ANTIBIOTIC THERAPY FOR SUBCLINICAL MASTITIS IN EARLY LACTATION; EFFECTS ON INFECTION, SOMATIC CELL COUNTS AND MILK PRODUCTION

A THESIS PRESENTED IN PARTIAL
FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
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ABSTRACT

This study was conducted with the main purpose of investigating the effects of antibiotic therapy applied during lactation on the subclinically infected quarters in terms of bacterial infection, somatic cell count (S.C.C.), milk yield and composition (i.e. milkfat and protein percentage). The main objectives involved were:-

(1) To identify cows with high somatic cell counts (subclinical mastitis) in early lactation.

(2) To identify the individual quarters with high somatic cell counts and bacterial infection.

(3) To treat some of these high S.C.C. quarters with an antibiotic, and leave some of them untreated (control quarters).

(4) To measure the effects of treatment on S.C.C., bacterial infection, yield and composition of milk.

During weeks four to six of lactation 12 cows from a herd of 100 cows at No.3 Dairy Research & Development Unit, Massey University, were identified to have consistently high values for somatic cell counts. Milk samples were aseptically taken from the individual quarters of these cows and subjected to bacteriological tests. Eight subclinically infected quarters with high somatic cell counts were subsequently treated with sodium cloxacillin (Orbenin) formulated in a slow release base.

Before antibiotic treatment of any quarters, there was a close association between infection and somatic cell counts in individual quarters. Thus only 12% of the quarters which showed no infection had somatic cell counts higher than 300,000 cells/ml, whereas the corresponding proportion of infected quarters was 74%.

Out of the eight infected quarters which were treated with antibiotic, five were cured of infection (62.5% cure rate); in these quarters the average S.C.C. was greatly reduced from 4,207,000 cells/ml before treatment to about 160,000 cells/ml afterwards whereas the three quarters which were treated but not cured showed a slight decrease from 3,991,200 cells/ml before treatment to 2,638,800 cells/ml after treatment. The average somatic cell counts of the five infected control quarters increased slightly from their original value of about 2,061,500 cells/ml up to 2,111,000 cells/ml.

There was no significant or consistent effect of antibiotic therapy, successful or otherwise, on milk yield from individual quarters. However adjusted means showed a 10% non-significant difference between the successfully treated quarters and the untreated control quarters in favour of the cured quarters. The effects of treatment on milk composition were small and were significant only for protein percentage.

The practical implications of the results were discussed. From the results obtained firm conclusions on the effects of antibiotic therapy of subclinically infected quarters on milk yield, and composition were not possible. The major benefit is likely to be the reduction in the number of infected quarters, and the consequent reduction in the risk of new infection in other quarters. It is however suggested that more work is required to establish the actual effect of antibiotic therapy by obtaining information on the performance of the successfully treated quarters in the following lactation.

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CHAPTER ONE

1:0 INTRODUCTION

The use of somatic cell counting systems for monitoring mastitis status (the subclinical form) of herds and the udder health of individual cows is being widely accepted in many countries throughout the world. A large amount of survey data and analytical results is available from many countries that show the relation between udder infection and somatic cell count (S.C.C.). Studies in New Zealand (review by Holmes, 1981), for example, have shown that there is a clear association between udder infection and somatic cell count in the milk from individual cows, and that on average, cows with high S.C.C. produce less milk than cows with lower S.C.C. Also work in U.S.A. (Schultz, 1977) has shown that loss of milk production due to subclinical mastitis is related to somatic cell counts. The report indicates that on a quarter basis, loss starts at 500,000 cells/ml, progresses to 7.5% at 1,000,000 cells/ml and 30% at 5 million cells/ml. Barnard (1981) reports that as a general rule, for each increase of 100,000 cells/ml, there will be about a 3% increase in infected quarters and a decrease of about 0.45 kg of milk per cow daily. Recent information presented by George Shook of University of Wisconsin, U.S.A. at the annual meeting of the National Mastitis Council held in mid February, 1982 in Louisville, Kentucky, U.S.A. (Hoard's Dairyman, March 25, 1982), showed that average daily production of Wisconsin cows dropped from 21.9 kg to 21.3 kg as the somatic cell count rose from 50,000 to 100,000 cells/ml. Ali *et al* (1980) have reported that rennet clotting time (R.C.T.), loss of fat in whey, curd moisture and losses in curd yield and rigidity were all greater in the milk of higher somatic cell counts. Since somatic cell standards may be applied to milk used in the manufacture of dairy products, it might in the long run become increasingly important to limit the number of these cells in milk.

It is on the basis of such findings that an increase in the somatic cell count in the milk of an individual cow is now used as a measurement of subclinical mastitis, and with the introduction of individual cow S.C.C. service in countries like New Zealand, the

farmer can use the cow's S.C.C. to detect cows which are subclinically affected and take appropriate control measures. The loss in milk production cited above and the probable reduced yield and quality of dairy products (such as cheese) made from milk of high somatic cell counts is of great concern to the dairy farmer and the dairy industry at large and calls for measures to avoid such a loss.

The effect of treating subclinical mastitis during lactation has been investigated in some overseas countries. The response obtained in U.K. with antibiotic therapy given to subclinically affected quarters has shown that subclinical mastitis can be treated with antibiotic quite successfully, with the success varying between different causal organisms (Griffin, 1971). Such information is scanty and seems to be lacking in New Zealand. Additional information on the contribution of this method of mastitis control is therefore desirable, particularly when considering the loss caused by subclinical mastitis in a lactating dairy cow. Kingwill *et al* (1979) suggested that another method of reducing the duration of infections is to treat all new infections during lactation. This study was therefore intended to provide data which should provide information about the response to treatment in terms of bacterial infection, somatic cell count, milk yield, milk fat and milk protein percentages. This was accomplished by treating with an antibiotic some of the quarters found to be subclinically affected and then measuring the subsequent changes in the somatic cell count, bacterial infection, milk production and percent of both milk fat and milk protein of these quarters and comparing them with quarters which were subclinically affected but not treated.

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CHAPTER TWO

2:0 LITERATURE REVIEW

2:1 INTRODUCTION

This chapter is divided into two parts, I and II. Part I is devoted to a brief discussion of mastitis in general, whereas Part II deals with subclinical mastitis in some greater detail. The available literature on mastitis is voluminous and therefore only representative reports will be cited in this review.

2:1:0 PART I - MASTITIS IN GENERAL

2:1:1 DEFINITION: Mastitis, which can generally be defined as an inflammation of the mammary gland, is a complex disease having different causes, degrees of intensity and variations in duration and residual effects (Carrol, 1977; Schalm and Woods, 1953). The definition of mastitis arrived at by the International Dairy Federation (IDF) requires the finding of pathogenic micro-organisms in the milk and also a concentration of somatic cells in excess of 500,000 cells/ml (Int. Dairy Fed., 1971).

Tolle (1971) further elaborates on the definition that: "Mastitis is an inflammatory change of the mammary gland which, along with physical, chemical and microbiological changes, is characterized by an increase of somatic cells, especially leucocytes in milk, and by pathological changes in the mammary tissue."

Objections have been raised to the criteria embodied in this definition (see Proc. IDF Seminar on Mastitis Control, 1975: Giesecke, 1974); nevertheless the criteria draw special attention to two aspects of mastitis which are of major importance, namely:

a) the response by or damage to, the gland tissue

b) the presence of pathogenic bacteria in the gland.

The International Dairy Federation (IDF) definition can be summarized as shown in Table 2.1.

TABLE 2.1: ASSESSMENT OF CYTOLOGICAL-BACTERIAL FINDINGS IN MASTITIS DIAGNOSIS (TOLLE, 1975).

Cell Count per ml milk	Pathogenic Micro-organisms	
	Not Isolated	Isolated
<500,000	normal secretion	latent infection
>500,000	non-specific mastitis	mastitis

This definition applies to the examination of quarter milk samples taken at the usual milking time from the foremilk after discarding the first few squirts of milk.

The main drawback of this definition, however, is that udder infection is not defined and this has led to various bacterial sampling methods and cultural procedures (see Part II) resulting in considerable confusion between workers in the diagnosis of the disease. Despite the disparity in the sampling techniques involved it can be concluded from the different definitions appearing in the literature that the diagnosis of mastitis in lactating cows is based on:

(i) the clinical examination of the udder and its secretion;

(ii) the bacteriological examination of milk;

(iii) the somatic cell count of milk.

2:1:2 CAUSE: Most commonly the disease begins as a result of penetration of pathogenic bacteria through the teat duct into the interior of the gland where, if conditions are favourable, they multiply and

their by-products of growth and metabolism irritate the delicate tissues causing an inflammatory response (Schalm *et al*, 1971). The inflammation grossly characterized by high temperature, redness, pain and swelling together with alterations in the consistency and appearance of the milk is known as "clinical mastitis", whereas the existence of inflammation in the absence of observable signs or any visual abnormalities in the milk is referred to as "subclinical mastitis" (Duirs, 1980).

Detection of clinical mastitis is much simpler because the visual symptoms can be easily recognized. The main pathogenic bacteria of economic importance in mastitis are of the Streptococci group (i.e. *Streptococcus agalactiae*, *Streptococcus dysgalactiae* and *Streptococcus uberis*) and *Staphylococcus aureus* (Neave, 1975).

2:1:3 CLINICAL FORMS OF MASTITIS:

(i) Peracute Mastitis - In addition to the gross signs of swelling, heat, redness and pain, this form is accompanied by systemic signs of fever, depression, shivering, loss of appetite and rapid loss of weight.

(ii) Acute Mastitis - This is also characterized by the gross signs of swelling, heat, redness and pain which are accompanied by fever and mild depression.

(iii) Sub-acute or Mild Mastitis - This is the form of mastitis in which milk shows abnormal consistency or colour of milk. In this form the gross signs of mastitis are subdued and not accompanied by systemic effects.

(iv) Chronic Mastitis - This is the form of mastitis in which the cow's milk periodically shows abnormalities and then returns to normal i.e. the condition periodically flares-up and then returns to normal after some time (Schmidt, 1971). It occurs when a quarter fails to respond to treatment over a period of time. The quarter may atrophy or show abnormal clinical changes for the rest of the animal's life.

Other forms of mastitis such as latent infections, in which milk

shows the presence of pathogenic micro-organisms but has a normal cell count or non-specific or aseptic mastitis in which there is no recognisable infection and the symptoms may be subclinical or clinical, could probably be classed under the category of subclinical mastitis because they often do not show overt signs. Subclinical form of mastitis is discussed in Part II but it could simply be defined here as the form of mastitis that shows no macroscopic evidence of inflammation but examination of the milk reveals udder infection, increased cell count and also alterations in the chemical properties of milk.

2:1:4 DIAGNOSIS: Since mastitis is a disease of the udder, the milk can be used to diagnose the disease. The results of cytological, enzymatic, biochemical and bacteriological analysis of milk will show the responses of the udder to mastitis and effects of the disease on the udder. Some workers use bacterial examination almost exclusively in the diagnosis of mastitis (e.g. Neave, 1975) whereas others use a combination of somatic cell count and bacteriological information (e.g. Pearson and Greer, 1974).

Early literature suggests that clinical mastitis in cows caused problems serious enough to attract the attention of scientists. The scientific literature reveals a distinct tendency to describe, classify and define mastitis in terms of available diagnostic techniques and information on the pathogenesis (see review by Giesecke and Van den Heever, 1974). Early observations on the disease were therefore limited to the clinical signs observed by examination of udder tissue and secretion. Schalm *et al* (1971) described a method of physically examining the lactating udder immediately after milking. They reported that thorough evacuation of the udder is necessary because the presence of milk reduces the chances of detecting scar tissues and fibrosis.

King (1974) using clinical examination by manual palpation showed that quarters with distinct or marked fibrosis gave lower yields. The author states that the effect on yield was due to udder damage. He further cites the work of Udall & Johnson (1931) who consider that the value of a physical examination in the diagnosis of mastitis rates above any other single method. Switzer and Gates (1931) cited by King (1974)

compared the results of post-mortem examination of udders with the physical examination notes on these cows as made in routine field reports. Results obtained proved that a physical examination is a very efficient means of detecting clinical mastitis under practical field conditions.

Giesecke & Van den Heever (1974) have cited the work of a number of research workers who regarded clinical examination of the udder to be very useful as it detected not only acute conditions but also permanent udder changes due to chronic mastitis. Narayan and Iya (1954) showed that tissue abnormalities could be detected by deep palpations of the udder. In contrast, however, Giesecke & Van den Heever (1974) have reported work of others who recommended clinical diagnosis mainly for acute mastitis and laboratory tests as supplementary methods.

Some workers have cast doubts about the efficiency of physical examinations. Giesecke & Van den Heever (1974) report the work of Heidrich and Renk (1963) which showed that with few exceptions, the physical examination of the udder is inadequate for diagnosis of mastitis because quarters with no induration may be bacteriologically positive. Giesecke & Van den Heever (1974) noted that discrepancies between chronic tissue alterations and the presence or absence of inflammatory changes in the udder seem to result from the fact that in some indurated udders epithelial lesions persist whereas in others they heal completely. It is therefore possible that an incorrect diagnosis of mastitis may be made in udders in which inflammation has subsided and the quarters have simply become indurated rather than mastitic.

The strip-cup represents another form of clinical examination in which the foremilk is examined using a black plate placed within the cup and the presence of small flakes, milk clots and water-like consistency suggest the presence of mastitis. Neave *et al* (1954) reported that 12 - 13% of quarters showing clots in milk were not infected with the more common pathogenic organisms and hence its reliability as an indication of infection is questionable. This

indicates that there may be instances where clots occur in milk without actual infection.

The most recently developed technique of clinical examination of mastitis is the individual in-line filters which show clots suggesting the presence of mastitis in the herd. The only drawback of this method is that it involves going back to the cow at milking time to trace the quarter(s) from which the clots might have come. Nevertheless this would probably seem to be the most acceptable method of clinical diagnosis of mastitis in big herds.

It would appear from this discussion that clinical examination of the udder is a very subjective technique and depends on the experience, skill and judgement of the examiner. However when considering individual farm conditions it is probably the only practicable method for diagnosing mastitis though it might soon become a lost art in big herds where the milker cannot afford the time required to carry out an effective clinical examination of the udder of each cow in the herd.

The importance of early diagnosis of mastitis is to alert the farmer of impending problems before they become acute clinical cases, and since such clinical cases develop from subclinical ones, it might be beneficial to put more emphasis on the diagnosis of mastitis at subclinical level. Bacterial assessment and somatic cell counting are the two main diagnostic criteria used for the assessment of sub-clinical mastitis. This type of diagnosis will be discussed at length in Part II.

2:1:5 PREDISPOSING CAUSES: The severity of mastitis is determined to a considerable extent by the nature of the infecting bacteria, by natural mechanisms of resistance of the cow, and to some degree by stresses placed on the mammary glands by milking practices and environmental factors (Schalm *et al*, 1971). Stresses placed on the mammary glands by the milking practices and environmental factors are the main factors considered as predisposing causes in this context.

(i) Milking Machine

There is good practical and scientific evidence to show that the milking machine influences mastitis on farms in other ways than the obvious one of transferring bacteria to the outside of the teat from one cow to another or from one teat to another teat (Thiel, 1975). Machine effects can be loosely classified as those associated with over milking, with internal and external damage to teats and with vacuum fluctuations.

Udder damage can come from over milking, high vacuum levels and inadequate squeeze phase (e.g. Petersen, 1964). Constant exposure of the teats to vacuum levels above those normally used in milking machines i.e. above 380 mm Hg (above 15" Hg) causes damage to the teat skin particularly when the teat-cup liners do not collapse completely during each pulsation (Kingwill *et al*, 1979). Plastridge (1958) cites data reported by Burkey & Sanders (1949) which suggested that a vacuum level of over 380 mm Hg tends to increase the incidence of mastitis. Because higher operating vacuum levels will result in increased teat apex damage and/or teat sinus damage at the end of milk flow, it is reasonable to expect that this will lead to a rise in new infections. The possible explanation for that is bacteria might find a ready lodgement through teat erosions where they could multiply and thereby increase the risk of infection of the udder. Petersen (1964) postulated that severe teat injury could predispose the udder to infection and indicated that the teat canal could remain partly open if the milking machine was left on the teat after the end of milkflow thus allowing easy penetration of any bacteria around. Langlois *et al* (1982) observed a significant increase in teat end score as vacuum increased from 42.5 to 51 kPa. Total plate counts were significantly higher (1667 c.f.u/ml milk) with 51 kPa than with 34 or 42 kPa vacuum level (771 and 723 c.f.u/ml milk, respectively). The authors have also reported that 48.9% of quarter milk samples from cows milked at 51 kPa were positive for *Staphylococcus aureus* (the predominant cause of mastitis) vs 19.9 and 16% for 34 and 42.5 kPa, respectively.

Reports on the effects of over milking as a predisposing cause of mastitis are contradictory. Mochrie *et al* (1953a, 1953b, 1955), for

example, failed to relate the vacuum level and duration of milking with udder health. Petersen (1964) reported that repeated over-milking (on 16 to 26 occasions) for three to five minutes caused damage to the lining of the teat sinus. Milking cows for eight minutes every milking for a full lactation caused an increase in mastitis when compared with four minutes (Dodd *et al*, 1950), whereas milking for 6.5 minutes for a full lactation had no effect when compared with cups being removed when milkflow stopped (O'Shea, 1974). Natzke *et al* (1978) reported more new infections in the group of cows overmilked for an average of 6.5 minutes compared with the control group.

Another area of risk is the ability of the milking machine to transmit bacteria between cows' quarters. This may occur through reverse flow of milk brought about by cup-slip, vacuum instability or flooding cups with milk.

In Irish work (O'Callaghan *et al*, 1976) teat cup slip has been implicated as a major factor influencing the incidence of mastitis in a herd of cows. This is in support of a short experiment conducted in U.S.A. (Thompson and Schultze, 1975). Australian work (Gibb and Mein, 1976) found that the percentage of cows on which liner slip occurred varied between 4% to 59% for individual liners. Although no individual factors could apparently be related to the incidence of slip, it appeared that the liner with the barrel of widest diameter slipped least frequently and older liners slipped less frequently than new liners. Other work in U.S.A. and Ireland showed that narrow bore liners fell off during milking more frequently than wider bore liners and liners with no mouthpiece cavity also fell off more frequently (Schmidt *et al*, 1963; McGrath and O'Shea, 1972). Unstable vacuum within the machine might be expected to lead to an increase in cup slip or fall and there is some evidence which shows that this is indeed true (O'Callaghan *et al*, 1976). Another possible factor is the careless removal of teat-cups which may cause a drop in vacuum or may have effects within the milking unit equivalent to liner slip. Vacuum should therefore be cut-off from the milking unit before any cups are removed. Most of the research reports indicate that heavy milking units, narrow bore liners with poorly designed necks and

unstable vacuum in the machine are the factors likely to cause cup slip. Many specific references and comments on the role of machine milking in relation to udder health and teat damage are given in the book titled "Machine Milking" published by National Institute for Research in Dairying, Reading (NIRD, 1979), edited by C.C. Thiel and F.H. Dodd.

It would appear from this brief discussion that the milking machine factors predisposing cows to mastitis are more complex than is at first apparent and this explains why the literature appears to be confusing and contradictory, particularly the literature which deals with the importance of most physical settings like type and design of liners. Nevertheless, there is enough evidence to show that many individual milking machine factors predispose cows to udder infection. In general terms the effect of the milking machine factors on incidences of mastitis was demonstrated by Foot (1934) and Ward (1943, 1944) cited by Oliver (1955). Foot's data showed a higher herd wastage due to mastitis in machine- than in hand-milked herds and, Ward showed a mastitis incidence of 12.5% and 8.9% for cows in machine- and hand-milked herds, respectively. Results reported by Pearson *et al* (1972) indicate clearly the importance of machine efficiency in relation to udder health as shown by the fact that 68% of the low mastitis incidence herds had milking machines which were totally efficient, in contrast to only 16% in the high incidence herds, although the herds with efficient machines were probably well-managed in all other ways as well, which could be important.

