Record 80 : 649-653.

Cullen, G.A. ( 1968 )

Cell counts throughout lactation. The Veterinary Record 83 : 125-128.

Curran, R.C. and Harnden, D.G. ( 1972 )

The pathological basis of medicine. London: William Heinemann Medical Books Limited : 681p.

Daniel, R.C.W. and Fielden, E.D. ( 1971 )

The relationship between Wisconsin Mastitis Test scores on cow composite milk samples and milk production. New Zealand Veterinary Journal 19 : 157-159.

Daniel, R.C.W., Smith, G.C. and Barnum, D.A. ( 1966 )

The relationship of California Mastitis Test ( CMT ) scores with leukocyte counts on bucket milk samples. Canadian Veterinary Journal 7 : 80-83.

Daniel, R.C.W., Fielden, E.D. and Munford, R.E. ( 1971 )

An evaluation of the Wisconsin Mastitis Test as an aid in mastitis control programmes. New Zealand Veterinary Journal 19 : 155-156.

Daniel, S. and Pavithran, K. ( 1985 )

Chemical constituents affecting osmolality of milk in cross-bred cattle. Kerala Journal of Veterinary Science 16 : 49-60.

Davies, W.L. ( 1938 )

The chloride content of milk. Journal of Dairy Research 9 : 327-335.

Davis, J.G. ( 1947 )

The rapid abnormality detector. Dairy Industries 12 : 35-40.

Davis, J.G. ( 1975 )

The detection of subclinical mastitis by electrical conductivity measurements. Dairy Industries International 40 : 286-291.

Deeth, H.C. and Fitzgerald, C.H. ( 1975 )

Factors governing the susceptibility of milk to spontaneous lipolysis. International Dairy Federation Bulletin, Document Number 85 : 24-34.

De Langen, H. ( 1967 )

Determination of DNA in milk. Australian Journal of Dairy Technology 22 : 36-40.

De Rham, O. and Andrews, A.T. ( 1982 )

Qualitative and quantitative determination of proteolysis in mastitic milks. Journal of Dairy Research 49 : 587-596.

Dijkman, A.J., Breunissen, H., van der Laar, J.M.J., Perriens, J., Verbene, H. and Jaartsveld, F.H.J. ( 1979 )

Entrapped air as a cause of erroneous milk cell counts. Netherlands Milk and Dairy Journal 33 : 155-157.

Dill, C.W., McGill, R., Lane, G.T., Bryant, J.N. and Thompson, Y. ( 1972 )

Distribution changes in bovine skim milk proteins during forced milk release. Journal of Dairy Science 55 : 200-203.

Dobbins, C.N. ( 1977 )

Mastitis losses. Journal of the American Veterinary Medical Association 170 : 1129-1132.

Dodd, F.H. ( 1971 )

The strategy of mastitis control. The Control of Bovine Mastitis. Edited by Dodd, F.H. and Jackson, E.R. The National Institute for Research in Dairying, Shinfield, Reading, England. p 7-13.

Dodd, F.H. ( 1981 )

Laboratory methods for use in mastitis work. International Dairy Federation Bulletin, Document Number 132 : 1-27.

Dodd, F.H. and Neave, F.K. ( 1970 )

Mastitis control. National Institute for Research in Dairying, Biennial Reviews Number 3559 : 21-60.

Dohoo, I.R. and Meek, A.H. ( 1982 )

Somatic cell counts in bovine milk. Canadian Veterinary Journal 23 : 119-125.

Duirs, G.F. and MacMillan, K.L. ( 1979 )

Interrelationships between somatic cell counts, production, age and mastitis organisms in individual cows. In Proceedings of the New Zealand Society of Animal Production 39 : 175-179.

Duitschaeffer, C.L. and Ashton, G.C. ( 1972 )

Variations of somatic cells and neutrophils in milk throughout lactation. Journal of Milk and Food Technology 35 : 197-202.

Dulin, A.M., Paape, M.J. and Capuco, A.V. ( 1984 )

Activity of NAGase in milk. Contribution by milk somatic cells. Journal of Dairy Science 67 suppl 1 : 253.

Du Preez, J.H. ( 1987 )

Comparison of various criteria for determining the health status of the bovine udder. Journal of the South African Veterinary Association 58 : 9-14.

Du Preez, J.H., Greeff, A.S. and Eksteen, N. ( 1981 )

Isolation and significance of anaerobic bacteria isolated from cases of bovine mastitis. Onderstepoort Journal of Veterinary Research 48 : 123-126.

Easterday, B.C., Simon, J. and Hanson, R.P. ( 1958 )

The use of the modified Whiteside test as a screen test for bovine mastitis. Journal of the American Veterinary Medical Association 133 : 470-473.

Eberhart, R.J. ( 1977 )

Coliform mastitis. Journal of the American Veterinary Medical Association 170 : 1160-1163.

Edwards, S.J. ( 1933 )

A selective medium for the diagnosis of streptococcus mastitis. Journal of Comparative Pathology and Therapeutics 46 : 211-217.

Edwards, S.J. ( 1938 )

The diagnosis of streptococcal mastitis by cultural methods. Journal of Comparative Pathology and Therapeutics 51 : 250-263.

Elliot, R.E.W., Tattersfield, J.G. and Brookbanks, E.O. ( 1976 )

New Zealand National Mastitis Survey: 1965-1966. 2 Measures of mastitis prevalence. New Zealand Veterinary Journal 24 : 40-44.

Emanuelson, U. and Persson, E. ( 1984 )

Studies on somatic cell counts in milk from Swedish dairy cows. 1) Non-genetic causes of variation in monthly test-day results. Acta Agricultura Scandinavica 34 : 33-44.

Emanuelson, U., Olsson, T., Holmberg, O., Hageltorn, M., Mattila, T., Nelson, L. and Åström, G. ( 1987 )

Comparison of some screening tests for detecting mastitis. Journal of Dairy Science 70 : 880-887.

Emanuelson, U., Olsson, T., Mattila, T., Åström, G. and Holmberg, O. ( 1988 )

Effects of parity and stage of lactation on adenosine triphosphate, somatic cell count and antitrypsin content in cow's milk. Journal of Dairy Research 55 : 49-55.

Euger, J.R. and Brunner, J.R. ( 1979 )

Determination of lactose in milk products by high performance chromatography. Journal of Dairy Science 62 : 685-690.

Fay, A.C., Cave, H.W. and Atkeson, F.W. ( 1938 )

Detection of mastitis by the brom-thymol-blue test, leukocyte count and the microscopic examination of incubated samples of milk. Cornell Veterinarian 28 : 40-50.

Feagan J.T., Griffin, A.T. and Lloyd, G.T. ( 1966a )

Effects of subclinical mastitis on heat stability of skimmilk and skimmilk powder. Journal of Dairy Science 49 : 940-944.

Feagan, J.T., Griffin, A.T. and Lloyd, G.T. ( 1966b )

Effects of subclinical mastitis on heat stability of fluid milk. Journal of Dairy Science 49 : 933-939.

Fernando, R.S. and Spahr, S.L. ( 1983 )

Effects of milking interval on selected milk constituents from normal and infected quarters. Journal of Dairy Science 66 : 1155-1161.

Fernando, R.S., Rindsig, R.B. and Spahr, S.L. ( 1981 )

Effect of length of milking interval and fat content on milk conductivity and its use for detecting mastitis. Journal of Dairy Science 64 : 678-682.

Fernando, R.S., Rindsig, R.B. and Spahr, S.L. ( 1982 )

Electrical conductivity of milk for detection of mastitis. Journal of Dairy Science 65 : 659-664.

Fernando, R.S., Spahr, S.L. and Jaster, E.H. ( 1985 )

Comparison of electrical conductivity of milk with other indirect methods for the detection of subclinical mastitis. Journal of Dairy Science 68 : 449-456.

Finch, L.A. and Martin, D.R. ( 1984 )

Human and bovine group B streptococci: two distinct populations. Journal of Applied Bacteriology 57 : 273-278.

Fitzgerald, C.H., Deeth, H.C. and Kitchen, B.J. ( 1981 )

The relationship between the levels of free fatty acids, lipoprotein lipase, carboxyesterase, NAGase, somatic cell count and other mastitis indices in bovine milk. Journal of Dairy Research 48 : 253-265.

Fleet, I.R., Linzell, J.L. and Peaker, M. ( 1972 )

The use of an autoanalyser for the rapid analysis of milk constituents affected by mastitis. British Veterinary Journal 128 : 297-300.

Forbes, D. and Herbert, C.N. ( 1968 )

Studies in the pathogenesis of staphylococcal mastitis. The Veterinary Record 82 : 69-73.

Forster, T.L., Montgomery, M.W. and Montoure, J.E. ( 1961 )

Some factors influencing the activity of A-, B-, and C- esterases of bovine milk. Journal of Dairy Science 44 : 1420-1430.

Forster, T.L., Ashworth, U.S. and Luedecke, L.O. ( 1967 )

Relationship between California Mastitis Test reaction and production and composition of milk from opposite quarters. Journal of Dairy Science 50 : 675-682.

Fox, L.K., Timms, L.L. and Schultz, L.H. ( 1986 )

Changes in bovine milk secretion following intramammary infusions of concanavalin A, oyster glycogen, or water. Journal of Dairy Science 69 : 1259-1269.

Fox, L.K., Hancock, D.D., McDonald, J.S. and Gaskins, C.T. ( 1988 )

N-acetyl- $\beta$ -D-glucosaminidase activity in whole milk and milk fractions. Journal of Dairy Science 71 : 2915-2922.

Funk, C.D., Schultz, L.H. and Barr, G.R. ( 1967 )

Investigations on possible use of mastitis screening tests in Dairy Herd Improvement Association Central Laboratories. Journal of Dairy Science 50 : 47-52.

Gebre-Egziabher, A., Wood, H.C., Rober, J.D. and Blankenagel, G. ( 1979 )

Evaluation of automatic mastitis detection equipment. Journal of Dairy Science 62 : 1108-1114.

Giesecke, W.H. ( 1978 )

Therapy of bovine mastitis: the intramammary tissue compatibility of mastitis remedies administered intracisternally to cows. Onderstepoort Journal of Veterinary Research 45 : 107-118.

Giesecke, W.H. and van den Heever, L.W. ( 1967 )

The diagnosis of mastitis by direct and indirect cytological methods. Journal of the South African Veterinary Medical Association 38 : 16-21.

Giesecke, W.H. and van den Heever, L.W. ( 1974 )

The diagnosis of bovine mastitis with particular reference to subclinical mastitis: A critical review of relevant literature. Onderstepoort Journal of Veterinary Research 41 : 169-212.

Giesecke, W.H. and Viljoen, M.H. ( 1974 )

The diagnosis of subclinical mastitis in lactating cows: a comparison of cytological methods and a monovalent radial immunodiffusion test. Onderstepoort Journal of Veterinary Research 41 : 51-74.

Gill, M.S. and Holmes, C.W. ( 1978 )

Somatic cell counts, mastitis and milk production in dairy herds. New Zealand Journal of Dairy Science and Technology 13 : 157-161.

Ginn, R.E., Packard, V.S. and Thompson, D.R. ( 1979 )

Comparison of the automated with the semi-automated Coulter counter method and the Direct Microscopic Somatic Cell Count on raw milk samples. Journal of Food Protection 42 : 567-568.

Grandison, A.S. and Ford, G.D. ( 1986 )

Effects of variations in somatic cell count on the rennet coagulation properties of milk and on the yield, composition and quality of cheddar cheese. Journal of Dairy Research 53 : 645-655.

Grappin, R., Packard, V.S. and Ginn, R.E. ( 1980 )

Repeatability and accuracy of the dye-binding and infrared methods for analysing protein and other milk constituents. Journal of Food Protection 43 : 374-375.

Gray, J.D.A. ( 1935 )

An aerobic acid-fast actinomyces in cow's milk due to an udder infection. Journal of Dairy Research 6 : 49-55.

Greatrix, G.R., Quayle, J.C. and Coombe, R.A. ( 1968 )

The detection of abnormal milk by electrical means. Journal of Dairy Research 35 : 213-221.

Green, T.J. and Middleton, L. ( 1984 )

Evaluation of LATA mastitis detector. The Veterinary Record 14 : 616.

Greer, D.O. and Pearson, J.K.L. ( 1976 )

Reproducibility of electronic cell counts in milk; a study of 5 further factors. Journal of Dairy Research 43 : 371-379.

Griffin, T.K., Natzke, R.P. and Neave, F.K. ( 1974 )

A comparative assessment of bovine intramammary infection by bacteriological and somatic cell determination. In Proceedings of the 19th International Dairy Congress, New Delhi, Vol 1E, p 126.

Griffin, T.K., Dodd, F.H., Neave, F.K., Westgarth, D.R., Kingwell, R.G. and Wilson, C.D. ( 1977 )

A method of diagnosing intramammary infection in dairy cows for large experiments. Journal of Dairy Research 44 : 25-45.

Griffin, T.K., Morant, S.V. and Dodd, F.H. ( 1987 )

Diagnosing infectious subclinical mastitis in surveys or large scale experiments. International Dairy Federation Bulletin, Document Number 211 : 9-24.

Gudding, R. ( 1982 )

Increased free fatty acid concentrations in mastitic milk. Journal of Food Protection 45 : 1143-1144.

Haenlein, G.F.W., Schultz, L.H. and Zikakis, J.P. ( 1973 )

Composition of proteins in milk with varying leukocyte counts. Journal of Dairy Science 56 : 1017-1024.

Hale, H.H., Helmbolt, C.F., Plastringe, W.N. and Stula, E.F. ( 1962 )

Bovine mastitis caused by a mycoplasma species. Cornell Veterinarian 52 : 582-591.

Hamer, W.H. and Jones, T.H. ( 1909 )

Report on an epidemic of scarlet fever in London and Surrey due to an infected milk supply. Journal of Comparative Pathology and Therapeutics 22 : 363-377.

Harmon, R.J. and Newbould, F.H.S. ( 1980 )

Neutrophil leukocyte as a source of lactoferrin in bovine milk. American Journal of Veterinary Research 41 : 1603-1606.

Harmon, R.J., Schanbacher, F.L., Fergusson, L.C. and Smith, K.L. ( 1975 )

Concentration of lactoferrin in milk of normal lactating cows and changes occurring during mastitis. American Journal of Veterinary Research 36 : 1001-1007.

Harmon, R.J., Schanbacher, F.L., Fergusson, L.C. and Smith, K.L. ( 1976 )

Changes in lactoferrin, immunoglobulin-G, bovine serum albumin and  $\alpha$ -lactalbumin during acute experimental and natural coliform mastitis in cows. Infection and Immunity 13 : 533-542.

Heald C.W. ( 1982 )

Checking electronic somatic cell count machines. Dairy and Food Sanitation 2 : 276-279.

Heald C.W., Jones, G.M., Nickerson, S.C., Patterson, W.N., Vinson, W.E. ( 1977 )

Preliminary evaluation of the Fossomatic somatic cell counter for analysis of individual cow samples in a central testing laboratory. Journal of Food Protection 40 : 523-526.

Heeschen, W. ( 1975 )

Determination of somatic cells in milk ( technical aspects of counting ). International Dairy Federation Bulletin, Document Number 85 : 79-92.

Herrington, B.L. and Kleyn, D.H. ( 1960 )

A potentiometric method for the determination of chloride in milk. Journal of Dairy Science 43 : 1050-1057.

Hinton, C.L. and Macara, T. ( 1927 )

The determination of aldose sugars by means of chloramine-T, with special reference to the analysis of milk products. The Analyst 52 : 668-688.

Hoare, R.J.T. ( 1982 )

Mastitis: Its effect on milk yield, composition and quality. In Proceedings of the Conference on Dairy Production from Pasture, New Zealand and Australian Societies of Animal Production p 85-103.

Hodges, R.T., Jones, Y.S. and Holland, J.T.S. ( 1984 )

Characterisation of staphylococci associated with clinical and subclinical bovine mastitis. New Zealand Veterinary Journal 32 : 141-145.

Hodges, R.T., Holland, J.T.S., Neilson, F.J.A. and Wallace, N.M. ( 1985 )

Prototheca zopfii mastitis in a herd of dairy cows. New Zealand Veterinary Journal 33 : 108-111.

Hodgson, D.J. ( 1978 )

Calibration of the Fossomatic cell counter. In Proceedings of the XXth International Dairy Congress, Volume E, p 188-189.

Hogan, J.S., White, D.G. and Pankey, J.W. ( 1987a )

Effect of teat dipping on intramammary infections by staphylococci other than Staphylococcus aureus. Journal of Dairy Science 70 : 873-879.

Hogan, J.S., Pankey, J.W. and Duthie, A.H. ( 1987b )

Growth responses of Staphylococcus aureus and Streptococcus agalactiae to Corynebacterium bovis metabolites. Journal of Dairy Science 70 : 1294-1301.

Holmes, C.W. and Gill, M.S. ( 1978 )

Seminar on Mastitis by Glaxo ( New Zealand ) Ltd, Hamilton, New Zealand ( unpublished ).