(ii) Milking Routine Hygiene

Bacteria are ubiquitous i.e. widelyspread in the cow's environment. Thus the level of hygiene practiced during every milking will influence the spread of bacteria from cow to cow. The possible sources of microbial contamination and transmission from cow to cow at milking time are: (a) the milking unit (i.e. teat-cup liners, claw and long milk tubes), (b) skin on the end of the teats, (c) udder washing cloths and (d) hands of the milker.

Contaminated udder, teat-cup liners, claw and milk tubes and teat washing all cows with a common cloth from a bucket of diluted

disinfectant solution, disseminate most of the micro-organisms between mammary quarters of each cow and between cows (Bacic *et al*, 1968; Johns, 1966; Newbould, 1965).

Since intramammary infection results from microbial invasion into the gland via the teat canal (Giesecke *et al*, 1968), it would appear that if no micro-organisms contact the teat end, intramammary infection would not result. McDonald (1969) reports that when bacterial population on the skin on the teat end was reduced to a very low degree, intramammary infection was prevented. Other reports (e.g. McDonald, 1970; Neave, 1971) indicate that certain milking-time hygienic procedures (such as washing udders with running water and drying them with individual towels before each milking, and teat dipping in disinfectant solution after milking coupled with personal cleanliness) prevent transmission of micro-organisms on the teat skin. However the use of single-service paper towels might probably prove to be economically not a practical proposition in terms of expenses involved. Washing udders with running water from a hose would seem preferable to washing with udder cloths because cloths can be a potent source of cross-infection particularly when only one cloth is used for all cows (Benson *et al*, 1974). A study by Hoare (1974) has shown that there is a lower incidence of mastitis in herds where udders are washed with running water than where buckets and cloths are used. The author reports that there is even less mastitis if udder soap is used in conjunction with the running water. The incidence of subclinical mastitis was 25% of quarters in herds washed with buckets and cloths, 21% with running water, and 12% with running water and udder soap. It has been suggested in other reports (Neave, 1971) that since the usual methods of washing udders are not very effective in washing the ends of teats, washing should be restricted to the teats and particularly the tips of teats because washing the udder is likely to spread micro-organisms residing on the surface of the udder (this is with particular reference to *Streptococcus uberis* and coliforms).

The milker's hands are also a possible source of micro-organisms that can be easily spread to the teats if not frequently disinfected

during the milking process. Bacteria capable of causing intramammary infection have been isolated from the milker's hands (Newbould, 1965). An average of 30% of hands yielded positive swabs after being washed with disinfectant compared to 95% when no disinfectant was used (Report Natn. Inst. Dairy. U.K. 1962).

Since mastitis is sometimes regarded as an environmental disease (Fisher, 1981), cleanliness of the dairy premises and sanitation of milking equipment are some of the factors that would greatly help in controlling mastitis (Dunsmore, 1981).

2:1:6 MASTITIS CONTROL: In dealing with control measures, farmers must realize that mastitis not only causes financial losses due to (a) reduced milk secretion, (b) discarded milk, (c) increased replacement costs, (d) drug costs, (e) veterinary fees, and (f) high culling rate; but it is also an increasingly important milk-quality problem (Brookbanks *et al*, 1971; Ali *et al*, 1980).

Most of the studies reported in the literature indicate that an effective mastitis control program includes the following five major points, namely:-

1. Milking machine efficiency check.
2. Use of a teat sanitizer after milking.
3. Dry-cow therapy.
4. Prompt treatment of clinical cases.
5. Culling of chronically infected cows.

The importance of the milking machine in mastitis control can not be over emphasized. The dairyman should select a milking machine system designed to ensure vacuum stability. Fluctuating vacuum has been found to be the common fault of the milking system in the causation of mastitis (Hopkirk *et al*, 1943; Nyham & Cowhig, 1967). Thus periodic evaluations of all parts of the milking system are needed to maintain it in a properly functioning condition.

Disinfection of milking teat-cups between milking of cows can not be accomplished in the time available or with systems currently

in use, particularly in large herds. However, disinfection of teats after milking is regarded as an effective means for reducing the spread of infectious mastitis (Neave *et al*, 1969; Neave, 1971; Sheldrake and Hoare, 1980).

Control of mastitis in problem herds by the intramammary infusion of antibiotics into all quarters of all cows as they enter the dry period has been advocated as the most practical and least costly means for reducing the incidence of mastitis (Roberts *et al*, 1969). To be much more effective, however, such a program must be associated with hygiene during milking, and use of good milking equipment. It is suggested in some literature (e.g. Schalm *et al*, 1971) that it would perhaps be better to select cows for dry therapy on the basis of either their reaction to California Mastitis Test (CMT) applied monthly throughout lactation or an average of somatic cell count levels regularly monitored during lactation. Such an approach would avoid infusing antibiotics into the uninfected cows and thus reduce expenses incurred in the control program as well as reducing the probability that bacteria will become resistant to the antibiotic.

Assuming that cows are being tested routinely for mastitis so that subclinical and chronic problems are identified, rigorous culling programs can eliminate chronic mastitis cows from the herd. Mellenberger (1982) suggests that older cows that are unresponsive to two or three series of antibiotic treatment for mastitis during lactation should receive serious consideration for culling. A report from Dorset, U.K. (Farmers Weekly, November 27, 1981) shows that high culling rates coupled with strict hygiene have brought mastitis levels down from 526,000 to 283,000 cells/ml.

There is experimental evidence to show that the net gain in productivity in the first year of the control program is small but it increases in subsequent years if the farmer perseveres (Asby *et al*, 1975).

2:2:0 PART II - SUBCLINICAL MASTITIS

2:2:1 INTRODUCTION

Subclinical mastitis is a widespread problem in dairy cows and the fact that it is virtually without readily detectable symptoms means that the farmers are unaware of its presence. It has been estimated that subclinical mastitis occurs in 28% of quarters and in 55% of cows (Dodd and Jackson, 1971) and chemical analysis of milk indicates a similar proportion (Linzell and Peaker, 1972). New Zealand results (Moller, 1978) showed that in an average herd without mastitis control, 13% of quarters get treated for clinical mastitis and 26% of the quarters have subclinical mastitis. A report from Sri Lanka (Rupasinghe and Rulasegaram, 1982) indicates that the incidence of subclinical mastitis ranges from 39.5 to 92.2% of cows in Government farms with large units of European breed cows, and from 27.8 to 90% of cows in private farms with smaller units of cross-bred cows. The average prevalence rate of subclinical mastitis in Norwegian dairy cows (Bakken, 1982) is reported to be 31% of the cows and 11.6% of quarters. Stiles and Rodenburg (1981) have reported that 97% of mastitis infections in Ontario, Canada, are subclinical involving no visible changes. Also a recent report compiled by Holmes (1981) on behalf of the Livestock Improvement Association Movement in New Zealand indicates that about 20 to 40% of all cows in seasonal supply herds are affected by subclinical mastitis; and a survey of mastitis in the British herd (Wilson & Richards, 1980) revealed that the national prevalence of subclinical mastitis as defined by the International Dairy Federation was 9.6% of all quarters. These few cited reports indicate that the condition is numerically important.

2:2:2 ECONOMIC IMPORTANCE OF SUBCLINICAL MASTITIS

Subclinical mastitis as reflected in an elevated somatic cell count, causes decreases in the milk yield of cows. Estimated loss reported from Canada (Stiles & Rodenburg, 1981) ranged from more than 15% in herds with cell counts above 800,000 cells/ml to less than 2.5% in herds below 300,000 cells/ml. A recent report from Wisconsin, U.S.A. (Hoard's Dairyman, March 25, 1982) shows that of the infected

quarters, 75% do not show symptoms other than high cell counts and that for every quarter which shows clinical signs such as flakes in milk or actual flare-ups, there are at least 20 or 25 other infected quarters showing no visible symptoms at all. Since subclinical mastitis has no visible symptoms, cows may be culled for low production without the farmer suspecting the presence of infection. The forced culling of these animals substantially reduces farmers' opportunities to cull for low production based on genetic potential (Hoare, 1982). The economic importance of subclinical mastitis can be assessed from its effect on yield and composition of milk and the quality of products made from milk with high somatic cell counts. Stiles and Rodenburg (1981) have reported that subclinical mastitis results in a decrease in production representing an average loss of more than $100 per cow per year in Ontario, Canada.

(i) Effect on milk yield - Individual quarters which have a high somatic cell count (S.C.C.) produce less milk than other quarters on the same udder but with a low S.C.C. (Holmes, 1981); and similarly individual cows which have a high S.C.C. produce less milk than other cows in the same herd but with a low S.C.C. (see Table 2.2 and Figure 2.1). Since a high S.C.C. is indicative of subclinical mastitis, it can be concluded that subclinical mastitis causes a decrease in milk yield.

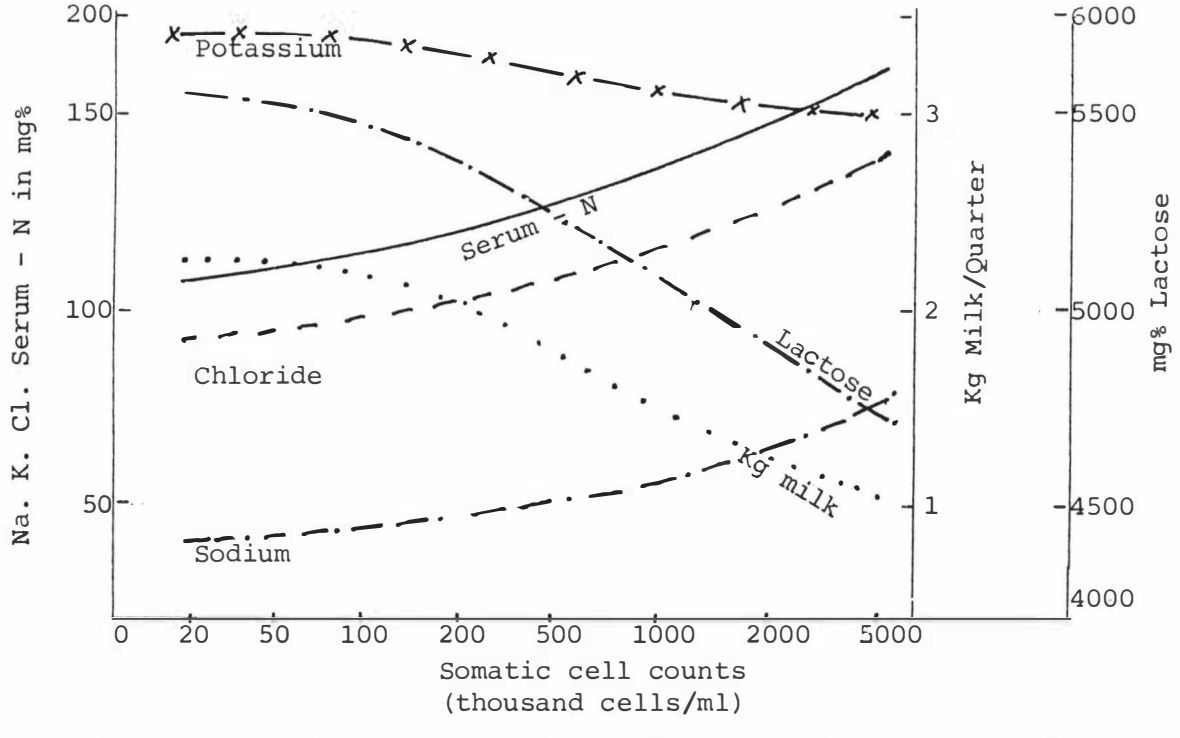

FIGURE 2.1: RELATIONS BETWEEN SOMATIC CELL COUNT, MILK COMPOSITION AND MILK PRODUCTION FROM INDIVIDUAL QUARTERS (HEESCHEN, 1975).

As regards the correlation between milk quantity and cell content, it can be seen from Figure 2.1 that the milk yield begins to decrease at a relatively low S.C.C. in agreement with the bio-chemical changes. This inherent relationship between severity of disease and milk yield allows a statistical estimate to be made of economic losses due to mastitis per quarter, animal and herd with sufficient accuracy. An increased absolute number of somatic cells in milk is a sensitive indicator of changes in chemical composition of milk or lower milk yield (Heeschen, 1975).

TABLE 2.2: THE RELATION BETWEEN S.C.C. OR INFECTION, AND MILK PRODUCTION FROM INDIVIDUAL QUARTERS OF AN UDDER OR FROM INDIVIDUAL COWS.

(i) INDIVIDUAL QUARTERS (FORSTER, 1964; U.S.A.)

California Mastitis Test Result		Decrease in yield per quarter
Trace of slime	(approx. 300,000 cells/ml)	- 0.3 kg per quarter per day
Moderate slime	(approx. 900,000 cells/ml)	- 1.0 kg " " " "
Thick slime	(approx. 2,000,000 cells/ml)	- 1.8 kg " " " "
Very thick slime		- 2.7 kg " " " "

(ii) INDIVIDUAL QUARTERS (MORRIS, 1973; AUSTRALIA)

	Decrease in yield by an infected quarter
Cows in 1st Lactation	38%
Cows in 2nd Lactation	57%
Cows in 3rd Lactation	67%

Note: The S.C.C. from these quarters was increased.

(iii) INDIVIDUAL COWS (DANIEL & FIELDEN, 1971; N.Z.)

Somatic Cell Count Level	Percentage decrease in yield
214,000 cells/ml	- 5%
647,000 cells/ml	- 11%
1,480,000 cells/ml	- 15%

(iv) INDIVIDUAL COWS (GILL & HOLMES, 1978; MACMILLAN & DUIRS, 1980; N.Z.)

Somatic Cell Count	1978	1980
0 to 249,000 cells/ml	0	0
250 to 449,000 cells/ml	- 2%	- 3%
500 to 749,000 cells/ml	- 5%	- 5%
Greater than 750,000 cells/ml	- 7%	- 10%

(v) INDIVIDUAL COWS (STILES & RODENBURG, 1981; CANADA)

	% Estimated Milk Production Loss
Less than 300,000 cells/ml	0 - 2.5
300,000 - 500,000 cells/ml	2.5 - 7.5
500,000 - 800,000 cells/ml	7.5 - 15.0
Greater than 800,000 cells/ml	15.0 - 25.0

(vi) AVERAGE MILK YIELD FROM INFECTED (1) AND OPPOSITE NORMAL QUARTERS - (N) (KING, 1978)

Cell count per ml for Infected Quarters	No of cows	Yield (kg) 1	Yield (kg) N	Decrease in yield (kg) i.e. 1 - N
200,000 to 500,000	83	2.04	2.11	- 0.07
500,000 to 1,000,000	85	1.83	2.09	- 0.26
1,000,000 to 2,000,000	81	1.74	2.13	- 0.39

Note: All the cell counts from the opposite healthy (normal) quarters were below 200,000 per ml.

The results in Table 2.2 show in general that the higher the cell count the greater the reduction in yield. Rako *et al* (1963) in a study of 1003 cows found that the yield of affected quarters was depressed (in comparison with opposite healthy quarters) by 12.2% for quarters which were bacteriologically positive but showed no clinical signs i.e. subclinical cases. Hoare (1982) cites the work reported by Reichmuth *et al* (1970) in which quarters with over 500,000 cells/ml of foremilk yielded 17.1% less than the opposite healthy quarters. Also data from 933 animals, mainly heifers, examined by quarter and cow comparison, demonstrated substantial loss due to subclinical mastitis (Meijering *et al*, 1978). The same authors cite the work of

de Rooy & Jaartsveld (1969) who assessed the mean loss over the whole lactation for cows with subclinical mastitis at 7 - 9% (1.2 to 1.5 kg/cow/day). Using fixed models to examine the partial influence of cell count on quarter-milk yield, Reichmuth (1968) cited by Meijering *et al* (1978) found that the drop in yield became significant when the cell count exceeded 500,000 cells/ml. He reported that cows with a cell count in excess of 500,000 cells/ml in at least one quarter produced 9% less milk compared with healthy animals. Waite & Blackburn (1957) and McKenzie *et al* (1958) noted a drop in the daily milk yield per cow when the total cell count increased to an average of 500,000 cells/ml. Janzen (1970) reports that infected quarters whether showing mastitis symptoms or not, usually yield less milk of lower total solids than the corresponding uninfected front or hind quarters of the same udder.

All these reports confirm the effect of subclinical mastitis on milk yield which, as it can be deduced from Table 2.2, appears to be proportional to the severity of the changes occurring in the udder as judged by cell count.

(ii) Effect on milk composition - The composition of milk from individual quarters is known to change in association with increases in somatic cell count (see Figure 2.1). The following changes have also been reported to occur in herd bulk milk as shown in Table 2.3.

TABLE 2.3: CHANGE IN % COMPOSITION ASSOCIATED WITH AN INCREASE OF 100,000 CELLS/ML IN BULK SOMATIC CELL COUNT.

Milk Constituent	Percentage loss	Reference
Fat %	- 0.010%	(Asby *et al*, 1977)
Protein %	- 0.001%	(Gill, 1977)
Solids-not-fat %	- 0.019%	(Gill & Holmes, 1978)
	- 0.015%	(Asby *et al*, 1977)

These are all relatively small changes in milk composition, nevertheless they represent adverse effects of high somatic cell counts (i.e. subclinical mastitis).

Udder infections change milk composition by altering membrane permeability within the mammary gland and by reducing synthesis of milk components (Schalm, 1977). There are two general physiological explanations for changes in milk composition associated with mastitis and elevated somatic cells, namely:-

a) injury to the udder cells which reduces the synthesis of those milk components synthesized in the udder; typical examples are lactose and most of the casein, and

b) changes in permeability of membrane which permit increased "leakage" of materials from the blood to the milk. There appears to be increased permeability of both the vascular and secretory epithelia. Typical examples of materials which increase due to this phenomenon are sodium, chloride and immunoglobulins (see Figure 2.1). When the concentration of lactose is decreased, compensation must be made to ensure that milk and blood maintain the same osmotic pressure. Most of this compensation is accomplished by increases in sodium and chloride (Schalm, 1977).

The changes in composition are directly proportional to the severity of infection as measured by cell count or CMT (Waite & Blackburn, 1963; Foster *et al*, 1966; McKenzie and Anderson, 1981). King (1978) observed significant changes in composition when the infected quarters showed cell counts of 500,000 cells/ml or above. On a between herd basis Asby *et al* (1977) demonstrated a relationship between bulk milk composition and cell count; total solids, solids-not-fat and fat concentration tending to decrease as cell count increased.

(iii) Effect on yield and quality of dairy products - High somatic cell levels are also associated with the development of quality defects in dairy products, particularly on storage (Brus and Jaartsveld, 1971) and present problems in the manufacture of cheese by depressing acid production and reducing the firmness of the curd

(Hampton & Randolph, 1969). The most recent information comes from a study which showed that milk with high S.C.C. had a slower clotting reaction with rennet and gave a lower curd yield per 100 kg milk (Ali *et al*, 1980). Work in Wisconsin, U.S.A. showed that decreases in cheese yield per 100 kg milk occurred in association with increases in bulk somatic cell count (B.S.C.C.); see Table 2.4. A high somatic cell count may affect the growth rate of bacteria added as starter cultures to milk. In his review Guthy (1979) cites the work reported by Kastli *et al* (1966) in which the growth of *Streptococcus lactis* was retarded when the somatic cell count was between 200,000 and 500,000 cells/ml and the retardation was even more pronounced when the cell count was above 500,000 cells/ml. He also reports that butter made from milk of high somatic cell count has a less pronounced aroma and an impaired taste both of which become marked on storage.

TABLE 2.4: THE RELATION BETWEEN B.S.C.C. AND CHEDDAR CHEESE YIELD (HOARD'S DAIRYMAN, MARCH 25, 1980).

B.S.C.C. (cells/ml)	Cheddar cheese yield (kg cheese/100 kg milk)
240,000	9.75
496,000	9.69
640,000	9.43

The cell count in milk is used in some countries as one of the criteria of the hygienic quality of the milk (Dijkman *et al*, 1969). Mastitic milk has a high cell count and this also leads to lowering of quality (Schalm *et al*, 1971). The Standard Plate Count has now become the basic bacterial test for raw milk in the New Zealand Dairy Industry and this may be affected by the growth of mastitis-causing organisms which may lead to down-grading of the milk thus lowering income if rate of payment is based on grade. It may therefore be necessary to identify infected cows, both clinical and subclinical, in order to prevent milk from being down-graded.

Milk quality is of national importance because most of New Zealand's milk, for example, is processed into products which must

meet rigid standards before they can be sold and exported. Improved udder health should lead to the production of good quality milk and dairy products.

2:2:3:0 DIAGNOSIS

Subclinical mastitis usually has no apparent signs and can be detected only by special tests. Measuring the number of somatic cells in milk is one of such tests. Somatic or "body" cells found in milk include small numbers of cells released as part of the normal process of replacing milk producing tissue. The epithelial cells of the mammary gland are subjected to continuous wear during their specific activities of the synthesis of milk and its constituents. The worn cells and the cell debris are discharged with the milk (Heeschen, 1975) and thus are a regular and normal component of it. The remainder of the somatic cells in the milk are leucocytes or white blood cells whose role in the body is to: (a) destroy bacteria, (b) prevent or eliminate infection and (c) repair damaged tissue.

Increased somatic cell counts result when the udder is injured, severely stressed or when it becomes infected with mastitis (Schalm & Lasmanis, 1968). These authors report that the content of cells increases in mastitic milk primarily due to an overwhelming number of leucocytes infiltrating from the blood and so, by counting the cells an assessment of the probability of bacterial infection can be made (MacMillan & Duirs, 1980; Schalm & Lasmanis, 1968).

There are two commonly accepted diagnostic signs of subclinical mastitis: infection of the foremilk and a raised milk cell count (Linzel, 1975). The use of a somatic cell counting system for monitoring mastitis status of herds and the udder health of individual cows is being widely accepted in many countries throughout the world. Cell counts provide a measure of inflammatory reaction in the udder and taken together with the isolation of bacteria, they are a guide to the prevalence of subclinical mastitis where there are no gross changes in the milk or clinical changes in the udder. The International Dairy Federation (see Section 2:1:1) states that where the milk and udder are macroscopically normal a cell count of more than 500,000 cells/ml

together with the presence of pathogenic bacteria signifies subclinical mastitis. Isolation of pathogenic bacteria in the milk sample as well as high somatic cell levels would therefore seem to be an important criterion in arriving at a correct diagnosis of subclinical mastitis due to the fact that exudation of leucocytes into the milk could also be caused by any mechanical injury of the mammary tissue as well as stress (Afifi, 1968; Schalm & Lasmanis, 1968; Whittlestone *et al*, 1969; Pearson *et al*, 1971). It is reported in literature (Schalm & Lasmanis, 1968) that leucocyte exudation into the milk may continue for long periods after the cause of mastitis has been removed. The relationship between the presence of pathogens and cell count is shown in Tables 2.5, 2.6, 2.7 and 2.8.

TABLE 2.5: THE RELATIONSHIP BETWEEN THE INCIDENCE OF MASTITIS PATHOGENS AND CELL COUNT IN INDIVIDUAL COWS (MACMILLAN & DUIRS, 1980; N.Z.).

	Infection Status			
Cell Count (thousands/ml)	Primary Pathogen (1)	Secondary Pathogen (2)	Non-infected	All cows
200 or less	18%	74%	86%	66%
201 - 500	30%	21%	12%	19%
501 - 1,000	20%	5%	1%	7%
More than 1,000	32%	0%	1%	8%
	100%	100%	100%	100%
No. of cows	79	99	152	300
(%) of all cows)	(24)	(30)	(46)	

(1) *Staphylococcus aureus*, Streptococcus sp.

(2) *Corynebacterium bovis*, Micrococcus sp.

It can be seen from the above table that primary pathogens known to cause mastitis were isolated from only 79 (24%) of the 330 samples; and that 82% of these 79 infected cow samples also contained over 200,000 cells/ml. In contrast, there were milk samples from 152 cows

(46%) from which no bacteria were isolated; 86% of these samples contained less than 200,000 cells/ml. The secondary pathogens are bacteria present in the milk but do not usually cause mastitis. Results in Table 2.5 show that they do not greatly elevate cell counts. Some of the samples from which primary pathogens were isolated as shown in Table 2.5 also had low cell counts (18%). It is reported that these were probably cows with a less severe infection in only one quarter, and that higher cell count from that quarter was diluted by milk from the other three quarters. The authors postulated that since cell count levels are constantly changing, these samples may have been obtained from cows with an early infection, or the non-infected samples which contained high cell counts may have been obtained just when the leucocytes had overcome the infection (Schalm & Lasmanis, 1968). It is for such reasons that more than one cell count for each individual cow can prove more meaningful than a result from a single sample (Griffin, 1971; Natzke *et al*, 1972; Neave, 1975; MacMillan & Duirs, 1980). Cows with persistently high cell counts are most likely to be those with infected udders, and be the cause of herd's high bulk milk cell count (Holmes, 1981).

TABLE 2.6: MEAN GEOMETRIC S.C.C. FOR QUARTERS OF KNOWN INFECTION STATUS (CELLS/ML) X 10^3 (SHELDRAKE, 1982; AUSTRALIA).

Herd V			Herd D			Herd W		
Staph. aureus	Minor pathogens	Free	*Staph. aureus*	Minor pathogens	Free	*Staph. aureus*	Minor pathogens	Free
860	250	116	2525	303	140	705	193	76
b	c	f	a	c	e,f	b	e	f
Number								
122	1719	600	122	224	13	138	509	16

Note: Different letters indicate significant difference using student's t - test, P <0.001.

The above table shows different levels of S.C.C. in quarters affected by *Staphylococcus aureus* or minor pathogens infections in different herds, indicating the problem encountered in coming up with

a standard threshold of S.C.C. at which bacterial infection of economic importance can be expected with certainty. Sheldrake (1982) suggests that if a common threshold is to be selected for herds like those presented in Table 2.6, then the lower level of S.C.C. should be chosen. This would result in a high proportion of false positive predictions in Herd D, which nevertheless would be preferable to a high proportion of false negative predictions in the remaining two herds (Sheldrake, 1982).

TABLE 2.7: THE RELATIONSHIP BETWEEN SOMATIC CELL COUNT AND INFECTION (HOARD'S DAIRYMAN, MARCH 25, 1982; VIRGINIA POLYTECHNIC INSTITUTE & STATE UNIVERSITY, U.S.A.).

Somatic cell count range in foremilk	Percentage of cows	Percent Positive*
<100,000	37	37.8
100,000 - 200,000	20	61.0
200,000 - 300,000	12	66.6
300,000 - 400,000	8	69.0
400,000 - 500,000	6	73.8
500,000 - 700,000	7	72.9
700,000 - 1,000,000	6	75.8

[*Bacteria isolated from foremilk in the data given in Table 2.7 included the Streptococci sp. (i.e. *Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis*), *Staphylococcus aureus, Escherichia coli; Staphylococcus epidermidis* and *Corynebacterium bovis*]

The results presented in Table 2.7 show that as the somatic cell counts rose, the percentage of cows infected rose. Similar results (see Table 2.8) have been reported from a study in Pennsylvania (Stiles & Rodenburg, 1981).

The percentage of infection shown in Table 2.7 seems to be very high particularly under the <100,000 cells/ml range. Although nothing about productivity appears in the table, one would expect a reduction in milk yield particularly if a greater proportion of the pathogens involved are the primary ones i.e. *Staph. aureus* and the Streptococci

group. That being the case then it could be deduced that a mild elevation in somatic cell concentration is likely to have an adverse effect on milk production.

TABLE 2.8: INFECTION RATE OF COWS WITHIN VARIOUS SOMATIC CELL COUNT RANGES IN A PENNSYLVANIA STUDY (STILES & RODENBURG, 1981).

Somatic cell count	% of cows infected with Major Pathogen
<100,000	6
100,000 - 200,000	17
200,000 - 300,000	34
300,000 - 400,000	45
400,000 - 500,000	51
500,000 - 600,000	67
>600,000	79

The percentage of cows in which major pathogen was isolated in the data presented in Table 2.8 seems to suggest a subclinical mastitis problem at a rather low level of somatic cell count. The problem is much more obvious at a level >500,000 cells/ml where 67% of the cows were found to be infected with a major pathogen.

Duirs (1980) gave some recommendations about the level of somatic cell count to be adopted as a basis for diagnosis of subclinical mastitis (see Table 2.9).

TABLE 2.9: CELL COUNT LEVELS RELATED TO SUBCLINICAL MASTITIS (DUIRS, 1980; N.Z.).

Cell count (cells/ml)	Bulk-cell count from vat	Individual cow cell counts
<250,000	Indicates a low level of subclinical mastitis (satisfactory).	Indicates a healthy udder.
250,000 - 500,000	Indicates that the herd has a moderate subclinical mastitis problem. Action required to contain problem.	Individual cows with cell counts consistently in this category have a high chance of being infected.
>500,000	Indicates a serious subclinical mastitis problem.	Indicates that individual cows are infected with mastitis.

In his review, Holmes (1981) concluded that:-

- all cows with S.C.C. which are consistently higher than about 200,000 to 300,000/ml, but without clinical signs, have subclinical mastitis.
- the majority of cows which have bacterial infection will have high S.C.C.
- the majority of cows which have low S.C.C. will be uninfected.

These conclusions are in agreement with the data presented in Tables 2.7 and 2.8. In view of this and the fact that mastitis accounts for 80% of the variation in somatic cell count (Duirs, 1980), one would be tempted to conclude that somatic cell counts in milk appear to be more important in subclinical mastitis detection than probably bacteriological identification.

However, the interpretation of somatic cell count results needs careful thought and has to take into consideration the other factors likely to cause an increase in cell counts. These factors are as indicated in the following discussion.

2:2:3:1 FACTORS INFLUENCING SOMATIC CELL COUNTS

(i) Stage of Lactation

It is generally agreed that the somatic cell count is high in colostrum but soon decreases and remains low for the first few weeks after which it gradually increases until the end of lactation (Cullen, 1966; Schalm *et al*, 1971). The average cell count according to Waite & Blackburn (1957) was lowest and least variable from day 70 to day 130 of lactation. The reported increase in cell counts in the beginning of lactation is due to the presence of large amounts of epithelial cells and polymorphonuclear (PMN) leucocytes (Cullen, 1966); see Table 2.10.

TABLE 2.10: CELL COUNTS IN NORMAL MILK (MILLIONS/ML) (CULLEN, 1966).

Stage of Lactation	Epithelial cells	Polymorphs	Lymphocytes	No. of Samples
Colostrum (0.5 day)	1.19	2.28	0.72	44
Early (5 days - 8 weeks)	0.14	0.14	0.09	40
Mid-lactation	0.12	0.10	0.05	84
Late - last 4 weeks Before drying	0.85	0.28	0.59	36

Blackburn (1966) reported that the increase in cell count at the end of lactation is probably due to the shedding of epithelial cells from the alveoli into milk during the course of involution of the udder which seems to be in agreement with the data presented in Table 2.10. This together with the deceasing volume of milk towards the end of lactation results in the exaggeration of cell concentration in the smaller volume of milk (Schalm *et al*, 1971).

Natzke *et al* (1972) using the presence of bacteria in two consecutive samples as indicator of infection showed that in late lactation uninfected quarters did not necessarily show elevated cell counts in composite udder milk. This confirmed the suggestion of Schalm and Lasmanis (1968) that the increase in cell counts at drying

off was the result of new infections. The reported trend in increase in cell counts with advancing lactation is probably due to an increase in new infection rate through lactation. Thus a sudden rise in cell numbers may sometimes be attributed to a new infection.

(ii) Age or Lactation Number

It is indicated in literature that the average cell count increases with the lactation age of a cow (Blackburn, 1966, 1968; Cullen, 1966; Weihe, 1969). This could probably be a reflection of the past infection history of the udder as Natzke *et al* (1972) found that uninfected animals showed no upward trend over five lactations. Earlier, Blackburn (1966) over a seven year period found that the increase in cells from one lactation to the next was mainly due to an increase in PMN leucocytes. This increase was associated with bacterial infection resulting in inflammation of the ducts and also an increase in the severity of lobular lesions.

Tolle (1971) reports that there is a decrease in cell count averages in cows older than six years. Sheldrake (1982) reports that if S.C.C. is to be used to predict the presence of intramammary infection the effects of stage and number of lactation may be safely ignored. In his study the effects of stage and number of lactation upon S.C.C. in quarters free from infection were found to be small when compared to the elevation in S.C.C. caused by infection with *Staphylococcus aureus*. It would appear from this discussion that the increase in cell count with age is small in uninfected cows but past infections play an important role.

(iii) Diurnal Variation and Milking Interval

Considerable variations in cell counts have been observed between morning and evening milk samples particularly when there are irregular milking intervals (Cullen, 1967). These variations were found at both high and low somatic cell counts; although the greatest variation occurred when the counts were greater than one million.

Schalm *et al* (1971) are of the opinion that the diurnal variation in cell count is the result of pressure changes in the alveoli. With

the fall in internal gland pressure, as milk is removed during the milking process, cells are released into the milk. Thus, milk taken from the gland several hours after a milking contains the greatest concentration of cells of any of the milk fractions. In his review Gill (1977) cites the work of Hoare (1977) who reported that there may not be a true diurnal variation in cell counts. Hoare (1977) showed that the number of cells shed per milking tends to be constant and that the higher milk production generally obtained at the morning milking results in a lower cell concentration (see Table 2.11).

TABLE 2.11: SOMATIC CELL COUNTS IN GLENFIELD DAIRY AVERAGE (HOARE, 1977 CITED BY GILL, 1977).

	a.m.	p.m.
Production (litres)	7.8	4.1
Log cell count (per ml x 10^3)	123	246
Total No. of cells/milking	9.84×10^8	10.8×10^8

It would appear from the above table that the somatic cell count at evening milkings will be higher than at morning milkings particularly when there is a long interval preceding the morning milking. Bulk samples for testing should thus be composites of both morning and evening milkings.

(iv) Milk Fraction

The somatic cell count of milk varies among different fractions of milk obtained at a single milking (Schalm *et al*, 1971). These authors reported that cell counts in foremilk are usually lower than those in strippings milk and the counts in middle milk are the lowest. Both PMN leucocytes and mononuclear cells are greater in strippings than in foremilk but the differences with regard to mononuclear cells are not as great as with PMN leucocytes.

Paape and Tucker (1966) showed that the cell counts in residual milk are higher than in foremilk but lower than in strippings. On

the contrary Natzke & Schultz (1967) reported higher counts in residual milk than in strippings.

In view of this it would seem important to standardize the time of sampling in the milking routine when taking samples from individual cows.

(v) Stress Related Factors

The effect of "stress" on the cell counts in cow's milk is quite controversial. Macadam (1954) reported a five-fold increase in cell counts when milk was drawn from cows after their transportation by truck to an abottoir. Whittlestone *et al* (1970) showed that stressful situations such as isolation from the herd, chasing by a dog and sudden thunderstorms can result in an increase in cell counts. These studies however have not indicated that cows were free of infection. Stress results in the release of ACTH from the anterior pituitary gland which activates the adrenal cortex to release corticosteroids (Paape *et al*, 1973). Thus the administration of ACTH mimics some of the physiological responses to stress.

Schalm *et al* (1965) found that injecting corticoids caused a rise in leucocyte in circulating blood but not in the milk. Paape *et al* (1973) observed no increase in cell counts in non-infected quarters when cows were injected with ACTH. Earlier Whittlestone *et al* (1970) had shown that quarters which had a previous history of mastitis resulted in increases in milk somatic cell counts following injection of ACTH. Wegner *et al* (1976) recorded positive correlations between blood leucocytes and somatic cell counts of milk in mastitis-free cows injected with corticotropin and between percent blood neutrophils and somatic cell counts of milk in environmental-heat stressed cows with no evidence of current mastitis.

It seems from some of the work cited above that stress may have an effect on cell count of the udder with previous history of infection or existing infection. This means that under stress, infections otherwise not detected will show up as increases in somatic cell level. It would thus appear that stress-induced cell count increases have

diagnostic value because they occur in animals harbouring infection.

It is apparent from this review of factors affecting cell counts that there is a general lack of agreement about conclusions to be drawn from somatic cell counts. Nevertheless there is substantial experimental evidence to show that high cell counts are associated with reduced milk yield regardless of whether or not they are caused by pathogenic organisms. This together with changes in milk composition indicates that there is some disturbance in the milk secretory tissues. Cell numbers in milk should therefore be considered as a measure of the functional status of the secretory tissue. For secretory activity, mastitis is probably the most important factor causing disturbances in the secretory tissue. It would appear that the sources of error in interpreting cell counts can be reduced if cell counts are carried out at regular intervals over a period and running means calculated to minimize the anomalies.

2:2:3:2 DIAGNOSTIC METHODS

Various methods have been developed for estimating the cell content of milk (Schalm *et al*, 1971). Depending on whether they are used for precise counting or as screening tests, the methods are referred to as direct or indirect methods.

Direct methods involve either the microscopic or electronic counting of somatic cells. The indirect methods depend on tests for the detection of DNA from cellular nuclear material or the reaction between cell DNA and certain chemical reagents which brings about a marked change in viscosity. Other indirect methods test for change in milk composition, electrical conductivity, pH measurements and enzymatic procedures. Some of these methods are briefly described in the following discussion; for details see Schalm *et al* (1971).

2:2:3:2:1 DIRECT METHODS FOR SOMATIC CELL DETERMINATION

(i) Microscopic Counting

This is a direct visual method for determining the number of cells in milk. It involves spreading 0.01 ml of milk sample over

1 cm^2 of a glass slide and staining the dried smear with methylene blue. The cells are then counted in a number of fields with an oil immersion lens and expressed as the number of cells per ml. Schalm *et al* (1971) have reviewed this technique fully. The method was first described by Prescott and Breed in 1910. They expressed each cell counted to represent 5000 cells/ml. An improved version of this method is now referred to as the Direct Microscopic Somatic Cell Count (D.M.S.C.C.). It involves counting the stained cells present in strips across the milk film rather than selected microscopic fields and uses a system of counting dependent on an estimation of cells present in the sample (Brazis *et al*, 1967; Ward & Postle, 1970).

The direct visual identification and counting of somatic cells appears to be the best reference method. There is however, an inherently large variation within one sample associated with microscopic counting due to the fact that the smears may not be homogeneous (Heeschen, 1975). Moreover the optical and manual operations involved are tedious and time consuming which virtually tend to preclude their use in large scale screening programmes.

(ii) Electronic Somatic Cell Counting

Electronic cell counting is now often used for estimating somatic cells in cow's milk. The method has been successfully used by several workers and is reported to be a very rapid and sufficiently accurate procedure. The electronic counters include the particle counter which uses current interruption (Coulter System) or light beam interruption (Auto analyser) and Fossomatic for detection of stained cells.