Holt, C. ( 1985 )

The milk salts: Their secretion, concentrations and physical chemistry. Developments in Dairy Chemistry - 3. Edited by Fox, P.F. Elsevier Applied Science Publishers, London. p 143-181.

Honkanen-Buzalski, T. and Bramley, A.J. ( 1984 )

Observations on Corynebacterium bovis infection of the bovine mammary gland. II. Experimental infection. Journal of Dairy Research 51 : 379-385.

Honkanen-Buzalski, T. and Sandholm, M. ( 1981 )

Trypsin-inhibitors in mastitic milk and colostrum: Correlation between trypsin-inhibitor capacity, bovine serum albumin and somatic cell counts. Journal of Dairy Research 48 : 213-223.

Honkanen-Buzalski, T., Griffin, T.K. and Dodd, F.H. ( 1984 )

Observations on Corynebacterium bovis infection of the bovine mammary gland. I. Natural infection. Journal of Dairy Research 51 : 371-378.

Hopkirk, C.S.M. ( 1972 )

Prevention of mastitis in cows. New Zealand Veterinary Journal 20 : 43-46.

**Hume, C.M. ( 1941 )**

Studies on the detection of mastitis in New Zealand dairy herds. I A field outfit for the bromthymol blue test for mastitis. New Zealand Journal of Science and Technology 22 : 322-327.

**Hutjens, M.F., Schultz, L.H., Ward, G.E. and Yamdagni, S. ( 1970 )**

Estimation of somatic cells in milk using membrane filter separation and DNA determination with diphenylamine. Journal of Milk and Food Technology 33 : 227-231.

**Hwang, M.N. and Ederer, G.M. ( 1975 )**

Rapid hippurate hydrolysis method for the presumptive identification of group B streptococci. Journal of Clinical Microbiology 1 : 114-115.

**International Dairy Federation ( 1971 )**

A monograph on bovine mastitis. Part 1 - Economics, aetiology and diagnosis. p 1-32.

**International Dairy Federation ( 1987 )**

Bovine mastitis. Definition and guidelines for diagnosis. International Dairy Federation Bulletin Number 211 : 3-17.

**International Union of Biochemistry ( 1979 )**

Enzyme Nomenclature. New York : Academic Press, 606 pages.

**Jaartsveld, F.H.J., van Puffelin, E., Oskam, J., Tielen, M.J.M., Verstegen, M.W.A. and Albers, G.A.A. ( 1983 )**

Somatic cell counts in milk of dairy cows in relation to stage of lactation, age, production level and presence of pathogens. Netherlands Milk and Dairy Journal 37 : 79-90.

**Janzen, J.J. ( 1969 )**

Evaluation of five screening tests used for estimating leukocyte counts in bulk milk. Journal of Dairy Science 52 : 329-334.

**Janzen, J.J. ( 1970 )**

Economic losses resulting from mastitis. A review. Journal of Dairy Science 53 : 1151-1161.

Janzen, J.J. ( 1972 )

The effect of somatic cell concentration in the raw milk on shelf life of the processed product. Journal of Milk and Food Technology 35 : 112-114.

Janzen, J.J. and Northern, W.L. ( 1972 )

The role of somatic cell concentration in milk flavour preference and acceptance. In Proceedings of the 67th annual meeting of the American Dairy Science Association : 1-3.

Jasper, D.E. ( 1976 )

Coliform mastitis in pastured herds. Theriogenology 6 : 175-181.

Jasper, D.E. ( 1977 )

Mycoplasma and mycoplasma mastitis. Journal of the American Veterinary Medical Association 170 : 1167-1172.

Jaynes, H.O. and Asan, T. ( 1973 )

Determination of lactose in milk by gas-liquid chromatography. Journal of Milk and Food Technology 36 : 333-336.

Jeffrey, D.C. and Wilson, J. ( 1987 )

Effect of mastitis-related bacteria on total bacterial count of bulk milk supplies. Journal of the Society of Dairy Technology 40 : 23-26.

Kangasniemi, R., Pelkonen, S. and Tanhuanpää, E. ( 1986 )

Absolute and relative effects of bacteria on the level of cell count, NAGase, BSA, Na, K and electrical conductivity in quarter foremilk of Ayrshire-cows. In Proceedings of the Symposium on Mastitis Control and Hygienic Milk Production, Espoo, Finland : 173-178.

Kennedy, B.W., Sethar, M.S., Tong, A.K.W., Moxley, J.E. and Downey, B.R. ( 1982 )

Environmental factors influencing test day somatic cell counts in Holsteins. Journal of Dairy Science 65 : 275-280.

Kiddy, C.A., Groves, M.L., McCann, R.E. and Treece, J.M. ( 1968 )

Effect of udder inflammation on electrophoretic pattern of cow's milk protein. Journal of Dairy Science 51 : 954.

Kisza, J. and Kruk, A. ( 1970 )

Changes in the heat stability of the proteins in mastitic milk. In Proceedings of the XVIIIth International Dairy Congress, Vol 1E, p 527.

Kitchen, B.J. ( 1976 )

Enzymic methods for the estimation of the somatic cell count in bovine milk. Journal of Dairy Research 43 : 251-258.

Kitchen, B.J. ( 1981 )

Review of the progress of dairy science: Bovine mastitis: Milk compositional changes and related diagnostic tests. Journal of Dairy Research 48 : 167-188.

Kitchen, B.J. and Middleton, G. ( 1976a )

A rapid enzymic test for the estimation of the somatic cell count of milk. Australian Veterinary Journal 52 : 102.

Kitchen, B.J. and Middleton, G. ( 1976b )

Enzymic methods for the estimation of the somatic cell count in bovine milk. II N-acetyl- $\beta$ -D-glucosaminidase test for routine estimation of the somatic cell count in milk. Journal of Dairy Research 43 : 491-494.

Kitchen, B.J., Taylor, G.C. and White, I.C. ( 1970 )

Milk enzymes - their distribution and activity. Journal of Dairy Research 37 : 279-288.

Kitchen, B.J., Masters, C.J. and Winzor, D.J. ( 1973 )

Effects of lipid removal on the molecular size and kinetic properties of bovine plasma aryl-esterase. Biochemistry Journal 135 : 93-99.

Kitchen, B.J., Durward, I.G., Middleton, G. and Andrews, R.J. ( 1978a )

Untitled paper. In Proceedings of the XXth International Dairy Congress, Paris, France. p 179-180.

Kitchen, B.J., Middleton, G. Salmon, M. ( 1978b )

Bovine milk N-acetyl- $\beta$ -D-glucosaminidase and its significance in the detection of abnormal udder secretions. Journal of Dairy Research 45 : 15-20.

Kitchen, B.J., Middleton, G., Durward, I.G., Andrews, R.J. and Salmon, M.C. ( 1980 )

Mastitis diagnostic tests to estimate mammary gland epithelial cell damage. Journal of Dairy Science 63 : 978-983.

- Kitchen, B.J., Seng Kwee, W., Middleton, G. and Andrews, R.J.T. ( 1984a )  
Relationship between the level of N-acetyl- $\beta$ -D-glucosaminidase ( NAGase ) in bovine milk and the presence of mastitis pathogens. Journal of Dairy Research 51 : 11-16.
- Kitchen, B.J., Middleton, G., Seng Kwee, W. and Andrews, R.J. ( 1984b )  
N-acetyl- $\beta$ -D-glucosaminidase ( NAGase ) levels in bulk herd milk. Journal of Dairy Research 51 : 227-232.
- Kossila, V. ( 1967 )  
Effect of lactation period, age and udder inflammation on the pH, titratable acidity and mastitis reaction in freshly drawn milk. Karjantuote 50 : 450-456.
- Kostov, L. and Dzhurov, T. ( 1968 )  
Some biochemical and chemical changes in milk due to subclinical mastitis. Dairy Science Abstracts 31 : 1441.
- Kowalski, Z.E. and Giesecke, W.H. ( 1986 )  
A high performance liquid chromatographic method for the fluorimetric determination of lactose, galactose and glucose in normal and abnormal milk of cows. Onderstepoort Journal of Veterinary Research 53 : 225-229.
- Kramer, V.R., Lederer, J., Frank, W. and Seefeldt, G. ( 1980 )  
Lactose und zellgehalt von einzelgemelksproben in abhangigkeit von euterinfektionen und systematischen einflussen. Milchwissenschaft 35 : 136-140.
- Kroger, D. and Jasper, D.E. ( 1967a )  
Relationships between Wisconsin Mastitis Test scores and cell counts in milk. Journal of Dairy Science 50 : 1226-1233.
- Kroger, D. and Jasper, D.E. ( 1967b )  
Effect of milk age, storage, and testing temperature upon the Wisconsin Mastitis Test score. Journal of Dairy Science 50 : 833-836.
- Labuschagne, J.H. ( 1976 )  
Bepaling van die pH in suiwelprodukte. South African Journal of Dairy Technology 8 : 87-91.
- Lascelles, A.K. and Lee, C.S. ( 1978 )  
Involution of the mammary gland. In Lactation - a Comprehensive Treatise, Volume IV. Edited by Larson, B.L., Academic Press, New York p 115-177.