The significance of the Coulter principle lies in its ability to record single cells one by one without subjective errors when these cells are suspended as the only particles in their size range in an electrolyte (Heeschen, 1975). Since the main problem encountered in determining somatic cells in milk is the elimination of fat particles overlapping the size range of cells, it is necessary to ensure that fat globules are removed before somatic cells are counted. This is accomplished by either centrifuging (Cullen, 1965; Phipps & Newbould,

1965; Read *et al*, 1967) or dispersing the milkfat using a non-ionic wetting agent (Cullen, 1967). Chemical isolation of intact cells from the fat is considered more suitable than the centrifugal method for regular examination of large number of milk samples using the electronic cell count because it is quicker, more economical in terms of cost, and more adaptable to automation (Dijkman *et al*, 1969; Pearson *et al*, 1970).

A more descriptive account on how to measure somatic cells in cow's milk using a Coulter Counter has been reported by Tolle (1971). Other workers have reported the correlation coefficient between electronic cell counts and direct microscopic cell counts. Mitchel *et al* (1967) reported a correlation of 0.978; Pearson *et al* (1970) 0.978; Read *et al* (1967) 0.997; Philpot & Pankey (1973) 0.854 to 0.977 and Newbould (1974) 0.85 to 0.924.

Heeschen (1975) has reported a method whereby procedures using electronic counting can be standardized. He has also described the continuous flow analysis (Auto analyse System) which is essentially an Automated Coulter Counter and the Optical Fossomatic Instrument. The latter is an automated microscopic cell count using a DNA-specific fluorescent dye. Also Schmidt Madsen (1975) compared the Fossomatic with the Coulter Counter and direct microscopic count. Results obtained indicate that the Fossomatic appears slightly superior to Coulter in precision and in ease of operation.

Although the electronic counting system of somatic cells by different instruments is comparatively more accurate and capable of handling large numbers of samples in a day, standardization continues to be a problem in addition to the high initial cost of equipment.

2:2:3:2:2 INDIRECT METHODS FOR SOMATIC CELL DETERMINATION

(i) The Catalase Test

Catalase is an enzyme found in the cells of both animals and plants. Measurement of catalase activity was the first among the methods in use today for the indirect estimation of somatic cell

content of milk. The catalase content of normal milk is low except at the beginning and end of lactation; it increases in mastitis (Schalm *et al*, 1971).

This test is based on the principle that catalase liberates molecular oxygen and water from hydrogen peroxide. This gas is measured and expressed in volume percentage which is correlated with somatic cell count (Paape *et al*, 1965; and Schalm *et al*, 1971) as shown in Table 2.12. For detailed description of the test see Schalm *et al* (1971).

TABLE 2.12: RELATIONSHIP BETWEEN % GAS AND SOMATIC CELL NUMBERS PER ML (FROM SCHALM *et al*, 1971).

% Oxygen	Correlated Somatic Cell Range (cells/ml)
<20	<500,000
20 - 30	500,000 - 1,000,000
30 - 40	1,000,000 - 2,000,000
over 40	over 2,000,000

Normal milk generally yields less than 10%. The method is not suited for use at the side of the cow but is a useful laboratory test.

(ii) Whiteside Test

The reaction between milk obtained from mastitic cows and sodium hydroxide (NaOH) resulting in the formation of a sticky mass was first described by Whiteside in 1939. At room temperature the reaction takes place rapidly but when mastitic milk is heated or when milk contains preservatives such as formalin, mercuric chloride and potassium dichromate, no reaction takes place (Schalm *et al*, 1971). The degree of reaction is graded from 0 - 4 and these grades are correlated with somatic cell count ranges as described by Schalm *et al* (1971).

Murphy and Hanson (1941) modified the test to the form in which it is used today which involves the addition of one drop of 4% sodium hydroxide to five drops of cold milk on a glass plate followed by

vigorous stirring for about 20 seconds. The reaction is based on the fact that nucleic acid from somatic cells forms a sodium salt in the presence of sodium hydroxide producing a gelatinous mass to which serum solids and fat globules become absorbed to produce a characteristic precipitate (Dun *et al*, 1943).

(iii) California Mastitis Test

The California Mastitis Test (CMT) is an indirect method widely used for routine purposes in laboratories and under field conditions. The test is subjective and has strict limitations mainly because of the wide overlap of cell ranges (see Table 2.13).

The test was first described by Schalm & Noorlander (1957) who showed that when an anionic surface active agent is added to milk and stirred it results in a viscous mixture. The degree of viscosity depends on the number of somatic cells present in the milk i.e. as the somatic cell content increases, viscosity increases. The reagent proposed by Schalm & Noorlander (1957) was alkyl-arylsulphonate, with the addition of bromcresol purple to indicate the pH of the mixture. The test was designed for use as a cow's side test where milk from four quarters is squirted into four different depressions on a plastic paddle. Thereafter an equal amount of CMT reagent is added and the paddle is swirled approximately 10 times and the degree of viscosity of the contents assessed as shown in Table 2.13.

TABLE 2.13: ESTIMATION OF THE CELL CONTENT OF MILK BY CMT SCORES (SCHALM *et al*, 1971).

CMT	Cell Range (cells/ml)	Description of Visible Reaction
-ve	0 - 200,000	No evidence of precipitate
Trace	150,000 - 500,000	Slight precipitate which may disappear.
1	400,000 - 1,500,000	Distinct precipitate, no gel formation.
2	800,000 - 5,000,000	Mixture thickens immediately with some gel formation.
3	5,000,000	A thick gel forms.

Pettersen (1981) found a highly significant correlation ($P<0.001$) between the cell count results obtained by the Direct Microscopic Count (DMC), Electronic Cell Count (ECC) and California Mastitis Test (CMT).

Other indirect tests include Brabant Mastitis Test, The Michigan Mastitis Test, The Wisconsin Mastitis Test and Rolling Ball Viscometer. All these tests work on a similar principle as the CMT i.e. assessing the viscosity that develops in mastitic milk following the addition of specific reagents to a measured quantity of the milk sample. The grades are then correlated with somatic cell count as described by Schalm *et al* (1971).

2:2:3:3 BACTERIOLOGICAL DIAGNOSIS

Bacteriological methods are advocated for diagnostic purposes because the cause of about 90% of mastitis of economic importance (Neave, 1975) is due to the main pathogens, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, and *Streptococcus uberis*; and the most common methods detect these bacteria only. These bacteria have been called 'primary mastitis pathogens' and the intramammary infections for which these organisms are responsible, as 'primary infections'. These organisms and the coliforms cause infections with marked cellular response, clinical symptoms and major changes in milk yield and composition (Griffin *et al*, 1977). The other bacteria such as coagulase-negative staphylococcus (e.g. *Staphylococcus epidermidis*), micrococci and corynebacterium have been referred to as 'secondary mastitis pathogens' and intramammary infections for which these organisms are responsible, "secondary infections'. These normally cause relatively small cellular reactions in udder quarters and are rarely associated with clinical disease (Griffin *et al*, 1977). As the terms 'primary' and 'secondary' could imply sequence rather than severity of infection of economic importance, these are gradually being replaced by the terms 'major' and 'minor' respectively (Griffin *et al*, 1977).

Pearson & Greer (1974) observed in their study that 80% of the pathogens were primary and 15% were secondary. In New Zealand,

Staphylococci and Streptococci have been reported to be more commonly associated with abnormal udders and high somatic cell counts than other bacteria (Elliot *et al*, 1976).

The aim in bacteriological examination is to obtain a milk sample free of contamination by extraneous organisms. Precautions must therefore be taken when milk samples are drawn to ensure that bacteria do not enter the milk from extraneous source and render it unsuitable for diagnostic purposes. A reliable method for determining whether pathogens are derived from an intramammary origin is by sampling through the teat wall by puncture (Neave, 1975). This method is however not very practical under field conditions.

Fraction of Milk to be collected for Bacteriological test

Opinions seem to differ concerning the fraction of milk to be taken for the bacteriological diagnosis of mastitis. The first streams of milk usually contain the greater number of infecting micro-organisms that colonize the streak canal (Schalm *et al*, 1971) and for this reason some give preference to strict foremilk when only fresh milk is to be cultured. Others prefer to discard the first streams under the assumption that this will wash extraneous bacteria from the streak canal and reduce false positive results. Neave (1975) states that the number of false negative samples can be decreased by taking strict foremilk. On the other hand, Schalm *et al* (1971) report that established infections by Streptococci and Staphylococci can be detected with a high degree of accuracy from either foremilk or strippings milk without adherence to the strict foremilk principle. There is however a general agreement on the idea of discarding the first few squirts of milk before collecting any milk sample for bacteriological test and also cleaning and disinfecting the teat end with cotton wool soaked with 70 - 80% ethanol before any sampling is done.

It seems that with reasonable care, both in disinfecting the teat end and in collecting the milk after discarding the first few squirts as well as replacing the cap as quickly as possible, contamination can be avoided and the milk will be satisfactory for bacteriological test.

Methods Employed in the Isolation of Pathogens from Milk

The methods commonly used in diagnosing bacteria in milk samples include: the pour-plate method and the surface culture method.

(i) The Pour-Plate Method

This involves the use of 1 ml milk diluted with sterile saline solution in the ratio of 1 : 20, 1 : 40 or 1 : 100. A 1.0 ml portion of diluted milk is placed into an empty, sterile petri-dish and melted blood agar is poured onto the plate as the plate is rotated to mix the milk and agar. The agar is left for some time to solidify. Thereafter the plates are placed at 37°C in an upside down position for 24 hours. The number of colonies developing times the dilution factor equals the bacteria count per ml milk (Schalm *et al*, 1971).

(ii) The Surface Culture Method

This method is probably more commonly used than the pour-plate method. Using a platinum loop, 0.01 ml of milk is spread evenly over either one-fourth, one-half or the entire surface of a blood agar plate. After incubation at 37°C for 24 hours, the number of colonies counted times 100 equals the bacteria count per ml milk (Schalm *et al*, 1971).

Neave (1975) reports that direct plating 0.01 of fresh milk, while having the advantage of fewer false positive samples than with larger volumes, fails to detect pathogens in about 10% of samples from established infections of *Staph. aureus* compared with 6% false negatives and 4% false positives resulting from plating 0.05 ml of milk. Thus plating 0.05 ml of milk appears to be a very practical and effective technique.

In routine diagnostic studies on milk it is more important to detect the presence of pathogens than to determine their actual numbers. This can be done by plating milk samples with swabs on blood agar and incubating at 37°C for 24 hours before identifying the bacteria grown in colonies. It is suggested in some literature (Schalm *et al*, 1971; Neave, 1975) that milk should be incubated for 16 to 20

hours before streaking on blood agar as this leads to the detection of many more pathogens than when fresh milk sample is employed. However, Schalm *et al* (1971) point out that an argument against increasing the bacterial numbers by incubation of the milk samples before plating is that when sampling technique is poor, contaminants outgrow and mask small numbers of pathogens during initial incubation of milk.

The presence of micro-organisms does not necessarily indicate an infection of the udder tissue. The teat orifice, skin and the teat canal may all yield organisms that are not necessarily associated with any overt inflammation process and therefore do not necessarily indicate their presence in the cistern or udder tissue (Munch-Peterson, 1971 cited by Giesecke and Van den Heever, 1974). Earlier, Newbould and Neave (1965) had demonstrated that the presence of organisms in milk might not indicate an infection of intramammary source unless the milk sample also contained a significantly higher number of cells than that found in a normal quarter of a cow. It would appear that the recovery of mastitis pathogens or any other micro-organisms from milk taken aseptically does not necessarily indicate that they are of intramammary origin. This has led to confusion about bacteriological diagnosis and efforts to develop a much more acceptable definition of infection.

Neave (1975) proposed a system of bacteriological test that requires the confirmation of infections, whenever possible, by two or more consecutive milk samples and demonstrated that consecutive samples from infected quarters are rarely both negative. Griffin *et al* (1977) confirmed and reported that quarters diagnosed as infected by this method nearly always give positive reactions just like with indirect tests indicating inflammation (i.e. cell count, California Mastitis Test etc.). Earlier, Griffin (1971) had reported that the criterion for detecting an infection before therapy could be instituted was based on the bacteriological examination of two foremilk samples taken with aseptic precautions. It would appear that the adoption of this method coupled with the use of cell counts is the best method of confirming infections of subclinical mastitis before administering any antibiotic.

The bacteriological tests are time consuming and costly but they are necessary for proper control of the treatment of infections with antibiotics and other therapeutic agents, particularly in experimental studies.

2:2:4 CONTROL METHODS

Although mastitis control is based on methods of preventing intramammary infection, hygiene and management practices are sometimes not sufficiently effective to reduce infection to very low levels (Dodd & Griffin, 1975). Therefore successful control has to incorporate methods of eliminating infections. It is suggested in some literature (Kingwill *et al*, 1971) that if a control system is to reduce infection levels in months rather than years, it is necessary to eliminate infections by treating subclinical cases of mastitis with antibiotics in addition to those infections that reveal themselves by clinical symptoms. This can be accomplished by the use of antibiotic therapy either during lactation or drying-off period (Griffin, 1971; Plommet & Le Louedec, 1975). The choice of method will depend on the level of infection in a herd, the probable benefit to be obtained from its use and important economic factors.

(i) Treatment of Subclincial Mastitis during Drying-off Period

Most of the reports appearing in the literature indicate that dry-cow therapy is preferable because it is a method which:-

a) does not contaminate saleable milk;

b) prevents most new infections from occurring in the dry period and is also effective in eliminating existing infections;

c) allows the regeneration of damaged udder tissue before the following calving;

d) gives the greatest proportion of uninfected cows at the time of highest milk yield.

Smith *et al* (1967) showed that the infusion of udder quarters with an antibiotic resulted in the elimination of 84% of all infections

present at drying-off, and greatly reduced new infection in the dry period. These workers have reported that the proportion of cows calving with infected quarters was reduced from 62% to 16%. Results reported by Kingwill *et al* (1971) demonstrate that a simple control system combining hygiene with therapy substantially reduces levels of subclinical and clinical mastitis. It was demonstrated, using penicillin, that the rates of elimination of *Staphylococcus aureus* infections were higher when antibiotics were infused at drying-off rather than in lactation (Schalm & Ormsbee, 1949) and that new infections in the dry period could be reduced (Dodd & Neave, 1951). That reducing the duration of infection was essential for effective control systems and that antibiotic therapy at drying-off was the best way of achieving this was first realized in 1966 (Neave, Dodd & Kingwill, 1966). Results reported by Bodoh *et al* (1975) indicate that the dairyman can maintain low cell numbers with good management, teat dipping and selective drying-off therapy. Table 2.14 shows the effect of drying-off antibiotic therapy on new infections in dry period and levels of infection at calving (Griffin, 1971). Similar results have recently been reported in New Zealand (Pankey *et al*, 1982); see Table 2.15.

TABLE 2.14: THE EFFECT OF ANTIBIOTIC (CLOXACILLIN) THERAPY AT DRYING-OFF ON INFECTIONS PRESENT AT DRYING-OFF, NEW INFECTIONS IN THE DRY PERIOD AND LEVELS OF INFECTION AT CALVING (GRIFFIN, 1971).

Treatment	No. of quarters	Infection type	% of total quarters infected at drying-off	% of total quarters infected at drying-off, persisting to calving	% of total quarters with new infection at calving	% of total quarters infected at calving
None (Control)	1144	Staphylococcal	10.3	9.5	5.3	14.8
		Streptococcal	13.7	11.1	4.3	15.4
		'Other'	0.7	0.4	1.3	1.7
Antibiotic (1 g of Benzathine Cloxacillin)	1196	Staphylococcal	11.2	1.8	0.3	2.1
		Streptococcal	13.1	0.4	1.8	2.2
		'Other'	1.7	0.5	0.8	1.3

Results presented in Table 2.14 show that drying-off therapy gave consistently high rates of elimination of infection caused by all types of pathogens and also prevented most of new infections during the dry period in contrast with untreated quarters.

Using two dry-cow therapy products (Neopen D.C. White and Orbenin), Pankey *et al*, (1982) recorded an elimination rate of more than 90% of *Streptococcus agalactiae*, 88% of *Streptococcus uberis* and 84% of *Staphylococcus aureus* with either product (see Table 2.15). These results further support the effectiveness of dry-cow therapy in reducing the level of subclinical mastitis in dairy herds by shortening the duration of intramammary infections.

TABLE 2.15: SUMMARY OF EFFICACY DATA ON DRY THERAPY (PANKEY *et al*, 1982; N.Z.).

Treatment	No. treated		Intramammary infections								
			Staph. aureus			*Strep. uberis*			*Strep. agalactiae*		
	Cows	Quarters	DO[a] No.	PP[b] No.	Cure %	DO No.	PP No.	Cure %	DO No.	PP No.	Cure %
Neopen[c]	229	893	37	6	83.8	30	0	100	215	15	93.0
Orbenin[d]	56	222	27	4	85.2	36	8	77.8	42	3	92.8
Control	45	178	39	27	30.8	8	4	50	14	10	28.6

a Denotes at drying off.

b Denotes within 14 days postpartum.

c Product manufactured by Smith-Biolab, Auckland, N.Z.

d Product manufactured by Beecham Veterinary Products, Auckland, N.Z.

Although treatment of udder infection at drying-off is now accepted as having a role in mastitis control, different people have different views on how drying-off therapy should be used. Some investigators advocate the treatment of all cows at drying-off (Smith, Neave, Dodd & Brander, 1966) and others recommend identifying and treating only infected cows (Rindsig *et al*, 1979). Both treatment regimens seem to have good points in that treating all quarters of all cows at drying-off provides all cows with a degree of protection against new infections

that may develop during the dry period and also permits treatment of certain latent infections that ordinarily go undetected (Fisher, 1981), whereas treating at drying-off only quarters of cows that showed abnormal cell count during lactation reduces expenses because cost of treatment is related to the number of high cell count cows (Dodd & Griffin, 1975).

It would appear that in deciding on the specific way in which drying-off therapy should be integrated into a control system, it is important to recognize the way it complements techniques of preventing new infection. Apart from the cost involved, the main possible long term disadvantage of indiscriminate use of drying-off therapy is that it may encourage the development of antibiotic resistant strains of pathogens or strains that cause infections very difficult to cure or that by reducing the levels of secondary infections, quarters may become more liable to infection with primary pathogens (Schalm, Lasmanis and Carroll, 1966). It should be remembered that the struggle against mammary infections is essentially an economic one and therefore the method of treatment used must be an effective one and the least costly possible.

(ii) Treatment of Subclinical Mastitis during Lactation

Another method of reducing the duration of infections in dairy herds would be to treat all clinical and subclinical infections during lactation. The advantage of such an approach would be an earlier treatment of most new infections and it is possible that the rates of elimination might be improved, compared with drying-off therapy, thus reducing the sources of pathogens for reinfecting other quarters. Dodd & Griffin (1975) contend that in order to reduce the duration of infection, it is necessary to treat subclinically infected quarters annually.

Literature in this area of subclinical mastitis control is very scanty probably because this method of control would be an unfavourable venture to most farmers because of the expense incurred through discarded milk of treated quarters. However it may be useful to treat cows infected in early lactation to prevent continued milk loss

throughout the rest of the lactation. The few reports recorded in the literature indicate that the response rates obtained with so-called "blitz" therapy are very good for Streptococcal and 'other' infections. Griffin (1971) reports that the response rate for sub-clinical Staphylococcal infections with blitz therapy was much higher than the response rates obtained when clinical infections were treated. With Staphylococci the average elimination in lactation was less than 30% for treatment given when clinical signs were present but exceeded 60% when treatment was given to subclinically infected quarters during lactation. Results of treatment of subclinically affected quarters are as given in Table 2.16.

TABLE 2.16: THE RESPONSE TO THERAPY GIVEN DURING LACTATION TO SUB-CLINICAL STAPHYLOCOCCAL INFECTION IN RELATION TO THE STAGE OF LACTATION AND AGE OF THE TREATED COWS AND THE WHITESIDE SCORE OF THE INFECTED QUARTER. THE RESULTS ARE THE MEAN OF VARIOUS SODIUM CLOXACILLIN FORMULATIONS (GRIFFIN, 1971).

Lactation age	1 - 3	4 - 6	7 - 9
No. treated	147	139	74
% Response	60	47	44

Lactation stage of treated cow, month	1 - 3	4 - 6	7 - 9
No. treated	89	114	157
% Response	43	49	59

Whiteside reading of treated quarter	0	1	2	3	4
No. treated	46	75	93	12	17
% Response	61	61	54	46	24

It would appear from Table 2.16 that quarters with high Whiteside readings had a low percentage of response to antibiotics. It may be conjectured that such quarters had a more severe type of mastitis that does not readily respond to treatment. Philpot (1969) reported that many strains of Staphylococci readily penetrate the duct walls of the udder and become established in numerous foci that are quickly walled off with fibrous tissue which makes it difficult to get drugs to the organisms in concentrations that are bactericidal.

Plommet and Le Louedec (1975) reported that the treatment of subclinical infections of the udder during lactation makes it possible to cure with a reasonable chance of success those due to Streptococci though the success is less certain with infection caused by Staphylococci and Gram-negative bacteria. They however commented that it is really only useful if appropriate measures of hygiene have first been taken to avoid rapid return to the original situation. They suggested that if there is no urgency it is more economic to defer this treatment until drying-off. Griffin (1971) seems to share the same opinion. He points out that blitz therapy can not be applied regularly in a herd as part of a control system because of the expense incurred in contaminated milk and the cost of diagnosis of infection if infected quarters only are to be treated.

The most recent work by Freke & Bates (1981) on antibiotic residues in milk tends to suggest that the current withholding time of six milkings (i.e. 72 hrs) after the last treatment with an antibiotic may have to be extended to eight or even ten milkings (i.e. 96 to 120 hrs). The loss of milk incurred from such long withholding times will make farmers hesitate to opt for this method of subclinical mastitis control. There is thus need for more research work in this area to see whether blitz therapy would justify the expenses involved.

However the problems encountered in processing dairy products from milk with high somatic cell counts (see Section 2:2:2) might in the near future necessitate the use of this method of control particularly in herds with severe mastitis problem.

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CHAPTER THREE

3:0 MATERIALS AND METHODS

3:1 RATIONALE: The main objectives were:-

- To identify cows with high somatic cell counts (sub-clinical mastitis) in early lactation.
- To identify the individual quarters with high somatic cell counts (S.C.C.).
- To treat some of these high S.C.C. quarters, and leave some of them untreated.
- To measure the effects of treatment on S.C.C., bacterial infection, yield and composition of milk.

3:2 PROCEDURE AND PLAN

A brief outline: Composite milk samples were taken from each cow in the herd of 100 cows at No.3 Dairy Research & Development Unit, Massey University, and submitted to the Livestock Improvement Association's Laboratory in Palmerston North for somatic cell count determination. This was repeated on three separate occasions and 20 cows with consistently high value for S.C.C. were selected. After three further measurements with these cows, 12 cows with consistently high S.C.C. were finally selected.

Using the 12 selected cows (high S.C.C. cows) the following sequence of operations was performed:-

(1) (a) Individual quarters were sampled for

- bacterial analsysis (2 consecutive foremilk samples/ quarter before treatment);
- somatic cell count determination;
- milk composition (fat and protein concentration).

(b) Individual quarter milk yield recording.

(2) Quarters with high S.C.C. were paired, either within cow or between cows, and one quarter out of each pair was treated with an antibiotic.

(3) (a) Individual quarters were sampled for

- bacterial analysis (2 consecutive foremilk samples/ quarter 3 weeks after the end of treatment);
- somatic cell count determination;
- milk composition (fat and protein concentration).

(b) Individual quarter milk yield recording.

3:3 EXPERIMENTAL PROCEDURE

Twelve cows from No.3 Dairy Research & Development Unit, Massey University, selected on the basis of their high somatic cell counts, were used in the study to investigate the effect of treating sub-clinically affected cows with an antibiotic during lactation. Foremilk samples were taken aseptically from the individual quarters of these cows for bacteriological tests before carrying out any treatment. Quarters in which bacteria were isolated in two consecutive samples were considered to be infected. These cows were a mixture of Jersey or Jersey cross and Friesian or Friesian cross with ages ranging from three to 12 years (see Table 3.1 for details). Most of these cows were in their second month of lactation when the experiment started and had no immediate past history or evidence of clinical mastitis in current lactation.

After a preliminary period of three complete milking days, cows with high S.C.C. in more than two quarters formed the "within cow comparison group". One quarter from each cow in this group was treated with an antibiotic (treated quarter) and the other quarter forming a pair was left untreated (control). The other cows with high S.C.C. in at least one quarter formed a "between cows comparison

TABLE 3.1: BREED, AGE AND AVERAGE* S.C.C. IN INDIVIDUAL QUARTERS OF THE 12 SELECTED COWS.

Cow No.	Breed	Age	S.C.C. in individual quarters (thousand/ml) LF	RF	LR	RR
18	Jersey Cross	3 yrs	690.7 +ve	417.7 +ve	991 +ve	283 -ve
22	Friesian x Jersey	7 yrs	30.7 -ve	150 -ve	120.7 -ve	590.7 +ve
28	Jersey x Ayrshire	9 yrs	46.3 +ve	550 -ve	32.7 +ve	933.3 +ve
36	Jersey	5 yrs	207 -ve	35.3 -ve	53.3 -ve	49.3 -ve
43	Friesian	3 yrs	116.3 -ve	270.7 -ve	72 -ve	17835.3 +ve
46	Jersey	4 yrs	775.7 -ve	444 -ve	210 -ve	258.3 -ve
72	Jersey	3 yrs	61.7 -ve	775 +ve	67 -ve	147.3 -ve
81	Friesian Cross	4 yrs	179.7 -ve	52 -ve	49.3 -ve	1142.7 +ve
112	Jersey	6 yrs	28 -ve	26.3 -ve	18.3 -ve	5387 -ve
114	Jersey x Friesian	12 yrs	6537.7 +ve	1732 +ve	2371 +ve	2429.3 +ve
134	Jersey x Friesian	11 yrs	110.3 +ve	153.3 +ve	7287.3 +ve	230.3 +ve
144	Friesian	8 yrs	28.7 -ve	2257 +ve	687.3 +ve	1320.7 -ve

Note: +ve denotes bacterial infection

-ve denotes no bacterial infection (as defined by Griffin, 1971 and Neave, 1975).

* Average of three individual measurements of S.C.C.

group". Two quarters from these cows were paired (see Table 3.2) and one quarter out of each pair received antibiotic treatment. The selection of quarters for antibiotic treatment in each pair was done by using the random numbers table. The quarters thus selected were treated with three doses of an antibiotic (Sodium Cloxacillin 200 mg) by intramammary infusion at 48 hour intervals. The choice of antibiotic was made after performing a sensitivity test on the bacteria isolated from the foremilk samples.

TABLE 3.2: PAIRING OF QUARTERS IN EACH TREATMENT GROUP.

(a) Within cow comparison group

Quarter	S.C.C. level	Infection	Remarks
18 LF	690,700 cells/ml	+ve	Treated
18 RF	417,700 cells/ml	+ve	Control
43 RF	270,700 cells/ml	-ve	Control
43 RR	17,835,000 cells/ml	+ve	Treated
114 LR	2,371,000 cells/ml	+ve	Control
114 RR	2,429,300 cells/ml	+ve	Treated
144 LR	687,300 cells/ml	+ve	Control
144 RF	2,257,000 cells/ml	+ve	Treated

(b) Between cows comparison group

Quarter	Av.S.C.C. level	Infection	Remarks
22 RR*	590,700 cells/ml	+ve	Treated
28 RR	933,300 cells/ml	+ve	Control
72 RF*	775,000 cells/ml	+ve	Treated
46 LF*	775,700 cells/ml	-ve	Control
81 RR	1,142,700 cells/ml	+ve	Treated
36 LF	207,000 cells/ml	-ve	Control
134 LR*	7,287,300 cells/ml	+ve	Treated
112 RR	5,387,000 cells/ml	-ve	Control

* LF = Left front; LR = Left rear
RF = Right front; RR = Right rear

Data collection on individual quarter milk yield, somatic cell count, fat and protein concentration was done on all 48 quarters of the 12 cows during both pre- and post-treatment periods. The second bacteriological tests were done on foremilk samples taken aseptically

from all individual quarters three weeks after the end of the treatment period to determine the effect of the antibiotic on the treated quarters and check any new infection that might have developed in other quarters.

The cows were grazed and milked with the rest of the herd except on two complete milking days of the week (from Tuesday evening to Thursday morning) when they were milked separately with two quarter-milking buckets in order to collect data on the parameters studied (i.e. individual quarter milk yield, S.C.C., fat and protein concentration). The study was carried out from October, 1981 to 25 February, 1982.

3:4 METHODS

1. Milking

The cows were milked in a walk-through shed with two special quarter-milking buckets. Cows were checked for clinical mastitis and washed with hose water before attaching the milking machine to the udder. Teats were sprayed with a sanitizer immediately after milking as routinely done with the rest of the herd.

2. Milk Yields

Individual quarter yields were weighed to the nearest 1 g and samples withdrawn for somatic cell count, fat and protein percentage determinations.

3. Milk Composition

Samples drawn from individual quarter yields were used for the determination of fat and protein percentages using Milko-Tester MK III and Pro-Milk MK İI (A/S. N Foss Electric), respectively.

4. Somatic Cell Count Determination

Two milk samples of about 10 - 15 ml were drawn from each individual quarter's yield on two consecutive evening milkings per week for somatic cell count. The samples were syringed into special

sample bottles containing Potassium dichromate as a preservative and submitted to the Livestock Improvement Association Laboratory in Palmerston North for Electronic Somatic Cell Counting. Prior to analysis, somatic cell counts were transformed to a log scale from the complete data set. Straight arithmetic means were also calculated and presented as such (see Chapter Four).

5. Bacteriological Examination

Aseptically drawn foremilk samples were taken from each quarter immediately prior to milking on two consecutive days for bacteriological test both before treatment and three weeks after the end of treatment period. On both occasions the cows' teats were cleaned with paper towels and thoroughly wiped with cotton wool soaked with methylated spirit before the milk was drawn directly into labelled sterile screw-capped sample bottles after discarding the first few squirts of milk and the sample bottles were capped immediately.

The samples were immediately taken to the Microbiology Laboratory at the Faculty of Veterinary Science, Massey University, for bacteriological tests. The aim of these bacteriological tests was to isolate and identify the major pathogenic bacteria commonly diagnosed in New Zealand dairy herds i.e. *Staphylococcus aureus* and the Streptococci group (i.e. *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, and *Streptococcus uberis*. The procedure involved in performing the tests was:-

(a) Plated milk samples on blood agar using swabs, and incubated at 37°C for 24 - 48 hours.

(b) After the incubation period, plates were examined for the presence of either Staphylococcus or Streptococcus bacteria.

Staphylococcus bacteria can be readily identified as they appear as large greyish white colonies and hemolyze blood agar thus creating a clear zone around such colonies. In Streptococcus bacteria, however, there is no such conspicuous zone of clearing around the colonies and the colonies appear much smaller than those of Staphylococcus.

(c) The presence of *Staphylococcus aureus* was confirmed by performing a DNase Test and the presence of Streptococci was confirmed by both Gram's Stain and Catalase Test as briefly described in this text.

A quarter was considered subclinically infected when the same primary pathogen was isolated from it on two consecutive foremilk samples (Griffin, 1971; Neave, 1975; Griffin *et al*, 1977).

DNase Test

Using a platinum loop, the organism was aseptically picked from one of the colonies suspected to be of *Staphylococcus aureus* and streaked onto DNase agar in defined areas of the plate; and Oxford Staph was used as a control organism on the same DNase agar plate. The plates were then incubated at 37°C for 24 - 48 hours.

Staphylococcus aureus hydrolyses DNase agar and the presence of a clear zone around the organism when the incubated DNase agar culture is flooded with IN HCl indicates a positive reaction. Results obtained with this test did not require any further tests on the Staphylococcus colonies cultured.

Gram's Stain

This test was performed in order to distinguish Streptococci from Diptheroides. Using a platinum loop, slide smears were aseptically made from the colonies suspected to be of Streptococci; fixed over the flame and stained with the Gram's Stain and examined under the microscope. Diptheroides appear as "Chinese lettering" rods whereas Streptococci appear in chains and are purple in colour i.e. gram positive.

Catalase Test

This test was done to identify Streptococci from fields of plates which had both Staphylococcus and Streptococcus colonies. The organism was aseptically picked with a platinum loop from a colony suspected to be that of Streptococcus and streaked on to a nutrient

agar slope in a test-tube placed in a slanting position in a test tube rack. The test tubes were then incubated at 37°C for 24 - 48 hours. After the incubation period hydrogen peroxide was poured over the nutrient agar of the test organism and observed for production of gas bubbles. If no gas bubbles were produced the organism isolated was Streptococcus.

The basis of this test is that Catalase, an enzyme which liberates molecular oxygen as gas bubbles from hydrogen peroxide ($2H_2O_2$ + Catalase = $O_2\uparrow$ + $2H_2O$), is not found in Streptococcus bacteria but is present in Staphylococcus bacteria.

6. Antibiotic Treatment

Orbenin (Sodium Cloxacillin 200 mg) was used in this study. The choice of the antibiotic was made after performing a sensitivity test on the bacteria isolated from the foremilk samples. Each of the eight selected quarters (subclinically affected) was infused with three doses of the antibiotic at 48 hour intervals. No data was collected during the treatment period.

7. Statistical Analysis

Statistical analyses were carried out by means of Analysis of Variance (ANOVA) and Analysis of Covariance. In the ANOVA, the disproportionate subclass numbers of a 2 x 2 design was used after a preliminary analysis of variance to estimate the interaction between before and after treatment because of the unproportionality of the number of treated and control quarters used in the experiment (Snedecor, 1956). The analsysis of covariance was used in some of the statistical analyses in order to adjust for sources of bias such as age, stage of lactation and yield (Snedecor & Cochran, 1980). The t - test was also used in some tests.

* * * * * * * * * * *

CHAPTER FOUR

4:0 RESULTS

4:1 INTRODUCTION:

Originally the plan was to identify quarters with high cell counts, pair them, treat one quarter from each pair and leave the other quarter untreated (control quarter). This plan was however changed after obtaining bacteriological results of the foremilk samples aseptically taken from individual quarters. It was then decided that only quarters with bacterial infection and high cell counts should be treated. Thus treatment was restricted to quarters with subclinical infection and high somatic cell counts. The analysis of the effect of antibiotic treatment was also restricted to quarters with subclinical infection and high cell counts. A quarter was considered subclinically infected when the same pathogen was isolated from it on two consecutive foremilk samples.

In this text the terms preliminary and experimental periods refer to a period before any treatment was carried out to any of the quarters, and a period starting immediately after a course of antibiotic treatment up to the end of the experiment respectively; whereas treatment period refers to those days when quarters selected for treatment were given a course of antibiotic therapy without any data collection on any of the parameters studied.

4:2 SOMATIC CELL COUNTS AND INFECTION:

Results of both somatic cell counts determinations and bacteriological tests carried out during the preliminary period show that quarters identified with bacterial infection from two consecutive foremilk samples had an average cell count of 2,362,100 cells/ml and non-infected quarters had an average of about 178,400 cells/ml whereas the doubtful cases contained 2,844,600 cells/ml (see Table 4.1). These results revealed that nine out of the 12 cows selected for this study on the basis of high cell counts were infected with a primary (major) pathogen in one or more quarters (75% infection).

TABLE 4.1: MEAN CELL COUNTS OF QUARTERS IDENTIFIED AS INFECTED, NON-INFECTED AND DOUBTFUL PRIOR TO ANTIBIOTIC THERAPY (AVERAGE OF 3 INDIVIDUAL MEASUREMENTS OF SOMATIC CELL COUNTS TAKEN DURING THE PRELIMINARY PERIOD).

Infected* Quarters		Non-infected Quarters		Doubtful** Quarters	
Quarter	S.C.C. ('000 cells/ml)	Quarter	S.C.C. ('000 cells/ml)	Quarter	S.C.C. ('000 cells/ml)
28 LR	32.7	112 LR	18.3	46 LF	775.7
28 LF	46.3	112 RF	26.3	114 LR	2371.0
134 LF	110.3	112 LF	28.0	112 RR	5387.0
134 RF	153.3	144 LF	28.7		
134 RR	230.3	22 LF	30.7		
18 RF	417.7	36 RF	35.3		
22 RR	591.3	28 RF	46.3		
144 LR	687.3	36 RR	49.3		
18 LF	690.7	81 LR	49.3		
72 RF	775.0	81 RF	52.0		
28 RR	933.3	36 LR	53.3		
18 LR	991.0	72 LF	61.7		
81 RR	1142.7	72 LR	67.0		
114 RF	1732.0	43 LR	72.0		
144 RF	2257.0	43 LF	116.3		
114 RR	2429.3	22 LR	120.7		
114 LF	6537.2	22 RF	150.0		
134 LR	7287.3	81 LF	179.7		
43 RR	17835.3	36 LF	207.3		
		46 LR	210.0		
		46 RR	258.3		
		43 RF	270.7		
		18 RR	383.0		
		72 RR	442.0		
		46 RF	444.0		
		144 RR	1320.7		
Overall mean	2,362.1		178.4		2,844.6

* The same pathogen isolated in samples on two consecutive days.

** A pathogen isolated in the sample on only one day.

Table 4.2 shows the relationship between the incidence of mastitis primary pathogen and cell counts. The pathogen was isolated from only 19 (39.6%) of the 48 quarters. About 74% of these 19 infected quarters had an average cell count above 300,000 cells/ml and 21% contained less than 200,000 cells/ml. Some of the remaining 29 quarters had the primary pathogen in one of the two consecutive foremilk samples taken before treatment or subsequently. Quarters shedding the same type of primary pathogen in one of the two consecutive samples taken before treatment or subsequently were regarded as doubtful. Three such cases were identified and by using a different definition of subclinical mastitis these quarters could have been considered as infected thus leaving 26 non-infected quarters. Of these 26 quarters, 18 (69.2%) contained less than 200, cells/ml and four (15.4%) had cell counts above 300,000 cells/ml.

TABLE 4.2: THE RELATIONSHIP BETWEEN THE INCIDENCE OF MASTITIS PATHOGENS AND CELL COUNTS.

Quarter milk cell count	Infection Status: Primary Pathogen Positive		Non-infected		Doubtful		All Quarters
('000 cells/ml)	No. of Quarters	Percent	No. of Quarters	Percent	No. of Quarters	Percent	Percent
200 or less	(4)	21	(18)	69.2			45.8
201 - 300	(1)	5.3	(4)	15.4			10.4
301 - 500	(1)	5.3	(3)	11.5			8.3
501 - 1,000	(6)	31.6			(1)	25	14.6
Over 1,000	(7)	36.8	(1)	3.9	(2)	75	20.8
		100%		100%		100%	99.9%
No. of Quarters	19		26		3		48
(% of Quarters)	(39.6)		(54.2)		(6.3)		

These results show a close association between infection and somatic cell counts in individual quarters. They also demonstrate that using somatic cell count (taking say 300,000 cells/ml and above as indicative of subclinical infection) as the only criterion for

diagnosing subclinical mastitis in these quarters would have resulted in about 10.4% false negatives and 8.3% false positives giving a prediction accuracy of about 90%. These results tend to suggest that a threshold of about 300,000 cells/ml in the milk from an individual quarter can reasonably be depended upon in predicting the prevalence of subclinical mastitis in cows which also seems to confirm one of the conclusions made by Gill & Holmes (1978) that an average cell count that is higher than about 300,000 cells/ml in the milk from an individual quarter is usually associated with the isolation of bacteria.