Lawrence, A.J. ( 1968 )

The determination of lactose in milk products. Australian Journal of Dairy Technology 23 : 103.

Lee, C.S. and Frost, A.J. ( 1970 )

Mastitis in slaughtered dairy cows. Australian Veterinary Journal 46 : 201-203.

Lee, C.S., McDowell, G.H. and Lascelles. A.K. ( 1969 )

The importance of macrophages in the removal of fat from the involuting mammary gland. Research in Veterinary Science 10 : 34-38.

Lennette, E.D. ( 1980 )

Manual of clinical microbiology. Washington D.C. : American Society for Microbiology. Third edition, 1044 p.

Lindström, U.B., Kenttämies, H., Arstilla, J. and Tuovila, R. ( 1981 )

Usefulness of cell counts in predicting bovine mastitis. Acta Agricultura Scandinavica 31 : 199-203.

Linzell, J.L. ( 1975 )

The physiological background to subclinical mastitis. Veterinary Annual 15 : 42-46.

Linzell, J.L. and Peaker, M. ( 1971 )

Early detection of mastitis. The Veterinary Record 89 : 393-394.

Linzell, J.L. and Peaker, M. ( 1972 )

Day to day variations in milk composition in the goat and cow as a guide to the detection of subclinical mastitis. British Veterinary Journal 128 : 284-295.

Linzell, J.L. and Peaker, M. ( 1975 )

Efficacy of the measurement of the electrical conductivity of milk for the detection of subclinical mastitis in cows: Detection of infected cows at a single visit. British Veterinary Journal 131 : 447-460.

Linzell, J.L., Peaker, M. and Rowell, J.G. ( 1974 )

Electrical conductivity of foremilk for detecting subclinical mastitis in cows. Journal of Agricultural Science 83 : 309-325.

Little, T.W.A., Herbert, C.N. and Forbes, D. ( 1968 )

Electrical conductivity and the leukocyte count of bovine milk. The Veterinary Record 82 : 431-433.

Lutz, H., Breer, C. and Meyer, B. ( 1975 )

Counting somatic cells in milk with a flow-through cytophotometer. International Dairy Federation Bulletin, Document Number 85 : 130-132

McDermott, M.P., Erb, H.N. and Natzke, R.P. ( 1982 )

Predictability by somatic cell counts related to prevalence of intramammary infection within herds. Journal of Dairy Science 65 : 1535-1539.

McDonald, J.S. ( 1977 )

Streptococcal and staphylococcal mastitis. Journal of the American Veterinary Medical Association 170 : 1157-1159.

McDowell, F.H. ( 1941 )

Studies on the detection of mastitis in New Zealand dairy herds. II Factors influencing the bromothymol blue test for mastitis. New Zealand Journal of Science and Technology 22 : 328-337.

McDowell, F.H. ( 1945 )

Studies on the detection of mastitis in New Zealand dairy herds. New Zealand Journal of Science and Technology 27 : 258-269.

McEwen, A.D. and Cooper, M.B. ( 1947 )

Bovine mastitis. The Veterinary Record 59 : 655-664.

MacFaddin, J.F.

Media for the isolation, cultivation, identification and maintenance of medical bacteria. Baltimore : Williams and Wilkins. Volume 1, 928 p.

MacKenzie, D.D.S. and Lascelles, A.K. ( 1968 )

The transfer of <sup>131</sup>I-labelled immunoglobulins and serum albumin from blood into milk of lactating cows. Australian Journal of Experimental Biology 46 : 285-294.

McLeod, D.H. ( 1958 )

The effect of bovine mastitis on milk yield. State Veterinary Journal 13 : 62-66.

MacMillan, K.L., Duirs, G.F. and Hook, I.S. ( 1980 )

Relationships between milk somatic cell counts, production index and dry cow therapy in seasonal dairy herds. In Proceedings of the New Zealand Society of Animal Production : 180-188.

Mancini, G., Carbonara, A.R. and Heremans, J.F. ( 1965 )

Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 2 : 235-254.

Marquardt, R.R. and Forster, T.L. ( 1964 )

Relationship between aryl-esterase of milk and mastitis. Journal of Dairy Science 47 : 111-112.

Marquardt, R.R. and Forster, T.L. ( 1966 )

Variations in bovine plasma A-esterase activity and the relationship between plasma and milk A-esterase activities. Journal of Dairy Science 49 : 19-23.

Marquardt, R.R., Forster, T.L., Spencer, G.R. and Stabenfeldt, G.H. ( 1966 )

Aryl-esterase activities of milk from cows with experimentally induced mastitis. Journal of Dairy Science 49 : 631-635.

Marschke, R.J. and Kitchen, B.J. ( 1981 )

The pH of cow's milk and its use in mastitis detection. In Proceedings of the XX1st International Dairy Symposium, Moscow, Vol 1, p 203-204.

Marschke, R.J. and Kitchen, B.J. ( 1985 )

Detection of bovine mastitis by bromothymol blue pH indicator test. Journal of Dairy Science 68 : 1263-1269.

Marschke, R.J., Roberts, R. and Kitchen, B.J. ( 1987 )

The effect of sampling time on N-acetyl- $\beta$ -D-glucosaminidase ( NAGase ) levels in bovine milk and its relevance to mastitis diagnosis. Australian Journal of Dairy Technology 42 : 3-6.

Mattila, T. and Sandholm, M. ( 1985 )

Antitrypsin and N-acetyl- $\beta$ -D-glucosaminidase as markers of mastitis in a herd of Ayrshire cows. American Journal of Veterinary Research 46 : 2453-2456.

**Mattila, T. and Sandholm, M. ( 1986a )**

Milk plasmin, N-acetyl- $\beta$ -D-glucosaminidase, and antitrypsin as determinants of bacterial replication rates in whey. Journal of Dairy Science 69 : 670-675.

**Mattila, T., Saari, S., Vartiala, H. and Sandholm M. ( 1985 )**

Milk antitrypsin as a marker of bovine mastitis - correlation with bacteriology. Journal of Dairy Science 68 : 114-122.

**Mattila, T. Pyörälä, S. and Sandholm, M. ( 1986a )**

Comparison of milk antitrypsin, albumin, N-acetyl- $\beta$ -D-glucosamidase, somatic cells and bacteriological analysis as indicators of bovine subclinical mastitis. Veterinary Research Communications 10 : 113-124.

**Mattila, T., Syväjärvi, J., Jensen, N.E. and Sandholm, M. ( 1986b )**

NAGase and antitrypsin in subclinically infected quarter milk samples: Effect of bacteria, haemolysins, lactation stage and lactation number. American Journal of Veterinary Research 47 : 139-142.

**Mattila, T., Syväjärvi, J., Jensen, N. and Sandholm, M. ( 1986c )**

Determinants of bacterial replication rates in mastitic whey. Journal of Dairy Research 53 : 197-202.

**Mellors, A. ( 1968 )**

$\beta$ -N-acetylglucosaminidase in bovine milk. Canadian Journal of Biochemistry 46 : 451-455.

**Mellors, A. and Harwalkar, V.R. ( 1968 )**

Glycosidases in bovine milk:  $\alpha$ -mannosidase and its inhibition by zwitterions. Canadian Journal of Biochemistry 46 : 1351-1356.

**Merchant, I.A. and Packer, R.E. ( 1971 )**

Veterinary Bacteriology and Virology. The Iowa State University Press, Iowa, U.S.A. Seventh edition, 752p.

**Meyer, F. and Senft, B. ( 1979 )**

Investigations on the variations of concentrations of the whey proteins lactoferrin, blood serum albumin and lysozyme during milking. Milchwissenschaft 34 : 74-77.

Mielke, H. ( 1968 )

Investigations into the dose-dependant effect of oxytocin on the secretion and composition of cow's milk. II Effect on Na, K, Cl, Mg and lactose concentration and pH. Archives of Experimental Veterinary Medicine 22 : 547-561.

Mijnen, E., Jaartsveld, F.H.J., Albers, G.A.A., Verstegen, M.W.A. and Tielen, M.J.M. ( 1982 )

The value of cell count, lactose content, pH and conductivity of milk for mastitis detection in individual cows. Netherlands Milk and Dairy Journal 36 : 65-77.