4:2:1 THE RELATIONSHIP BETWEEN SOMATIC CELL COUNTS AND QUARTER MILK YIELDS:

Analysis of the somatic cell count data and quarter milk yields recorded during the preliminary period showed that higher somatic cell counts were associated with a lower quarter yield ($P<0.01$); see Table 4.3. The purpose of this kind of analysis was to determine the association between cell counts and milk yield.

TABLE 4.3: COVARIANCE ANALYSIS OF QUARTER MILK YIELD IN RELATION TO SOMATIC CELL COUNTS.

Summary

Source of variation	df	ss	ms	F
Unadjusted Σy^2	47	329.29615		
Adjusted by Regression	1	62.40653	62.40653	10.76**
Adjusted Σy^2	46	266.88962	5.8019	

$P<0.01$

Presenting in an equation form, this negative relation between somatic cell count and quarter milk yield was found to be:

$$\text{Daily milk yield} = 4.6 - 0.92 \times \text{S.C.C.} \times 10^5$$

Thus an increase in somatic cell counts from 100,000 to 1,000,000 was equivalent to a decrease of 0.9 kg milk i.e. an increase of

900,000 cells/ml caused a decrease of 0.9 kg milk quarter^{-1} day^{-1} which is equivalent to a decrease of 0.1 kg milk quarter^{-1} day^{-1} for an increase of 100,000 cells/ml.

4:3 Effects of Antibiotic Treatment:

4:3:1 Introduction:

The effects of antibiotic therapy on bacterial infection, somatic cell counts, milk yield and composition were assessed by covariance analysis in which the value of each quarter for the experimental period was adjusted to a common value for the preliminary period. The analysis of variance and t - test were used in testing the significance of the difference between the experimental and preliminary period for each quarter. Most of the examples of these analyses are presented in the appendix whereas means and adjusted means are included in the text.

4:3:2 Bacterial Infection:

Results of the bacteriological tests of the foremilk quarter samples performed three weeks after a course of antibiotic therapy on quarters selected for treatment showed that pathogens were eliminated in five out of the eight treated quarters - giving a cure rate of 62.5% (see Table 4.4). The three quarters which failed to respond to antibiotic treatment were from the oldest cows of the group. These cows had an infection in two or more quarters at the beginning of the experiment and the fact that the same pathogenic organisms were diagnosed in these quarters before and after treatment makes it difficult to tell whether the infection diagnosed three weeks after the end of the treatment period was due to re-infection of the same pathogens or not. Philpot (1969) indicated that individual cows infected in more than two quarters almost invariably remain infected and the quarters cleared of infection frequently become re-infected.

In this study, a quarter diagnosed as uninfected or non-infected during the pretreatment bacteriological tests but diagnosed with a major pathogen in the post treatment bacteriological tests was recorded to have developed a new infection. Similarly, a quarter diagnosed

with a major pathogen during the pretreatment bacteriological tests and not treated but identified as non-infected during the post-treatment bacteriological tests was recorded to have spontaneously recovered from the infection.

TABLE 4.4: THE RESPONSE TO ANTIBIOTIC THERAPY (SODIUM CLOXACILLIN 200 MG) GIVEN IN EARLY LACTATION TO SUBCLINICALLY INFECTED QUARTERS.

No. of quarters treated	Quarters cured	% Response
8	5	62.5

Results presented in Table 4.5 indicate that some spontaneous recoveries of infected quarters had occurred before the post-treatment bacteriological tests. These results show that of the 19 infections diagnosed during the preliminary period, three (15.8%) spontaneously disappeared from quarters without antibiotic therapy. Their somatic cell counts were lower than the previous values (see Table 4.5). Examination of the same table shows that five new infections developed during the treatment period. Of these five new infections, two were of Streptococcus bacteria, one was a mixed infection of *Staphylococcus aureus* and Streptococcus bacteria and the other two were of *Staphylococcus aureus*.

Two quarters, 18 LR and 114 LR, developed into clinical mastitis during the last two months of the experimental period and were successfully treated with Streptopen and Fort-V, respectively. This disqualified quarter 18 LR to be included in the control group. The quarter had been diagnosed with *Staphylococcus aureus* on both occasions i.e. before and three weeks after the end of the treatment period. Quarter 114 LR had been diagnosed with a mixed infection of *Staphylococcus aureus* and Streptococcus bacteria during the post-treatment bacteriological tests (Table 4.5).

4:3:3 Somatic Cell Counts:

Table 4.6 shows the average cell counts of treated and untreated (control) quarters before and after the treatment period. The average somatic cell counts of treated quarters decreased from 4,126,100 cells/ml to 1,089,500 cells/ml following treatment whereas the average cell counts of the control quarters increased from 2,061,500 cells/ml to 2,111,000 cells/ml.

Both covariance analysis of means and analysis of variance (ANOVA) of differences between means within quarters for the first month of the experimental period showed a highly significant difference ($P<0.01$) between the somatic cell counts of the treated and untreated quarters (Tables 4.7 and 4.8). However analysis of data of the experimental period as a whole showed no significant difference in cell counts of treated and untreated quarters at a 5% level of significance but the difference was significant at 10% level (see Table 4.9). Adjusted means showed a greatly reduced mean cell count of the treated quarters as compared to the control quarters (Table 4.6).

Examination of the results of the successfully and unsuccessfully treated quarters presented in Table 4.10 reveals that there was a marked decrease in somatic cell counts of the successfully treated quarters compared with the quarters that were not cured. The overall mean cell count of the successfully treated quarters decreased from 4,207,000 cells/ml to 159,900 cells/ml; whereas the overall mean cell count of the unsuccessfully treated quarters had a slight decrease from 3,991,200 cells/ml to 2,638,800 cells/ml. Individual somatic cell counts of the successfully treated quarters ranged from 110,800 cells/ml to 217,400 cells/ml as compared with 951,500 cells/ml to 5,285,700 cells/ml for the uncured quarters.

It is evident from the results presented in Table 4.10 that the high overall mean cell count of the treated quarters shown in Table 4.6 was inflated by the quarters which were not cured by the antibiotic treatment. Of interest from the results of this study is that quarters still having high somatic cell counts after treatment were

TABLE 4.5: SUBCLINICALLY INFECTED QUARTERS AND THEIR MEAN CELL COUNTS BEFORE AND AFTER TREATMENT (MEAN CELL COUNTS ARE AVERAGES OF 3 AND 27 INDIVIDUAL MEASUREMENTS OF S.C.C. TAKEN DURING THE PRELIMINARY AND EXPERIMENTAL PERIODS, RESPECTIVELY).

	Before Treatment			Treated	After Treatment			Cured
Cow No.	Quarter	10^3 cells/ml	Pathogen isolated	Yes/No.	Quarter	10^3 cells/ml	Pathogen isolated	Yes/No
18	RF	417.7	*Staphylococcus aureus*	No	18 RF	896.4	*Staphylococcus aureus*	No
	LF	690.7	" "	Yes	18 LF	217.4	Nil	Yes
	LR	991.0	" "	No	18 LR	1468.0	*Staphylococcus aureus*	No
22	RR	591.3	*Staphylococcus aureus*	Yes	22 RR	110.8	Nil	Yes
28	RF	46.3	Not infected	No	28 RF*	63.6	*Staphylococcus aureus*	No
	LF	46.3	*Staphylococcus aureus*	No	28 LF	41.1	Spontaneously recovered	
	LR	32.7	" "	No	28 LR	51.4	*Staphylococcus aureus*	No
	RR	933.3	" "	No	28 RR	3271.7	" "	No
43	RR	17835.3	*Staphylococcus aureus*	Yes	43 RR	155.3	Nil	Yes
72	RF	775.0	*Staphylococcus aureus*	Yes	72 RF	140.1	Nil	Yes
81	RR	1142.7	*Staphylococcus aureus*	Yes	81 RR	175.8	Nil	Yes
112	RR	5387.0	Not infected	No	112 RR*	3041.1	Streptococcus	No
114	RF	1732.0	*Staphylococcus aureus*	No	114 RF	2300.8	*Staphylococcus aureus*	No
	LF	6537.2	" "	No	114 LF	3211.1	" "	No
	LR	2371.0	Not infected	No	114 LR*	1229.5	Staphylococcus/Streptococcus	No
	RR	2429.3	*Staphylococcus aureus*	Yes	114 RR	1679.2	*Staphylococcus aureus*	No
134	RF	153.3	*Staphylococcus aureus*	No	134 RF	95.7	Spontaneously recovered	
	LF	110.3	" "	No	134 LF	94.9	" "	
	LR	7287.3	" "	Yes	134 LR	5285.7	*Staphylococcus aureus*	No
	RR	230.3	" "	No	134 RR	311.5	" "	No
144	RF	2257.0	*Staphylococcus aureus*	Yes	144 RF	951.5	*Staphylococcus aureus*	No
	LF	28.7	Not infected	No	144 LF*	98.8	" "	No
	LR	687.3	*Staphylococcus aureus*	No	144 LR	875.5	" "	No
	RR	1320.7	Not infected	No	144 RR*	1320.0	Streptococcus	No

* New infection.

TABLE 4.6: MEAN CELL COUNTS OF TREATED AND UNTREATED QUARTERS (AVERAGES OF 3 AND 27 INDIVIDUAL MEASUREMENTS OF S.C.C. TAKEN DURING THE PRELIMINARY AND EXPERIMENTAL PERIODS, RESPECTIVELY).

Untreated (Control) Quarters			Treated Quarters		
Preliminary Period		Experimental Period	Preliminary Period		Experimental Period
Quarter	10^3 cells/ml	10^3 cells/ml	Quarter	10^3 cells/ml	10^3 cells/ml
18 RF	417.7	896.4	22 RR	591.3	110.8
144 LR	687.3	875.5	18 LF	690.7	217.4
28 RR	933.3	3271.7	72 RF	775.0	140.1
114 RF	1732.0	2300.3	81 RR	1142.7	175.8
114 LF	6537.2	3211.1	144 RF	2257.0	951.5*
			114 RR	2429.3	1679.2*
			134 LR	7287.3	5285.7*
			43 RR	17835.3	155.3
Unadjusted means	2061.5	2111.0		4126.1	1089.5
Adjusted means		950.0			250.0

* Uncured quarters.

TABLE 4.7: EXAMPLE OF ANALYSIS OF COVARIANCE FOR TESTING THE DIFFERENCE BETWEEN ADJUSTED MEANS OF TREATED AND UNTREATED QUARTERS FOR S.C.C. (FOR DATA COVERING FIRST MONTH OF THE EXPERIMENTAL PERIOD).

Summary of Covariance Analysis

Source of variation	df	ss	ms	F
Total	11	3.51419		
Between Groups means	1	1.97929	1.97929	12.895**
Within Group means	10	1.5349	0.15349	

** P<0.01

TABLE 4.8: EXAMPLE OF ANALYSIS OF VARIANCE FOR TESTING THE DIFFERENCE BETWEEN ADJUSTED MEANS OF TREATED AND UNTREATED QUARTERS FOR S.C.C. (FOR DATA COVERING FIRST MONTH OF THE EXPERIMENTAL PERIOD).

Summary

Source of variation	df	ss	ms	F
Total	12	5.8423077		
Between Groups means	1	2.8297877	2.8297877	10.33**
Within Group means	11	3.01253	0.273865454	

** P<0.01

TABLE 4.9: SECOND EXAMPLE OF ANALYSIS OF COVARIANCE FOR TESTING THE DIFFERENCE BETWEEN ADJUSTED MEANS OF TREATED AND UNTREATED QUARTERS FOR S.C.C. (FOR DATA COVERING THE WHOLE EXPERIMENTAL PERIOD).

Summary

Source of variation	df	ss	ms	F
Total	11	3.99866		
Between Groups means	1	1.26641	1.26641	4.64
Within Group means	10	2.73225	0.273225	

P<0.1

from the oldest cows of the group (aged between 8 and 12 years), see Table 3.1.

Covariance analysis of means of the control quarters and successfully treated quarters showed a highly significant difference ($P<0.01$) between cell counts of the successfully treated and control quarters (Appendix 2:5). Comparison of mean cell counts of the control and successfully treated quarters shows that successful antibiotic treatment was highly effective in decreasing cell counts of the treated quarters (see Table 4.11). The table shows that the adjusted mean cell count of treated quarters was reduced to 100,000 cells/ml compared with 950,000 cells/ml for the untreated quarters.

4:3:4 MILK YIELD:

Results obtained indicate that antibiotic therapy was not accompanied by a significant increase in milk yield. Tests of means and mean differences by the various methods employed showed no significant difference between treated and control quarters (see Appendices 1:2 - 4:0). Comparison of successfully treated and control quarters showed no significant difference either (see Appendices 2:5 and 2:6).

Although adjustment of means did not change yield results from insignificance, the data presented in Tables 4.12 and 4.13 show that there was a trend for a slightly higher milk yield from treated quarters with a difference of about 10.3% which may be a real effect in milk production.

4:3:5 MILK COMPOSITION:

Analysis of data of both milkfat concentration (% Fat) and protein concentration (% Protein) showed no significant difference between treated and untreated quarters at a 5% level of significance (see Appendices 1:3 - 4:0). The difference in protein was however significant at 10% level (see Appendix 1:4). The analysis of covariance of data obtained from the first month's production following the antibiotic treatment showed a significant difference ($P<0.05$) in protein percentage (Appendix 2:5).

TABLE 4.10: MEAN CELL COUNTS OF SUCCESSFULLY AND UNSUCCESSFULLY TREATED QUARTERS (AVERAGES OF 3 AND 27 INDIVIDUAL MEASUREMENTS OF S.C.C. TAKEN DURING THE PRELIMINARY AND EXPERIMENTAL PERIODS, RESPECTIVELY).

Unsuccessfully Treated Quarters			Successfully Treated Quarters		
Preliminary Period		Experimental Period	Preliminary Period		Experimental Period
Quarter	10^3 cells/ml	10^3 cells/ml	Quarter	10^3 cells/ml	10^3 cells/ml
144 RF	2257.0	951.5	22 RR	591.3	110.8
114 RR	2429.3	1679.7	18 LF	690.7	217.4
134 LR	7287.3	5285.7	72 RF	775.0	140.1
			81 RR	1142.7	175.8
			43 RR	17835.3	155.3
Unadjusted means	3991.2	2638.8		4207.0	159.9
Adjusted means		1000.0			100.0

TABLE 4.11: MEAN CELL COUNTS OF UNTREATED (CONTROL) AND SUCCESSFULLY TREATED QUARTERS (AVERAGES OF 3 AND 27 INDIVIDUAL MEASUREMENTS OF S.C.C. TAKEN DURING THE PRELIMINARY AND EXPERIMENTAL PERIODS, RESPECTIVELY).

Control Quarters			Successfully Treated Quarters		
Preliminary Period		Experimental Period	Preliminary Period		Experimental Period
Quarter	10^3 cells/ml	10^3 cells/ml	Quarter	10^3 cells/ml	10^3 cells/ml
18 RF	417.7	896.4	22 RR	591.3	110.8
144 LR	687.3	875.5	18 LF	690.7	217.4
28 RR	933.3	3271.7	72 RF	775.0	140.1
114 RF	1732.0	2300.3	81 RR	1142.7	175.8
114 LF	6537.2	3211.1	43 RR	17835.3	155.3
Unadjusted means	2061.5	2111.0		4207.0	159.9
Adjusted means		950.0			100.0

TABLE 4.12: AVERAGE MILK YIELD OF TREATED AND CONTROL (UNTREATED) QUARTERS (KG/QUARTER/DAY).

(a) From data covering the first month of experimental period.

	Control	Treated	SE*
Unadjusted means	3.15	3.96	± 0.317
Adjusted means	3.43	3.79	± 0.316

SE* = Standard error

(b) From data covering the whole experimental period.

	Control	Treated	SE
Unadjusted means	2.79	3.43	± 0.303
Adjusted means	3.05	3.27	± 0.296

TABLE 4.13: AVERAGE MILK YIELD OF SUCCESSFULLY TREATED AND CONTROL QUARTERS (KG/QUARTER/DAY).

(a) From data covering the first month of experimental period.

	Control	Treated	SE
Unadjusted means	3.15	3.55	± 0.282
Adjusted means	3.13	3.61	± 0.283

(b) From data covering the whole experimental period.

	Control	Treated	SE
Unadjusted means	2.79	3.12	± 0.265
Adjusted means	2.77	3.13	± 0.266

Adjustment of means (Table 4.14) did not show much difference in milkfat concentration between treated and untreated quarters but there was a trend towards increased total protein concentration in untreated quarters as compared to treated quarters probably due to an increase in blood serum albumin infiltrating into infected quarters (Schalm, 1977). Analysis of data of successfully treated and control quarters did not show any significant difference either (Table 4.15).

TABLE 4.14: AVERAGE MILKFAT AND PROTEIN CONCENTRATION (%) OF TREATED AND UNTREATED (CONTROL) QUARTERS.

I Fat Concentration

(a) From data covering the first month of experimental period.

	Control	Treated	SE
Unadjusted means	5.21	5.20	± 0.194
Adjusted means	5.06	5.28	± 0.141

(b) From data covering the whole experimental period.

	Control	Treated	SE
Unadjusted means	5.44	5.27	± 0.126
Adjusted means	5.33	5.34	± 0.118

II Protein Concentration

(a) From data covering the first month of experimental period.

	Control	Treated	SE
Unadjusted means	4.06	3.79	± 0.042
Adjusted means	4.05	3.79*	± 0.042

(* $P<0.10$)

(b) From data covering the whole experimental period.

	Control	Treated	SE
Unadjusted means	4.02	3.78	± 0.045
Adjusted means	4.01	3.79	± 0.241

TABLE 4.15: AVERAGE MILKFAT AND PROTEIN CONCENTRATION (%) OF SUCCESSFULLY TREATED AND UNTREATED (CONTROL) QUARTERS.

I Fat Concentration

(a) From data covering the first month of experimental period.

	Control	Treated	SE
Unadjusted means	5.21	5.48	± 0.167
Adjusted means	5.21	5.48	± 0.167

(b) From data covering the whole experimental period.

	Control	Treated	SE
Unadjusted means	5.44	5.55	± 0.144
Adjusted means	5.44	5.55	± 0.144

II Protein Concentration

(a) From data covering the first month of experimental period.

	Control	Treated	SE
Unadjusted means	4.06	3.72	± 0.051
Adjusted means	4.06	3.72*	± 0.051

(* $P<0.05$)

(b) From data covering the whole experimental period.

	Control	Treated	SE
Unadjusted means	4.02	3.93	± 0.05
Adjusted means	3.99	3.96	± 0.05

On several occasions no milkfat percentage readings could be obtained from quarter 134 LR. As indicated in Table 4.10 this was one of the three quarters which were not cured by the antibiotic treatment. A similar case was experienced with quarter 144 RR on one occasion. On such occasions these quarters had very high cell count results. However untreated quarter 144 RR did not develop into a clinical case of mastitis.

* * * * * * * * * * *

CHAPTER FIVE

5:0 DISCUSSION

5:1 INTRODUCTION:

The final selection of quarters for antibiotic therapy and controls was based on both high cell count levels and the presence of bacterial infection; it has therefore been decided to start the discussion by relating somatic cell counts to bacterial infection of individual quarters before and after antibiotic therapy. This will be followed by a discussion on the effect of antibiotic therapy on subclinical infections, somatic cell counts, milk yield and composition with a final discussion on the practical application of antibiotic therapy for subclinical mastitis.