Miller, R.H. and Paape, M.J. ( 1988 )

Effects of parity, bacteriological status, stage of lactation, and dry period on N-acetyl- $\beta$ -D-glucosaminidase activity of milk and dry secretion. Journal of Dairy Science 71 : 2508-2512.

Miller, R.H., Paape, M.J. and Acton, J.C. ( 1986 )

Comparison of milk somatic cell counts by Coulter and Fossomatic counters. Journal of Dairy Science 69 : 1042-1046.

Milne, J.R. and Smyth, R. ( 1976 )

Rapid determination of somatic cells in milk. New Zealand Journal of Dairy Science and Technology 11 : 21-23.

Minett, F.C. ( 1937 )

Mastitis due to staphylococci. Journal of Comparative Pathology and Therapeutics 50 : 101-121.

Minett, F.C., Stableforth, A.W. and Edwards, S.J. ( 1930 )

The diagnosis of streptococcus mastitis. Journal of Comparative Pathology and Therapeutics 43 : 163-187.

Monlux, A.W. ( 1948 )

The catalase test in the diagnosis of infectious bovine mastitis. Cornell Veterinarian 38 : 389-405.

Morris, R.S. ( 1973 )

The depression of quarter milk yield caused by bovine mastitis, and the response of yield to successful therapy. Australian Veterinary Journal 49 : 153-156.

Muldoon, P.J. and Liska, B.J. ( 1971 )

Chloride ion activity electrode for the detection of abnormal milk. Journal of Dairy Science 54 : 117-119.

Mullen, J.E.C. ( 1950 )

The acid phosphatase of cow's milk. Journal of Dairy Research 17 : 288-295.

Munch-Petersen, E. and Mulgrave, L. ( 1969 )

Quarter versus bulk milk samples for the determination of staphylococci and leukocytes. Australian Journal of Dairy Technology 24 : 127-129.

Murphy, J.M. ( 1942 )

Further observations on the modified Whiteside test for the detection of chronic bovine mastitis. Cornell Veterinarian 32 : 439-444.

Murphy, P. ( 1976 )

The neutrophil. Plenum Medical Book Company, New York. 217 p.

Murphy, J.M. and Hanson, J.J. ( 1941 )

A modified Whiteside test for the detection of chronic bovine mastitis. Cornell Veterinarian 31 : 47-55.

Murphy, J.M. and Stuart, O.M. ( 1953 )

The effect of introducing small numbers of Streptococcus agalactiae ( Cornell 48 strain ) directly into the bovine teat cavity. Cornell Veterinarian 43 : 290-310.

Murthy G.K. and Rhea, U. ( 1967 )

Determination of major cations in milk by atomic absorption spectrophotometry. Journal of Dairy Science 50 : 313-317.

Murthy, G.K. and Whitney R.M. ( 1956 )

A plan for the rapid determination of major cations in milk. Journal of Dairy Science 39 : 364-373.

Mwakipesile, S.M. ( 1982 )

Antibiotic therapy for subclinical mastitis in early lactation: effects on infection, somatic cell count and milk production. Thesis, Massey University, Palmerston North, New Zealand, 142 pages.

Nagahata, H., Saito, S. and Noda, H. ( 1987 )

Changes in N-acetyl- $\beta$ -D-glucosaminidase and  $\beta$ -glucuronidase activities in milk during bovine mastitis. Canadian Journal of Veterinary Research 51 : 126-134.

National Mastitis Council ( 1968 )

Direct Microscopic Somatic Cell Count in milk. Journal of Milk and Food Technology 31 : 350-354.

Natzke, R.P., Schultz, L.H., Barr, G.R. and Holtmann, W.B. ( 1965 )

Variation in mastitis screening tests and milk composition of udder quarters under normal conditions and following omission of a milking. Journal of Dairy Science 48 : 1295-1299.

Natzke, R.P., Everett, R.W., and Postle, D.S. ( 1972a )

Normal milk somatic cell counts. Journal of Milk and Food Technology 35 : 261-263.

Natzke, R.P., Everett, R.W., Guthrie, R.S., Keown, J.F., Meek, A.M., Merrill, W.G., Roberts, S.J. and Schmidt, G.H. ( 1972b )

Mastitis control program: effect on milk production. Journal of Dairy Science 55 : 1256-1260.

Neave, F.K. ( 1975 )

Diagnosis of mastitis by bacteriological methods alone. International Dairy Federation Bulletin, Document Number 85 : 19-36.

Needs, E.C., Anderson, M. and Kirby S. ( 1988 )

Influence of somatic cell count on the whipping properties of cream. Journal of Dairy Research 55 : 89-95.

Nelson, F.E., Schuh, J.D. and Stott G.H. ( 1967 )

Influence of season on leukocytes in milk. Journal of Dairy Science 50 : 978-979.

Nelson, L., Olsson, T., Emanuelson, U., Holmberg, O., Sandholm, M. and Åström G. ( 1985 )

Adenosine triphosphate ( ATP ) as a detection method of mastitis. In Proceedings of the Seminar on Progress in the Control of Bovine Mastitis, Kiel, Germany. p 344-347.

Newbould, F.H.S. ( 1975 )

The possibility of eradicating specific infections in mastitis control. In Proceedings of the International Dairy Federation Seminar on Mastitis Control, Washington D.C. p 382-388.

Newman, L.E. and Kowalski, J.J. ( 1973 )

Fresh sawdust bedding - a possible source of klebsiella organisms. American Journal of Veterinary Research 34 : 979-980.

Ng-Kwai-Hang, K.F., Hayes, J.F., Moxley, J.E. and Monardes, H.G. ( 1982 )

Environmental influences on protein content and composition of bovine milk. Journal of Dairy Science 65 : 1993-1998.

Ng-Kwai-Hang, K.F., Hayes, J.F., Moxley, J.E. and Monardes, H.G. ( 1984 )

Variability of test-day milk production and composition and relation of somatic cell counts with yield and compositional changes of bovine milk. Journal of Dairy Science 67 : 361-366.

Nickerson G.C. ( 1985 )

Immune mechanisms of the bovine udder: An overview. Journal of the American Veterinary Medical Association 187 : 41-45.

Obara, Y. ( 1985 )

Diagnosis of bovine mastitis by determination of lysosomal enzyme activity in milk. Japanese Agricultural Research Quarterly : 298-304 .

Obara, Y. and Komatsu, M. ( 1984 )

Relationship between N-acetyl- $\beta$ -D-glucosaminidase activity and cell count, lactose, chloride or lactoferrin in cow milk. Journal of Dairy Science 67 : 1043-1046.

Obara, Y., Nakano, T., Kume, T. and Oshima, M. ( 1983 )

Mutual relationship between milk components and lysosome enzyme activity in abnormal milk. Japanese Journal of Veterinary Science 45 : 203-208.

O'Donovan, J., Dodd, F.H. and Neave, F.K. ( 1960 )

The effect of udder infections on the lactation yield of milk and milk solids. Journal of Dairy Research 27 : 115-120.

Okigbo, L.M., Sheliah, M.A., Richardson, G.H., Ernstrom, C.A., Brown, R.J. and Tippetts, E.L. ( 1984 )

Portable conductivity meter for detecting abnormal milk. Journal of Dairy Science 67 : 1510-1516.

Olson, J.C., Casman, E.P., Baer, E.F. and Stone, J.L. ( 1970 )

Enterotoxigenicity of Staphylococcus aureus cultures isolated from acute cases of bovine mastitis. Applied Microbiology 20 : 605-607.

Olsson, T. Sandstedt, O., Holmberg, O. and Thore, A. ( 1986 )

Extraction and determination of adenosine triphosphate ( ATP ) in bovine milk by the firefly luciferase assay. Biotechnology and Applied Biochemistry 8 : 361-369.

Onile, B.A. ( 1986 )

Is group B streptococcus a zoonosis ? Transactions of the Royal Society of Tropical Medicine and Hygiene 80 : 673-674.

Orchard, V.A. ( 1979 )

Nocardial infection of animals in New Zealand, 1976 - 1978. New Zealand Veterinary Journal 27 : 159-165.

Oshima, M. ( 1985 )

On the electrical conductivity criteria to be used to detect compositionally abnormal milk due to presumed subclinical mastitis. In Progress on the Control of Bovine Mastitis, Edited by Mann, V.T. p 360-363.

Oshima, M. and Fuse, H. ( 1977 )

Inverse relationship between the electrolyte concentration and the content of fat, protein and lactose in the normal milks of individual cows from the same herd. Journal of Dairy Research 44 : 347-350.

Paape, M.J. and Tucker, H.A. ( 1966 )

Somatic cell content variation in fraction collected milk. Journal of Dairy Science 49 : 265-267.

Paape, M.J., Snyder, W.W. and Hafs, H.D. ( 1962 )

Feulgen-DNA in milk as a measure of udder irritation. Journal of Animal Science 21 : 1028.

Paape, M.J., Hafs, H.D. and Snyder, W.W. ( 1963a )

Variation of estimated numbers of milk somatic cells stained with Wright's stain or with pyronin y-methyl green stain. Journal of Dairy Science 46 : 1211-1216.

Paape, M.J., Hafs, H.D. and Tucker, H.A. ( 1963b )

Relationship of Feulgen-DNA in milk to the number of somatic cells. Journal of Dairy Science 46 : 625.

Paape, M.J., Tucker, H.A. and Hafs, H.D. ( 1965 )

Comparison of methods for estimating milk somatic cells. Journal of Dairy Science 48 : 191-196.

Packard, V., Ginn, R.E., Gulden, D. and Arnold, E. ( 1986 )

An evaluation of reference and infra-red analyses of various components of raw milk. Dairy and Food Sanitation 6 : 430-435.

Packer, R.A. ( 1977a )

Bovine mastitis caused by Pseudomonas aeruginosa. Journal of the American Veterinary Medical Association 170 : 1166.

Packer, R.A.( 1977b )

Bovine mastitis produced by corynebacteria. Journal of the American Veterinary Medical Association 170 : 1164-1165.

Pankey, J.W., Nickerson, S.C., Boddie, R.L. and Hogan, J.S. ( 1985 )

Effects of Corynebacterium bovis infection on susceptibility to major mastitis pathogens. Journal of Dairy Science 68 : 2684-2693.

Patterson, D.S.P., Barrett, S. and Cullen, G.A. ( 1969 )

Oxidative enzymes in milk and their value as indicators of subclinical mastitis in the cow. The Veterinary Record 85 : 708-713.

Peaker, M. ( 1977 )

The aqueous phase of milk: Ion and water transport. In Symposium of the Zoological Society of London 41 : 113-134.

Peaker, M. ( 1978 )

The electrical conductivity of milk for the detection of subclinical mastitis in cows: Comparison of various methods of handling conductivity data with the use of cell counts and bacteriological examination. British Veterinary Journal 134 : 308-314.

Pearson, J.K.L., Wright, C.L. and Greer, D.O. ( 1970 )

A study of methods for estimating the cell content of bulk milk. Journal of Dairy Research 37 : 467-480.

Pearson, J.K.L., Greer, D.O. and Stewart, D.A. ( 1974 )

Reproducibility of the electronic cell count procedure for counting somatic cells in milk. Journal of Dairy Research 41 : 193-201.

Peridigon, G., Medici, M., Cecilia, M., Nader, M.E., de Macias P., Haedo, R., Oliver, G. and Pesce de Ruiz Holgado, A.A. ( 1986 )

Significance of the presence of bovine milk B-glucuronidase in mastitis detection. Journal of Dairy Science 69 : 27-31.

Philpot, W.N. and Pankey, J.W. ( 1973 )

Comparison of four methods for enumerating somatic cells in milk with an electronic counter. Journal of Milk and Food Technology 36 : 94-100.

Phipps, L.W. ( 1968 )

Electronic counting of cells in milk: examination of a chemical treatment for the dispersal of milk fat. Journal of Dairy Research 35 : 295-302.

Phipps, L.W. and Newbould, F.H.S. ( 1966 )

Determination of leukocyte concentrations in cow's milk with a Coulter counter. Journal of Dairy Research 33 : 51-64.

Pirisino, J.F. ( 1983 )

High performance liquid chromatographic determination of lactose, glucose and galactose in lactose reduced milk. Journal of Food Science 48 : 742-754.

Politis, I. and Ng-Kwai-Hang, K.F. ( 1988a )

Effects of somatic cell count and milk composition on cheese composition and cheese making efficiency. Journal of Dairy Science 71 : 1711-1719.

Politis, I. and Ng-Kwai-Hang, K.F. ( 1988b )

Association between somatic cell count of milk and cheese yielding ability. Journal of Dairy Science 71 : 1720-1727.

Postle, D.S. and Blobel, H. ( 1965 )

Studies of bulk milk screening procedures for mastitis. American Journal of Veterinary Research 26 : 90-96.

Poutrel, B., Caffin, J.P. and Rainard P. ( 1983 )

Physiological and pathological factors influencing the bovine serum albumin content of milk. Journal of Dairy Science 66 : 535-541.

Prentice, J.H. ( 1962 )

The conductivity of milk - the effect of the volume and degree of dispersion of the fat. Journal of Dairy Research 29 : 131-139.

Prescott, S.C. and Breed, R.S. ( 1910 )

The determination of the number of body cells in milk by a direct method. Journal of Infectious Diseases 7 : 632-640.

Pullinger, E.J. ( 1935 )

Mastitis streptococci in bulk milk. Journal of Dairy Research 6 : 369-382 .

Pyörälä, S. ( 1988 )

Indicators of inflammation to evaluate the recovery from acute bovine mastitis. In Clinical Aspects on Bovine Mastitis and Treatment During Lactation. Dissertation, College of Veterinary Medicine, Helsinki, Finland: 253 p.

Rainard, P. and Poutrel, B. ( 1982 )

Dynamics of nonclinical bovine intramammary infections with major and minor pathogens. American Journal of Veterinary Research 43 : 2143-2146.

Randolph, H.E. and Erwin, R.E. ( 1974 )

Influence of mastitis on properties of milk. X. Fatty acid composition. Journal of Dairy Science 57 : 865-868.

Read, R.B. and Bradshaw, J.G. ( 1966 )

Staphylococcal enterotoxin B - thermal inactivation in milk. Journal of Dairy Science 49 : 202-203.

Read, R.B., Reyes, A.L., Bradshaw, J.G. and Peeler, J.T. ( 1967 )

Electronic counting of somatic cells in milk. Journal of Dairy Science 50 : 669-674.

Read, R.B., Reyes, A.L., Bradshaw, J.G. and Peeler, J.T. ( 1969 )

Evaluation of seven procedures for detection of abnormal milk due to mastitis. Journal of Dairy Science 52 : 1359-1367.

Reichmuth, J. ( 1975 )

Somatic cell counting - interpretation of results. International Dairy Federation Bulletin, Document Number 85 : 93-109.

Reineccius, G.A., Kavanagh, T.E. and Keeny, P.G. ( 1970 )

Identification and quantitation of free neutral carbohydrates in milk products by gas-liquid chromatography and mass spectrometry. Journal of Dairy Science 53 : 1018-1022.

Rendos, J.J., Eberhart, R.J. and Kesler, E.M. ( 1975 )

Microbial populations of teat ends of dairy cows, and bedding materials. Journal of Dairy Science 58 : 1492-1500.

Renner, E. ( 1975 )

Investigations on some parameters of the milk for the detection of subclinical mastitis. International Dairy Federation Bulletin, Document Number 85 : 53-58.

Richardson, T., McGann, T.C.A. and Kearney, R.D. ( 1980 )

Levels and location of adenosine triphosphate in bovine milk. Journal of Dairy Research 47 : 91-96.

Rook, J.A.F. and Campling, R.C. ( 1965 )

Effect of stage and number of lactation on the yield and composition of cow's milk. Journal of Dairy Research 32 : 45-55.

Rottscheidt V.W. and Iben, B. ( 1981 )

Ist die bestimmung des lactosegehaltes mittels des infrarot milchanalysators geeignet zur diagnostik subklinischer galmastitiden. Praktische Tierarzt 62 : 756-764.

Ryniewicz, Z. and Wojcik, A. ( 1985 )

Lactose content in quarter milk during milking and in cow's milk. In Proceedings of the Vth International Symposium on Mastitis Control, Poland. p 601-609.

Safwate, A., Davicco, M., Barlet, J.P. and Delost, P. ( 1981 )

Sodium and potassium in blood and milk, and plasma aldosterone in high yield dairy cows. Reproduction, Nutrition and Development 21 : 601-610.

Sandholm, M. and Mattila, T. ( 1985 )

Merits of indirect tests in mastitis detection ( cell counting, NAGase, antitrypsin ). In Proceedings of the Seminar on the Progress of Mastitis Control, Kiel, Federal Republic of Germany, p 334-338.

Sandholm, M, Honkanen-Buzalski, T. and Kangasniemi, R. ( 1984 )

Milk trypsin-inhibitor capacity as an indicator of bovine mastitis - a novel principle which can be automated. Journal of Dairy Research 51 : 1-9.

Sbodio, O.A., Freyre, M.R., Rozyeki, V.R., Sabbag, N.G. and Weidman, P. ( 1985 )

Influence of lactation on the composition of individual cows milk. New Zealand Journal of Dairy Science and Technology 20 : 109-116.