5:2 THE ASSOCIATION BETWEEN S.C.C. AND BACTERIAL INFECTION:

Results of bacteriological tests and somatic cell count determinations performed during the preliminary period showed a close association between somatic cell count levels and bacterial infection of individual quarters. Quarters identified with a primary pathogen (i.e. infected quarters) from two consecutive foremilk samples had an average cell count of 2,362,100 cells/ml and the non-infected quarters had an average of about 178,000 cells/ml. The doubtful cases (i.e. quarters shedding the same primary pathogen in only one sample before treatment or subsequently) contained 2,844,600 cells/ml. About 74% of the infected quarters had cell counts above 300,000 cells/ml and only 21% contained less than 200,000 cells/ml.

A similar trend was observed after the treatment period. The successfully treated quarters showed an average cell count of about 160,000 cells/ml with the variation among individual quarters ranging from 110,800 to 217,400 cells/ml as compared to the uncured quarters which had an average of 2,638,800 cells/ml - showing a slight decrease from the original value of 3,991,200 cells/ml.

It would appear from the above account that cell count values of 300,000 cells/ml and above can be used with a high degree of accuracy in predicting bacterial infection in dairy herds. On comparing with the results obtained from other indicators of sub-clinical mastitis such as changes in lactose quantity, catalase activity, whey protein, chlorides, sodium and potassium, pH and rennetability, Renner (1975) concluded that a threshold value of 400,000 cells/ml provided a relatively high accuracy in diagnosing subclinical mastitis from aseptically taken foremilk samples. Table 5.1 shows the accuracy with which subclinically infected quarters/ cows could have been identified from somatic cell count values when using four suggested threshold values to indicate bacterial infection. The three doubtful cases i.e. quarters which shed bacteria in only one of the two consecutive samples were classified under the category of infected quarters.

TABLE 5.1: ACCURACY WITH WHICH SUBCLINICALLY INFECTED QUARTERS CAN BE IDENTIFIED FROM VALUES FOR S.C.C., USING FOUR THRESHOLD VALUES TO INDICATE BACTERIAL INFECTION, BASED ON THE AVERAGE S.C.C. FROM THREE SAMPLES.

Threshold value	Correctly diagnosed		Incorrectly diagnosed			
			Infected; but diagnosed as un-infected		Un-infected; but diagnosed as infected	
200,000 cells/ml	(36)*	75%	(4)	8.3%	(8)	16.7%
300,000 cells/ml	(39)	81.3%	(5)	10.4%	(4)	8.3%
400,000 cells/ml	(40)	83.3%	(5)	10.4%	(3)	6.3%
500,000 cells/ml	(41)	85.4%	(6)	12.5%	(1)	2.1%

( )* bracketed numbers represent the number of quarters classified under the respective categories out of the 48 quarters involved in the study.

Results presented in Table 5.1 indicate that using a threshold value of 300,000 cells/ml would result in a prediction accuracy of 81.3% with 10.4% false negatives and 8.3% false positives. The table also shows that using higher threshold values increases the percentage of false negatives while reducing the percentage of false positives. It would appear that a threshold value of 300,000 cells/ml could be considered as the minimum threshold value for predicting bacterial infection in individual quarters with the minimum risk of missing a high percentage of false negatives. These results confirm one of the conclusions made by Gill & Holmes (1978) that an average cell count that is higher than 300,000 cells/ml in the milk from an individual quarter is usually associated with the isolation of bacteria. The findings reported by Sheldrake (1982) show that based on foremilk samples from two herds, a level of approximately 300,000 cells/ml minimized the probability of misclassifying quarters on the basis of bacterial infection. He suggested that if a common threshold is to be selected for predicting the presence of subclinical infections, then a level of 300,000 cells/ml should be used instead of 600,000 cells/ml in order to minimize the probability of misclassifying quarters on the basis of bacterial infection.

It is reasonable to conclude from the evidence presented above that a threshold value of about 300,000 cells/ml will accurately distinguish between infected and uninfected quarters i.e. predict subclinical infection with a high degree of accuracy. However, data presented in Appendix 5:0 indicate that the value for somatic cell count of the cow's composite milk does not correlate much with the value of any of the individual quarters of the cow. The fact that the primary pathogen could be isolated from only one of the two consecutive samples in some quarters (i.e. quarters classified as doubtful cases in this study) with somatic cell counts above 300,000 cells/ml does not necessarily mean that such quarters were not infected. Many reports (e.g. Johns & Hastings, 1938; Griffin *et al*, 1977; MacMillan & Duirs, 1980) indicate that bacteria are not necessarily shed at each milking even when an infection is present. Neave (1975) pointed out that the concentration of bacteria in the milk of an infected quarter is not static, the numbers may fall so

low that occasionally they are not detected in the milk of single samples.

5:3 Effect of Antibiotic Therapy on Bacterial Infection:

The results of antibiotic therapy obtained from this study are similar to those reported by other workers. The rate of elimination of bacterial infection in subclinically affected quarters was 62.5% which compares well with the results reported by Griffin (1971) who obtained cure rates of just over 60%. Since the quarters which failed to respond to Cloxacillin (Orbenin - an antibiotic) therapy were from the oldest cows of the group it is possible that these quarters might have previously had chronic subclinical infections which are not easily cured probably because of the inaccessibility of the pathogens to the drug. This is possible especially when the pathogen buries itself into an abscess or deep into the host tissue thus affording itself protection or resisting the bactericidal activity of the antibiotic. Craven and Anderson (1980) indicated that in chronic subclinical infections a period of intracellular parasitism is a pathological feature and that killing of intracellular bacteria requires that the antibiotic should penetrate the host cell to a sufficient concentration and meet bacteria in a susceptible phase i.e. when their metabolic rate is high and they are actively dividing. In another study Craven & Anderson (1980) suggested that protection of intracellular *Staphylococcus aureus* from killing by Cloxacillin was due to the low metabolic rate of the intracellular pathogenic organisms. Results reported by Anderson (1977) indicate that intracellular location of the Staphylococci together with the contraction of the alveoli (rather than abscess formation, deep tissue penetration of organisms or duct occlusion by fibrosis and oedema which are all later manifestations of chronic infections) are sufficient to prevent effective therapy. It may be inferred from such findings that bacteria embedding themselves deep into the host cell or abscesses resulting from an infection are not accessible to the drugs. This would be a possible explanation for the failure of Cloxacillin in eliminating all *Staphylococcus aureus* infections despite the positive results of the *in vitro* sensitivity tests performed prior to the use

of the antibiotic on quarters infected with *Staphylococcus aureus* during this study.

Some workers (e.g. Griffin, 1971; Plommet & Le Louedec, 1975) have reported that lactation age is one of the factors affecting antibiotic therapy response of subclinically infected quarters. Poutrel (1980) observed highest cure rates for cows' quarters infected with subclinical mastitis in their first lactation. Results obtained from this study seem to suggest the same in that the antibiotic therapy failed to cure *Staphylococcus aureus* infection in quarters of cows aged between eight and twelve years. In view of all the above it is reasonable to postulate that the quarters of older cows might have had intermittent infections in previous lactations or as a result of possible frequent exposures to pathogenic organisms the quarters might have developed subclinical infections in which pathogens embedded themselves into host cells and in doing so became inaccessible to the antibiotic. The progressive pathology of subclinical mastitis emphasizes the need for initiating treatment before the organisms become established in inaccessible areas of the udder. In other words, efforts to control mastitis should not only be focussed on the treatment of clinical mastitis or treatment of subclinical cases during drying-off periods but should also aim to reduce the level of infection during lactation by reducing sources of pathogens for reinfecting other quarters/cows.

Type of pathogenic organism has also been reported as another factor affecting antibiotic therapy response of infected quarters. Griffin (1971) reported that with Staphylococci the average elimination rate in lactation was less than 30% for clinical cases of mastitis but exceeded 60% when treatment was given to quarters subclinically infected by the same pathogenic organisms during lactation. He also reported that this method of mastitis control enables the level of infection in a herd to be reduced at one time by 70 - 80% thus reducing the sources of pathogens for reinfecting other quarters. Plommet & Le Louedec (1975) also reported that the response of Staphylococci infections to cloxacillin was less than 30% for clinical mastitis during lactation and more than 60% for subclinical infection

during lactation or at the dry period. Table 5.2 shows the response of both *Staphylococcus aureus* and Streptococci to a slow release base of sodium cloxacillin (Orbenin).

TABLE 5.2: THE PROPORTION OF SUBCLINICAL INFECTIONS ELIMINATED WHEN TREATED*WITH SODIUM CLOXACILLIN FORMULATED IN A SLOW RELEASE BASE.

Pathogen	Dose (mg)	% Response	Reference
Staphylococcus aureus	200	62	Griffin (1971)
Staphylococcus aureus	200	62.5	Results from this study
Streptococcus agalactiae	200	100	Griffin (1971)
Streptococcus dysgalactiae	200	92	Griffin (1971)
Streptococcus uberis	200	72	Griffin (1971)

* Three infusions were given with intervals of 48 hours between doses.

It is evident from the above table that Streptococci cure rates were much higher than those of *Staphylococcus aureus*.

TABLE 5.3: THE PROPORTION OF CLINICAL INFECTIONS ELIMINATED WHEN TREATED*WITH SODIUM CLOXACILLIN FORMULATED IN FAST RELEASE BASES (GRIFFIN 1971).

Preparation	% Responses			
	Staph. aureus	*Str. agalactiae*	*Str. dysgalactiae*	*Str. uberis*
250 mg Sodium Cloxacillin fast release base	21	40	80	50
375 mg Sodium Cloxacillin fast release base	25	83	91	62

* See Table 5.2 above.

Results presented in Table 5.3 (from Griffin, 1971) show that cure rates of clinical mastitis caused by *Staphylococcus aureus* were much lower than those caused by Streptococci. Increasing the dosage of an antibiotic in clinical mastitis does not appear to increase the cure rates of *Staphylococcus aureus* to any significant level as compared to Streptococci. Comparison of the results presented in Tables 5.2 and 5.3 shows that treatment of *Staphylococcus aureus* at subclinical stage yields better results of cure rate than at clinical stage.

Failure to achieve a very high cure rate with Staphylococci as compared to Streptococci has generally been attributed to the intra-tissue and/or intra-cellular location of the Staphylococci (Pattison, 1958; Platanow & Blobel, 1963). It would appear that postponing treatment of Staphylococcal infections until dry periods or when they develop into clinical cases gives them time to establish themselves into such locations and thus become less accessible to drugs. Wilson and Kingwill (1975) reported that dry cow therapy eliminated over 80% of the Staphylococci in some herds but less than 50% in others. However recent work by Pankey *et al* (1982) shows that dry cow therapy eliminated about 84.5% of the *Staphylococcus aureus* infections. Since dry cow therapy is not 100% effective in eliminating infections particularly those of Staphylococci, it might be advisable to treat all subclinical infections occurring early in lactation. This would reduce the risk of infecting healthy cows in the herd. Norwegian work (Bakken, 1981) indicates that the chance of a quarter showing bacterial infection was increased seven-fold in those udders which had one infected quarter compared with those which had no infected quarter.

5:4 The Effects of Antibiotic Therapy on S.C.C., Milk Yield and Composition:

(i) Somatic cell counts

Results of bacteriological tests and somatic cell count determinations carried out after the treatment period showed a close association between somatic cell count levels of individual quarters

and cures of bacterial infection. Quarters cured by the antibiotic therapy had a dramatic reduction in somatic cell counts as compared to the uncured quarters whose somatic cell counts did not differ much from the untreated, control quarters (see Table 4.10). The average somatic cell counts of the control quarters increased slightly from their original value of about 2,061,500 cells/ml up to 2,111,000 cells/ml. It is evident from these results that successful antibiotic therapy of subclinically infected quarters was accompanied by a marked reduction of somatic cell counts. Unfortunately there are no other data for comparison because the few reported studies did not include the effect of antibiotic therapy on somatic cell counts. However these results support earlier reports which indicated that high cell counts are closely associated with intramammary infection and can therefore be used to predict the prevalence of mastitis in the herd. The results also tend to suggest that a farmer can, with certainty, regard all quarters or cows which show low cell counts following treatment to have been cleared of infection and vice-versa. In view of this it could be suggested that cows with quarters still harbouring pathogens and showing high cell counts after receiving one or two courses of antibiotic therapy should be candidates for culling (particularly the old ones) because such cows could be a continuing source of infection for the other cows in the herd.

(ii) Milk yield

Despite the fact that a bacterial cure was accompanied by a highly significant reduction in the somatic cell counts of the successfully treated quarters, the milk yield obtained from such quarters after treatment was not significantly different from that of the control quarters. However the successfully treated quarters produced about 10% more milk than the untreated (control) quarters. Smith *et al* (1968) reported that when infection is eliminated from a quarter during lactation the milk yield relative to uninfected quarters within the same udder does not recover until the following lactation when the yield shows a considerable, but not complete, recovery provided the quarter has remained uninfected during the dry period. Earlier Wheelock *et al* (1966) had reported that apart from a partial recovery

in certain experiments the yield of milk remained depressed throughout the lactation in quarters which the infection was eliminated spontaneously or by antibiotic therapy and that the recovery in milk yield was not complete even during the following lactation. Morris (1973) reported that when infections were successfully treated during lactation, the yield did not return to normal until the next lactation. It appears that elimination of infection is not necessarily accompanied by an immediate significant improvement in milk production probably because it takes so long for the damaged secretory tissues to fully recover and regenerate into a functional state.

Although the milk yield of the successfully treated quarters obtained from this study was not significantly different from that of the control quarters, the adjusted means showed a difference of about 10% in milk production which could be a real effect of the antibiotic therapy. However it is difficult to draw any conclusion from these results because the number of quarters used in this study was rather small. Nevertheless the estimated loss of 0.1 kg milk quarter^{-1} day^{-1} for each increase of 100,000 cells/ml over the range of 100,000 up to 1,000,000 cells/ml as revealed by the analysis of data collected during the preliminary period would call for an immediate action to prevent any further loss in milk production. The Department of Agriculture Mastitis Committee, Australia, (1970) reported that the average daily loss due to subclinical mastitis was about 0.9 kg milk quarter^{-1}, which would agree exactly with the present data if a quarter without mastitis had S.C.C. of 100,000 cells/ml and the subclinically infected had 1,000,000 cells/ml. The Committee also pointed out that when mastitis is cured the milk from the infected quarter does not revert to its previous level but may do so in the following lactation. Barnard (1981) estimated a loss of 0.45 kg milk cow^{-1} day^{-1} for each increase of 100,000 cells/ml but did not indicate whether all four quarters were subclinically infected.

It has been argued in some reports that since the advantage of treating subclinical infections during lactation is not immediately apparent, postponing the treatment of such infections until drying-off periods would be an economic proposition. But the problem is

some strains of bacteria, for example *Streptococcus agalactiae*, are too contagious (Plommet & Le Louedec, 1975) for deferred and non-synchronized treatments to lead to an effective mastitis control programme. Neave & Jackson (1971) indicated that there is a continuous high level of exposure to pathogens because normally about 50% of cows are infected at any one time and colonization of the teat orifice by pathogens is a common phenomenon. This is supported in the present experiment by the development of five new subclinical infections which were detected when carrying out bacteriological tests three weeks after the end of the treatment period. Thus failure to arrest subclinical infections in their early stages would permit pathogens to establish themselves into inaccessible areas of the udder which tend to afford them protection and hence remain sources of pathogens for reinfection or infecting other cows. Jackson (1971) indicated that elimination of infections occurs more frequently when therapy is used to treat the disease in a subclinical state. He reported that this difference in response to therapy of clinical and subclinical mastitis occurs with the three common types of Streptococci but is most obvious in the case of Staphylococcal infections. Although some subclinical infections are likely to recover spontaneously as observed from this study it may not be advisable to take chances as the percentage of such recoveries is small (only 15.8% of the subclinical infections recovered spontaneously during this study). However examination of results presented in Table 4.5 reveals that the quarters which had spontaneous recoveries during the treatment period are the ones which had relatively low cell counts when first identified with pathogenic organisms. It would therefore be reasonable to assume that the infection which had developed in these quarters was not severe, and if the suggested threshold value of 300,000 cells/ml had been used as the only criterion for predicting subclinical infection in the herd these quarters would have in fact been classified as uninfected. Thus depending on the level of mastitis in the herd it may be unwise to postpone treatment of subclinical infections particularly those detected early in lactation as they are likely to have small effects on milk yield throughout the rest of the lactation period and, more importantly, act as sources of pathogens

for infecting other quarters in the herd. Morris (1973) reported that infections lasting over three months depressed the yield of affected quarters by an average of about 35%, while transient infections depressed yield later in the same lactation by 13%. The aim of treating subclinical infections during lactation in a mastitis control programme should be to reduce the rate of new infection and the duration of each infection by reducing the source of pathogens for infecting other cows and thus improve both the quantitative and qualitative yield from the herd.

(iii) Milk composition

Analysis of data on milk composition (milkfat and protein percentage) showed no significant difference between treated and control quarters ($P>05$). But the comparison of the successfully treated and control quarters showed a significant difference ($P<0.05$) in protein concentration as revealed by the analysis of data covering the first month of the experimental period. However the interpretation of milk composition results particularly as far as fat and total protein percentages are concerned is rather difficult. This is due to the fact that in mastitis, whether clinical or subclinical, milk yield is reduced more than fat yield and fat percentage goes up (Schultz, 1977). Decreased fat content could be explained by reduced fat synthesis due to injury caused to secretory cells. Thus improvement in fat content following treatment would depend on how soon the secretory cells fully recover into a functional state. Wheelock *et al* (1966) reported that when infection was eliminated during the first lactation there was a complete recovery in composition of the milk in the second lactation.

Most experimental evidence suggests either no change or small increases in protein percentage which is contrary to a common belief that mastitis and associated increases in somatic cell counts are associated by a decrease in the percentage of total protein in milk (see review by Schultz, 1977). This apparent conflict arises from the fact that some protein components decrease while others increase. Results obtained from this study indicate that there was an increase in total protein percentage in untreated quarters as compared to the

successfully treated quarters. Wheelock *et al* (1966) reported that the most severe effects on composition were observed in those experiments in which there was the greatest depression in milk yield. They also reported that there was a more marked effect on the concentration of lactose than on the concentration of casein or fat, and that occasionally the contents of casein and fat were unaltered. In general, the components synthesized in the mammary gland (a_s-casein, β-casein, β-lactoglobulin, a-lactalbumin) decrease while those coming from the blood (mainly immunoglobulins and serum albumin) increase (Haenlein *et al*, 1973 and Randolph *et al*, 1974). From a processing point of view these changes are undesirable because the total casein is decreased while the less valuable whey proteins are increased. In view of this and the fact that it apparently takes time before significant improvement in production is realized, it may be difficult to draw any conclusion about the real effect of an antibiotic therapy on fat and protein percentage results obtained from this study.

5:5 The Practical Application of Antibiotic Therapy for Subclinical Mastitis.

Results of this study have shown that antibiotic therapy applied to subclinically infected quarters during lactation gives cure rates which are higher than those which would be expected from treatment of clinical cases particularly with Staphylococcal infections. The antibiotic therapy of subclinical infection achieved cure rates of 60% compared to cure rates of less than 30% recorded with clinical mastitis (Griffin 1971; Plommet & Le Louedec, 1975). The implication is that treating subclinical mastitis eliminates 60% of the infection in the herd at any one time whereas the treatment of clinical cases would eliminate less than 30% of the infection in the herd, indicating that treatment of only clinical cases of mastitis during lactation is not as effective as treating subclinical mastitis in reducing the prevalence of infections in a herd.

The cost of treating a subclinically infected quarter with a course of an antibiotic (Orbenin) and the loss suffered through withholding milk during the treatment period are shown in Table 5.4.