Schalm, O.W. ( 1977 )

Pathologic changes in the milk and udder of cows with mastitis. Journal of the American Veterinary Medical Association 170 : 1137-1140.

Schalm, O.W. and Lasmanis, J. ( 1968 )

The leukocytes: Origin and function in mastitis. Journal of the American Veterinary Medical Association 153 : 1688-1694.

Schalm, O.W. and Noorlander, D.O. ( 1957 )

Experiments and observations leading to the development of the California Mastitis Test. Journal of the American Veterinary Medical Association 130 : 199-204.

Schalm, O.W. and Woods, G.M. ( 1953 )

The mastitis complex. Journal of the American Veterinary Medical Association 122 : 462-467.

Schalm, O.W. and Ziv-Silberman, G. ( 1968 )

The incidence of mastitis: Comparison as indicated by California Mastitis Test applied to three different fractions of the same quarter milk samples. The Veterinary Record 82 : 184-188.

Schalm, O.W., Lasmanis, J. and Carroll, E.J. ( 1966 )

Significance of leukocytic infiltration into the milk in experimental Streptococcus agalactiae mastitis in cattle. American Journal of Veterinary Research 27 : 1537-1546.

Schalm, O.W., Carroll, E.J., and Jain, N.C. ( 1971 )

Bovine mastitis. Philadelphia : Lea and Febiger. 360 p.

Schleifer, K.H. ( 1986 )

Gram positive cocci. Bergey's Manual of Systematic Bacteriology, Volume 2, Section 12 : 999-1100. Williams and Wilkins, Baltimore, U.S.A.

Schmidt Madsen, P. ( 1968 )

Fluoro-opto-electronic cell counting on milk. Acta Veterinaria Scandinavica 9 : 376-377.

Schmidt Madsen, P. ( 1975 )

Fluoro-optical-electronic cell counting on milk. Journal of Dairy Research 42 : 227-239.

Schmidt Madsen, P., Klastrup, O., Olsen, S.J. and Pederson, P.S. ( 1974 )

Herd influence of bovine mastitis in four Danish dairy districts. Nordic Veterinary Medicine 26 : 473-482.

Schneider, R. and Jasper, D.E. ( 1966 )

Some factors responsible for variation in counting somatic cells on Prescott-Breed smears of milk. Journal of Milk and Food Technology 29 : 5-9.

Schultz, L.H. ( 1977a ).

Somatic cells in milk - physiological aspects and relationship to amount and composition of milk. Journal of Food Protection 40 : 125-131 .

Schultz, L.H. ( 1977b )

Somatic cell counting of milk in production testing programs as a mastitis control technique. Journal of the American Veterinary Medical Association 170 : 1244-1246.

Schultze, W.D. ( 1970 )

Field evaluation of an optical somatic cell counter for milk. Journal of Milk and Food Technology 33 : 562-567.

Sharp, P.F. and Struble, E.B. ( 1935 )

Period of lactation and direct titratable chloride value of milk. Journal of Dairy Science 18 : 527-532.

Shazly, A.E., Sharara, H.A. and Fahmi, A.H. ( 1974 )

Some factors affecting physical and chemical properties of Egyptian cow's and buffalo's milk. Milchwissenschaft 29 : 355-358.

Sheldrake, R.F. and Hoare, R.J.T. ( 1981 )

The detection of mastitis in individual quarters using electrical conductivity or somatic cell concentration. New Zealand Veterinary Journal 29 : 211-213.

Sheldrake, R.F., McGregor, G.D. and Hoare, R.J.T. ( 1983a )

Somatic cell count, electrical conductivity, and serum albumin concentration for detecting bovine mastitis. Journal of Dairy Science 66 : 548-555.

Sheldrake, R.F., Hoare, R.J.T. and McGregor, G.D. ( 1983b )

Lactation stage, parity, and infection affecting the somatic cells, electrical conductivity, and serum albumin of milk. Journal of Dairy Science 66 : 542-547.

Smit, L.E. and Nels, M.M. ( 1987 )

Vergelyking tussen die standaardkoper-reduksie, chloramien-T metodes en 'n gewysigde hoedrukvloeistofchromatografiese metode vir die bepaling van laktose in melk en melkprodukte. South African Journal of Dairy Technology 19 : 62-64.

Smith, A.M., Chesworth, J.M., Henderson, G.D. and Rodway, R.G. ( 1979 )

Use of Laurell electrophoresis for the quantitative measurement of albumin in mastitic milk. Journal of Dairy Research 46 : 547-550.

Smith, J.W. ( 1969 )

Development and evaluation of the Direct Microscopic Somatic Cell Count ( DMSCC ) in milk. Journal of Milk and Food Technology 32 : 434-441.

Spencer, G.R. and Simon, J. ( 1960 )

The catalase, California and cell count tests for detecting abnormalities in milk. American Journal of Veterinary Research 21 : 578-584.

Stabenfeldt, G.H. and Spencer, G.R. ( 1965 )

The lesions in bovine udders shedding haemolytic coagulase positive staphylococci. Pathologia Veterinaria 2 : 585-600.

Stabenfeldt, G.H. and Spencer, G.R. ( 1966 )

The lesions in bovine udders shedding nonhaemolytic coagulase negative staphylococci. Pathologia Veterinaria 3 : 27-39.

Stahlhut-Klipp, V.H. ( 1973 )

Beziehungen zwischen dem gehalt an somatischen zellen und dem lactosegehalt in viertelgemelken. Milchwissenschaft 28 : 766-768.

**Statistical Analytical Systems ( 1985 )**

SAS users guide: Statistics, version 5 edition. SAS Institute, Cary, N.C., U.S.A. 956 p.

**Steinsholt, K. and Calbert, H.E. ( 1960 )**

The quinhydrone electrode for pH determinations in milk and dairy products. Journal of Dairy Science 43 : 329-345.

**Stevenson, B.J. and McVeagh, T. ( 1983 )**

One mastitis outbreak cost a dairy farmer \$ 9,765. Surveillance 10 : 17-20.

**Strynadka, N.J. and Thornton, H.R. ( 1937 )**

The accuracy of the Direct Microscopic ( Breed ) Count of the bacteria and leukocytes in milk. Journal of Dairy Science 20 : 685-692.

**Symons, D.B.A. and Wright, L.J. ( 1974 )**

Changes in bovine mammary gland permeability after intramammary exotoxin infusion. Journal of Comparative Pathology 84 : 9-17.

**Szijarto, L.F. and Barnum, D.A. ( 1984 )**

Preparation, use and evaluation of standards developed for simultaneous monitoring of Coulter and Fossomatic electronic cell counting instruments in Ontario. Journal of Food Protection 47 : 227-231 .

**Systad O. and Røn, I. ( 1979 ),**

Variation in somatic cell counts of milk samples from individual cows. Acta Veterinaria Scandinavica 20 : 555-561.

**Tallamy, P.T. and Randolph, H.E. ( 1969 )**

Influence of mastitis on properties of milk. IV Hydrolytic rancidity. Journal of Dairy Science 52 : 1569-1572.

**Thompson, D.I. and Postle, D.S. ( 1964 )**

The Wisconsin Mastitis Test - An indirect estimation of leukocytes in milk. Journal of Milk and Food Technology 27 : 271-275.

**Thompson, D.R., Packard, V.S. and Ginns, R.E. ( 1976 )**

Electronic somatic cell count - chemical method, DMSCC and WMT tests for raw milk. Journal of Milk and Food Technology 39 : 854-858.

Thompson, G.E. ( 1988 )

Electrolytes and free fatty acids in milk during mammary involution in the cow. Research in Veterinary Science 44 : 175-177.

Timms, L.L., Sommer, D., Rothering, N. and Schultz, L.H. ( 1984 )

Lactation and dry period NAGase profiles. Journal of Dairy Science 67 : 166.

Tolle, V.A., Zeidler, H. and Heeschen, W. ( 1966 )

A method for the electronic cell count in milk. Milchwissenschaft 21 : 93-98.

Tucker, E.W. ( 1950 )

Pseudomonas infection of the bovine udder apparently contracted from contaminated treatment equipment and materials. Cornell Veterinarian 40 : 95-96.

Waite, R. and Blackburn, P.S. ( 1957 )

The chemical composition and the cell count of milk. Journal of Dairy Research 24 : 328-339.

Waite, R. and Blackburn, P.S. ( 1963 )

The relationship between milk yield, composition and tissue damage in a case of subclinical mastitis. Journal of Dairy Research 30 : 23-33.

Ward, G.E. and Schultz, L.H. ( 1972 )

Relationship of somatic cells in quarter milk to type of bacteria and production. Journal of Dairy Science 55 : 1428-1431.

Wegner, T.N. and Stull, J.W. ( 1978 )

Relation between mastitis test score, mineral composition of milk, and blood electrolyte profiles in Holstein cattle. Journal of Dairy Science 61 : 1755-1759.

Westgarth, D.R. ( 1971 )

Interpretation of bulk cell count data. The control of bovine mastitis. Edited by Dodd, F.H. and Jackson, E.R., The National Institute for Research in Dairying, Shinfield, Reading, England, p 105-111.

Wheelock, J.V., Rook, J.A.F., Neave, F.K. and Dodd, F.H. ( 1966 )

The effect of bacterial infections of the udder on the yield and composition of cow's milk. Journal of Dairy Research 33 : 199-215.

White, J.C.D. and Davies, D.T. ( 1958 )

The relation between the chemical composition of milk and the stability of the caseinate complex. Journal of Dairy Research 25 : 236-255.

Whiteside, W.H. ( 1939 )

Observations on a new test for the presence of mastitis in milk. Canadian Public Health Journal 30 : 44.

Wiesner, H.U. ( 1985 )

Concentrations of lactose in milk and urine from cows with and without udder infections. In Proceedings of the Conference on Progress in the Control of Bovine Mastitis, Kiel, Germany. Editor Mann, V.T. p

Wilkinson, H.W. ( 1978 )

Group B streptococcal infection in humans. Annual Review of Microbiology 32 : 41-57.

Willits, R.E. and Babel, F.J. ( 1965 )

Disc floatation method for measurement of catalase activity in milk. Journal of Dairy Science 48 : 1287-1289.

Wilson, C.D. and Richards, M.S. ( 1980 )

A survey of mastitis in the British dairy herd. The Veterinary Record 106 : 431-435.

Woolford, M.W. and Williamson, J.H. ( 1982 )

The electrical conductivity of milk as a diagnostic of subclinical mastitis. In Proceedings of the Conference on Dairy Production from Pasture, New Zealand and Australian Societies of Animal Production. p 114-115.

Woolford, M.W., Williamson, J.H., Copeman, P.J.A., Napper, A.R., Phillips, D.S.M. and Uljee, E. ( 1983 ).

An identical twin study of milk production losses due to subclinical mastitis. In Proceedings of the Ruakura Farmers Conference : 115-119 .

Yamamoto, M., Kume, T., Nakano, M., Obara, Y., Kudo, T., Ichikawa, T. and Notsuli, I. ( 1985 )

Automatic measurement of electrical conductivity for the detection of bovine mastitis. In Proceedings of the Conference on Progress in the Control of Bovine Mastitis, Kiel, Germany. p 364-369.

APPENDIXTHRESHOLD PROGRAM FOR THE DIAGNOSIS OF SUBCLINICAL MASTITIS

```
200 PRINT
210 PRINT
220 PRINT SPA(20): 'THRESHOLD PROGRAM FOR MASTITIS DIAGNOSIS'
240 PRINT
250 PRINT
260 PRINT SPA(20): 'WRITTEN BY ROBERT J. HOLDAWAY, '
270 PRINT
280 PRINT SPA(20): 'VETERINARY CLINICAL SCIENCES DEPARTMENT,'
290 PRINT
300 PRINT SPA(20): 'MASSEY UNIVERSITY, PALMERSTON NORTH.'
310 PRINT
320 PRINT SPA(20): 'JANUARY, 1989.'
330 PRINT
340 PRINT
350 PRINT SPA(38): 'MENU'
370 PRINT
610 PRINT SPA(16): 'OPTIMUM THRESHOLD ESTIMATION - SINGLE PARAMETER'
630 PRINT
650 REM *** THIS PROGRAM READS IN A BACTERIOLOGICAL RESULT AND ***
660 REM *** THE CORRESPONDING PARAMETER VALUE FOR A QUARTER. ***
670 REM *** THE VALUE IS COMPARED WITH A THRESHOLD, AND THE ***
680 REM *** ACCURACY OF THE DIAGNOSIS IS ESTABLISHED. ***
690 REM *** THE THRESHOLD INCREASES FROM AN INITIAL TO A ***
700 REM *** FINAL THRESHOLD VALUE BY INCREMENTS. ***
710 INPUT LINE "GIVE NAME OF DATA FILE: ",F1$
720 PRINT
730 INPUT 'ENTER PARAMETER NAME      ':A$
740 PRINT
750 INPUT 'ENTER INITIAL THRESHOLD  ':B
760 PRINT
770 INPUT 'ENTER FINAL THRESHOLD    ':C
780 PRINT
```

```
790 INPUT 'ENTER INCREMENT          ':D
800 PRINT
810 PRINT
815 INPUT 'DOES PARAMETER INCREASE WITH MASTITIS ? (Y/N)':R$
816 PRINT
820 INPUT 'ANY CORRECTIONS ? (Y/N)':E$
830 PRINT
840 IF E$="Y" THEN 712 ELSE 900
850 LET Q1=0
860 LET Q2=0
870 LET Q3=0
880 LET L=0
900 LET F0=0
910 LET F1=0
930 LET N=0
940 LET X=0
950 LET Y=0
960 LET Z=0
1000 PRINT
1010 DEFINE READ FILE #1 = F1$
1020 ON END #1 GOTO 1360
1030 IF B<C THEN DO
1040     READ #1, F, G
1050     IF R$="Y" THEN 1080 ELSE 1210
1080     IF F=1 THEN 1100 ELSE 1180
1100     LET F1=F1+1
1120     IF G=>B THEN X=X+1 ELSE Y=Y+1
1150     GOTO 1300
1180     LET F0=F0+1
1190     IF G<B THEN X=X+1 ELSE Z=Z+1
1200     GOTO 1300
1210     IF F=1 THEN 1215 ELSE 1260
1215     LET F1=F1+1
1220     IF G<B THEN X=X+1 ELSE Y=Y+1
1240     GOTO 1300
1260     LET F0=F0+1
1270     IF G=>B THEN X=X+1 ELSE Z=Z+1
1300     LET N=N+1
1340     GOTO 1040
1360     LET H=(X/N)*100
```

```
1370  CLOSE #1
1380  LET I=(Y/N)*100
1390  LET J=(Z/N)*100
1400  LET H1= INT(100*H + .5)/100
1410  LET I1= INT(100*I + .5)/100
1420  LET J1= INT(100*J + .5)/100
1430  LET V=(Y/F1)*100
1440  LET W=(Z/F0)*100
1450  LET V1= INT(100*V + .5)/100
1460  LET W1= INT(100*W + .5)/100
1465  IF R$="Y" THEN 1470 ELSE 1475
1470  IF V1>W1 AND T=0 THEN Q1=B ELSE 1500
1472  GOTO 1480
1475  IF V1<W1 AND T=0 THEN Q1=B ELSE 1500
1480  LET T=1
1490  LET Q2=B-D
1495  LET Q3=(Q1-Q2)/10
1500  PRINT 'FOR A ':A$ : 'THRESHOLD OF ':B
1510  PRINT
1520  PRINT 'TOTAL DIAGNOSES = ':N
1530  PRINT
1540  PRINT 'CORRECT DIAGNOSES = ':X, H1: '%'
1550  PRINT
1560  PRINT 'FALSE NEGATIVES = ':Y, I1: '%'
1570  PRINT
1580  PRINT 'FALSE POSITIVES = ':Z, J1: '%'
1590  PRINT
1600  PRINT 'TYPE 1 MISCLASS = ', V1: '%'
1610  PRINT
1620  PRINT 'TYPE 2 MISCLASS = ', W1: '%'
1630  PRINT
1640  PRINT 'TOTAL INFECTED = ':F1, 'TOTAL UNINFECTED ':F0
1650  PRINT
1660  PRINT
1700  LET B=B+D
1710  PRINT
1730  GOTO 900
1740  DOEND
1745  IF L=1 THEN 1900 ELSE 1750
1750  PRINT
```

```
1760 PRINT 'GOING TO SECOND STAGE'
1765 LET L=L+1
1770 PRINT
1780 PRINT 'INITIAL THRESHOLD = ':Q2
1790 PRINT
1800 PRINT 'FINAL THRESHOLD = ':Q1
1810 PRINT
1820 PRINT 'INCREMENT = ':Q3
1830 PRINT
1840 LET B=Q2
1850 LET C=Q1
1860 LET D=Q3
1870 GOTO 900
1900 PRINT
1910 PRINT 'END OF RUN'
1920 PRINT
1930 PRINT 'CRITICAL VALUE = THE THRESHOLD AT WHICH THE PROPORTION'
1940 PRINT 'OF MISCLASS 1 AND MISCLASS 2 ARE EQUAL.'
2000 END
```