Assuming that an average of about 10% difference in milk production revealed in this study is a real effect of the antibiotic therapy and using the data presented in Table 4.13(b), then a loss of only 20¢ is likely to be suffered by treating a subclinically infected quarter. However this loss is almost negligible when considering the risk of infecting other quarters or cows in the herd and the continued loss of milk production from the subclinically infected quarters.

TABLE 5.4: RETURNS FROM ANTIBIOTIC THERAPY OF SUBCLINICAL MASTITIS DURING LACTATION.

Assuming a 4% Butterfat content and a price of $3.20/kg fat.

		$
Income from a successfully treated quarter (3.13 kg milk x 0.04 kg fat x 30 days x 9 months x $3.20/kg fat)		108.17
Income from untreated quarter (2.77 kg milk x 0.04 kg fat x 30 days x 9 months x $3.20/kg fat)		95.73
Profit resulting from successful treatment		$ 12.44
Allowing a 60% cure rate (i.e. $12.44 x 0.6) gives		7.46
	$	
Cost of antibiotic therapy (Orbenin) 3 intramammary infusions @ $1.35	4.05	
Loss suffered through withholding milk during treatment (3.13 kg milk x 0.04 kg fat x 9 days x $3.20/kg fat)	3.61	
		7.66
Loss suffered		$ 0.20

Since treatment of clinical mastitis also entails production loss through withholding milk during the treatment period, it might therefore be reasonable to treat mastitis in the stage when there is the possibility of getting a higher response to antibiotic therapy.

The main benefit of treating subclinical mastitis during lactation is the reduction in the number of infected quarters and therefore reduced risk of infecting other quarters. This will be of particular importance in dairy herds where *Staphylococcus aureus* is the predominant pathogen. Bakken (1981) reported that the *Staphylococcus aureus* new infection rate was related to the prevalence of the *Staphylococcus aureus* mastitis in the herds. Pattison (1958) reported that if treatment is carried out early enough i.e. before duct-blockage and localisation of the Staphylococci in inaccessible parts of the gland, the antibiotic has a reasonable chance of reaching the invading organism. The other advantage of treating subclinical mastitis during lactation is the decrease in somatic cell count value which is associated with the successful treatment of the affected quarter as shown in this study. In addition to that dairy herds which maintain low cell counts through the control of mastitis will in general receive additional benefit from producing milk of high quality particularly if there is a premium for high quality milk or a penalty for low quality milk. The cell count in the milk is nowadays regarded as a general criterion of the health of the udder(s) from which the milk is withdrawn and in some countries it is used as one of the hygienic quality of the milk (Dijkman *et al*, 1969).

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CHAPTER SIX

6:0 CONCLUSION

This study was designed to investigate the effects of treating subclinically infected quarters with an antibiotic during lactation. From the results obtained firm conclusions on the effects of antibiotic therapy of subclinically infected quarters on milk yield, milkfat and protein percentage are not possible. However these results have confirmed earlier reports that antibiotic therapy can be used with a reasonable degree of success in eliminating subclinical mastitis during lactation which leads to a significant reduction in somatic cell counts of the cured quarters.

Although the recovery in infected quarters was not accompanied by a significant increase in milk production, it could be suggested from the results obtained that more work is required to establish the actual effect by obtaining information on the performance of the successfully treated quarters in the following lactation. This suggestion is based on the fact that results obtained from this study have shown a 10% non-significant difference in milk production between successfully treated and control quarters. If milk production is increased by 10%, the value of the extra milk is almost equal to the cost of the antibiotic therapy and the value of the withheld milk. The major benefit is the reduction in the number of infected quarters, and the consequent reduction in the risk of new infection.

The loss of milk production attributed to mastitis is quite considerable by any account and many studies including this have shown that the presence of subclinical infection of the udder is in no small part responsible for the reduction in milk production in an otherwise apparently healthy herd. It may therefore be useful to treat cows infected early in lactation to prevent continued milk loss throughout the rest of the lactation. However this method of mastitis control will only be useful if appropriate measures of hygiene are taken simultaneously.

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APPENDIX 1:0 FOR DATA COVERING FIRST MONTH OF EXPERIMENTAL PERIOD. SUMMARY OF ANALYSIS OF VARIANCE AND COVARIANCE ANALYSIS OF DATA OF TREATED AND UNTREATED (CONTROL) QUARTERS IN RESPECT OF SOMATIC CELL COUNT, MILK YIELD, FAT AND PROTEIN CONCENTRATION IN THE MILK.

APPENDIX 1:1 EFFECT OF TREATMENT ON S.C.C. OF SUBCLINICALLY INFECTED QUARTERS (LOG CELLS/ML).

	Infected but not treated			Infected and treated	
Data:	Preliminary Period	Experimental Period		Preliminary Period	Experimental Period
Quarter	Log cells/ml	Log cells/ml	Quarter	Log cells/ml	Log cells/ml
18 RF	2.57	3.03	18 LF	2.83	2.36
28 RR	2.70	3.44	22 RR	2.49	2.14
114 LF	3.60	3.40	43 RR	4.25	2.20
114 RF	3.21	3.20	72 RF	2.83	1.73
144 LR	2.78	2.85	81 RR	3.04	2.15
			114 RR	3.34	2.99
			134 LR	3.63	3.03
			144 RF	3.20	2.81
	$\Sigma X_1 = 14.86$	$\Sigma X = 15.92$		$\Sigma X_2 = 25.61$	$\Sigma X = 19.41$
	$\bar{X}_1 = 2.972$	$\bar{X} = 3.184$		$\bar{X}_2 = 3.20125$	$\bar{X} = 2.43$

METHODS: (i) ANALYSIS OF VARIANCE (ANOVA)

	Infected but not treated		Infected and treated	
	Preliminary Period	Experimental Period	Preliminary Period	Experimental Period
n	5	5	8	8
ΣX	14.86	15.92	25.61	19.41 GT = 75.8
$\bar{X}$	2.972	3.184	3.20125	2.43

PRELIMINARY ANALYSIS OF VARIANCE IGNORING THE DISPROPORTION

Source of variation	df	ss	ms
Total	25	7.554462	
Before vs After Trt	1	1.0161384	
Control vs Treated	1	0.4297112	
Individuals within groups (Error)	23	6.108124	0.265591843

DISPROPORTIONATE SUBCLASS NUMBERS: A 2 X 2 DESIGN (SNEDECOR, 1956, P.379)

	Infected but not treated		Infected and treated		$\frac{n_1 n_2}{n_1 + n_2} = W$	
					$\bar{X}_2 - \bar{X}_1 = D$	
	n_1	$\bar{X}_1$	n_2	$\bar{X}_2$	n	W
Before Trt	5	2.972	8	3.20125	13	3.0769
After Trt	5	3.184	8	2.43	13	3.0769

	W	$\bar{X}_2 - \bar{X}_1$ (D)	WD	WD^2
Before	3.0769	0.22925	0.705379325	0.16170821
After	3.0769	- 0.754	- 2.3199826	1.74926688
	$\Sigma W = 6.1538$		$\Sigma WD = -1.614603275$	$\Sigma WD^2 = 1.91097509$

$$\text{S.S. for interaction} = \Sigma WD^2 - \frac{(\Sigma WD)^2}{\Sigma W}$$

$$= 1.91097509 - 0.372779934$$

$$= 1.5382$$

$$F = \frac{1.5382}{0.26559}$$

$$= 5.791^* \qquad P<0.05$$

(ii) (a) ANALYSIS OF COVARIANCE

Source of variation	df	Σx^2	Σxy	Σy^2	Deviation from Regression df	ss	ms	F
Total	12	2.99568	0.29189	3.54263	11	3.51419		
Between Group means	1	0.16171	- 0.53451	1.76672				
Within Group means	11	2.83397	0.82640	1.77591	10	1.5349	0.15349	
Difference between adjusted means					1	1.97929	1.97929	12.895**

**P<0.01

(b) COMPARISON OF REGRESSIONS

Source of variation	df	Σx^2	Σxy	Σy^2	Deviation from Regression df	ss	ms	F
Control	4	0.72348	0.19586	0.24772	3	0.19470		
Treated	7	2.11049	0.63054	1.52819	6	1.33981		
Deviation from individual lines					9	1.53451	0.1705	
Common Regression	11	2.83397	0.82640	1.77591	10	1.53493		
Difference in slopes					1	0.00042	0.00042	0.0025 NS

(c) SIGNIFICANCE OF REGRESSION ADJUSTED BY COVARIANCE

Source of variation	df	ss	ms	F
Unadjusted Σy^2	11	1.77591		
Adjusted by Regression	1	0.24098	0.24098	1.57 NS
Adjusted Σy^2	10	1.53493	0.153493	

APPENDIX 1:2 EFFECT OF ANTIBIOTIC TREATMENT ON MILK YIELD OF SUBCLINICALLY INFECTED QUARTERS (KG QUARTER^{-1} DAY^{-1}).

	Infected but not treated			Infected and treated	
Data:	Preliminary Period	Experimental Period		Preliminary Period	Experimental Period
Quarter	Yield	Yield	Quarter	Yield	Yield
18 RF	3.11	2.49	18 LF	3.07	2.76
28 RR	3.28	2.63	22 RR	3.98	3.67
114 LF	2.62	2.44	43 RR	3.59	5.34
114 RF	2.39	2.19	72 RF	3.28	2.82
144 LR	6.56	6.01	81 RR	3.82	3.34
			134 LR	3.28	3.14
			144 RF	8.22	7.38
	$\Sigma Y_1 = 17.96$	$\Sigma Y = 15.76$		$\Sigma Y_2 = 32.85$	$\Sigma Y = 31.68$
	$\bar{Y}_1 = 3.592$	$\bar{Y} = 3.152$		$\bar{Y}_2 = 4.10625$	$\bar{Y} = 3.96$

METHODS: (i) ANALYSIS OF VARIANCE (ANOVA)

	Infected but not treated		Infected and treated		
	Preliminary Period	Experimental Period	Preliminary Period	Experimental Period	
n	5	5	8	8	
ΣY	17.96	15.76	32.85	31.68	GT = 98.25
$\bar{Y}$	3.592	3.152	4.10625	3.96	

PRELIMINARY ANALYSIS OF VARIANCE IGNORING THE DISPROPORTION

Source of variation	df	ss	ms
Total	25	63.0858654	
Before vs After Trt	1	0.4368037	
Control vs Treated	1	2.6897616	
Individuals within groups (Error)	23	59.9593001	2.606926091

DISPROPORTIONATE SUBCLASS NUMBERS: A 2 X 2 DESIGN

	Control		Treated			
					$\frac{n_1 n_2}{n_1 + n_2} = W$	
					$\bar{Y}_2 - \bar{Y}_1 = D$	
	n_1	$\bar{Y}_1$	n_2	$\bar{Y}_2$	n	W
Before Trt	5	3.592	8	4.10625	13	3.0769
After Trt	5	3.152	8	3.96	13	3.0769

	W	$\bar{Y}_2 - \bar{Y}_1$ (D)	WD	WD^2
Before	3.0769	0.51425	1.58230	0.81370
After	3.0769	0.808	2.48614	2.00880
	$\Sigma W = 6.1538$		$\Sigma WD = 4.06844$	$\Sigma WD^2 = 2.8225$

$$\text{S.S. for intereaction} = \Sigma WD^2 - \frac{(\Sigma WD)^2}{\Sigma W}$$

$$= 2.8225 - \frac{(4.06844)^2}{6.1538}$$

$$= 2.8225 - 2.689753328$$

$$= 0.132746672$$

$$F = \frac{0.13275}{2.60693}$$

$$= 0.0509 \quad NS^*$$

*NS = Not Significant

(ii) (a) ANALYSIS OF COVARIANCE

Source of variation	df	Σx^2	Σxy	Σy^2	Deviation from Regression df	ss	ms	F
Total	12	32.32123	28.87038	30.33429	11	4.54633		
Between Group means	1	0.80975	1.2754	2.0081				
Within Group means	11	31.51148	27.59498	28.32548	10	4.16023	0.416023	
Difference between adjusted means					1	0.3861	0.3861	0.93 NS

(b) COMPARISON OF REGRESSIONS

Source of variation	df	Σx^2	Σxy	Σy^2	Deviation from Regression df	ss	ms	F
Control	4	11.52828	10.81288	10.31128	3	0.16941		
Treated	7	19.9832	16.7821	18.0142	6	3.92042		
Deviation from individual lines					9	4.08983	0.45443	
Common Regression	11	31.51148	27.59498	28.32548	10	4.16023		
Difference in slopes					1	0.0704	0.0704	0.16 NS

(c) SIGNIFICANCE OF REGRESSION ADJUSTED BY COVARIANCE

Source of variation	df	ss	ms	F
Unadjusted Σy^2	11	28.32548		
Adjusted by Regression	1	24.16525	24.16525	58.09**
Adjusted Σy^2	10	4.16023	0.416023	

**P<0.01

APPENDIX 1:3 EFFECT OF ANTIBIOTIC TREATMENT ON MILKFAT CONCENTRATION (% FAT) OF SUBCLINICALLY INFECTED QUARTERS.

	Infected but not treated			Infected and treated	
Data:	Preliminary Period	Experimental Period		Preliminary Period	Experimental Period
Quarter	% Fat	% Fat	Quarter	% Fat	% Fat
18 RF	6.18	6.31	18 LF	5.50	6.21
28 RR	5.49	6.17	22 RR	4.85	5.71
114 LF	4.43	4.28	43 RR	4.21	4.61
114 RF	4.23	4.28	72 RF	5.68	5.81
144 LR	4.97	5.01	114 RR	4.20	5.01
			134 LR	4.33	4.28
			144 RF	4.89	4.88
	$\Sigma Y_1 = 25.3$	$\Sigma Y = 26.05$		$\Sigma Y_2 = 38.7$	$\Sigma Y = 41.57$
	$\bar{Y}_1 = 5.06$	$\bar{Y} = 5.21$		$\bar{Y}_2 = 4.8375$	$\bar{Y} = 5.19625$

METHODS: (i) ANALYSIS OF VARIANCE (ANOVA)

PRELIMINARY ANALYSIS OF VARIANCE IGNORING THE DISPROPORTION

Source of variation	df	ss	ms
Total	25	12.3466616	
Before vs After Trt	1	0.5040153	
Control vs Treated	1	0.0858678	
Individuals within groups (Error)	23	11.7567785	0.511164282

DISPROPORTIONATE SUBCLASS NUMBERS: A 2 X 2 DESIGN

	Control		Treated		$\frac{n_1 n_2}{n_1 + n_2} = W$	
					$\bar{Y}_2 - \bar{Y}_1 = D$	
	n_1	$\bar{Y}_1$	n_2	$\bar{Y}_2$	n	W
Before Trt	5	5.06	8	4.8375	13	3.0769
After Trt	5	5.21	8	5.19625	13	3.0769

	W	$\bar{Y}_4 - \bar{Y}_2$ (D)	WD	WD^2
Before	3.0769	- 0.2225	- 0.68461	0.15233
After	3.0769	- 0.01375	- 0.04231	0.00058
	$\Sigma W = 6.1538$		$\Sigma WD = - 0.72692$	$\Sigma WD^2 = 0.15291$

$$\text{S.S. for intereaction} = \Sigma WD^2 - \frac{(\Sigma WD)^2}{\Sigma W}$$

$$= 0.15291 - \frac{(- 0.72692)^2}{6.1538}$$

$$= 0.15291 - 0.85867705$$

$$= 0.06704$$

$$F = \frac{0.06704}{0.51116}$$

$$= 0.131 \quad NS$$

(ii) (a) ANALYSIS OF COVARIANCE

					Deviation from Regression			
Source of variation	df	Σx^2	Σxy	Σy^2	df	ss	ms	F
Total	12	4.93588	5.13254	6.90677	11	1.56973		
Between Group means	1	0.15233	0.00941	0.00058				
Within Group means	11	4.78355	5.12313	6.90619	10	1.41937	0.141937	
Difference between adjusted means					1	0.15036	0.15036	1.06 NS

(b) COMPARISON OF REGRESSIONS

Source of variation	df	Σx^2	Σxy	Σy^2	Deviation from Regression df	ss	ms	F
Control	4	2.5332	3.0206	3.9014	3	0.29962		
Treated	7	2.25035	2.10253	3.00479	6			
Deviation from individual lines					9	1.34000	0.2978	
Common Regression	11	4.78355	5.12313	6.90619	10	1.41937		
Difference in slopes					1	0.07937	0.07937	0.267 NS

(c) SIGNIFICANCE OF REGRESSION ADJUSTED BY COVARIANCE

Source of variation	df	ss	ms	F
Unadjusted Σy^2	11	6.90619		
Adjusted by Regression	1	5.48682	5.48682	
Adjusted Σy^2	10	1.41937	0.141937	38.657**

**P<0.01

APPENDIX 1:4 EFFECT OF ANTIBIOTIC TREATMENT ON MILK PROTEIN CONCENTRATION (% PROTEIN) OF SUBCLINICALLY INFECTED QUARTERS.

	Infected but not treated			Infected and treated	
Data:	Preliminary Period	Experimental Period		Preliminary Period	Experimental Period
Quarter	% Protein	% Protein	Quarter	% Protein	% Protein
18 RF	4.27	4.15	18 LF	4.20	3.99
28 RR	3.95	4.08	22 RR	3.77	3.53
114 LF	4.22	4.14	43 RR	4.48	3.35
114 RF	4.23	4.09	72 RF	3.96	3.95
144 LR	3.96	3.86	81 RR	3.84	3.78
			114 RR	4.34	4.09
			134 LR	4.00	3.82
			144 RF	3.96	3.82
	$\Sigma y_1 = 20.63$	$\Sigma y = 20.32$		$\Sigma Y_2 = 32.55$	$\Sigma y = 30.33$
	$\bar{Y}_1 = 4.126$	$\bar{y} = 4.064$		$\bar{Y}_2 = 4.06875$	$\bar{Y} = 3.79125$

METHODS: (i) ANALYSIS OF VARIANCE (ANOVA)

PRELIMINARY ANALYSIS OF VARIANCE IGNORING THE DISPROPORTION

Source of variation	df	ss	ms
Total	25	1.4883885	
Before vs After Trt	1	0.2461884	
Control vs Treated	1	0.1675385	
Individuals within groups (Error)	23	1.0746616	0.046724417

DISPROPORTIONATE SUBCLASS NUMBERS: A 2 X 2 DESIGN

	Control		Treated		$\frac{n_1 n_2}{n_1 + n_2} = W$	
					$\bar{Y}_2 - \bar{Y}_1 = D$	
	n_1	$\bar{Y}_1$	n_2	$\bar{Y}_2$	n	W
Before Trt	5	4.126	8	4.06875	13	3.0769
After Trt	5	4.064	8	3.79125	13	3.0769

	W	$\bar{y}_2 - \bar{y}_1$ (D)	WD	WD^2
Before	3.0769	- 0.05725	- 0.17615	0.01008
After	3.0769	- 0.27275	- 0.83922	0.22890
	$\Sigma W = 6.1538$		$\Sigma WD = - 1.01537$	$\Sigma WD^2 = 0.23898$

$$\text{S.S. for interaction} = \Sigma WD^2 - \frac{(\Sigma WD)^2}{\Sigma W}$$

$$= 0.23898 - \frac{(- 1.01537)^2}{6.1538}$$

$$= 0.23898 - 0.167534894$$

$$= 0.07144$$

$$F = \frac{0.07144}{0.04672}$$

$$= 1.529 \quad \text{NS}$$

(ii) (a) ANALYSIS OF COVARIANCE

Source of variation	df	Σx^2	Σxy	Σy^2	Deviation from Regression df	ss	ms	F
Total	12	0.53889	0,08523	0.70341	11	0.68993		
Between Group means	1	0.01008	0.04796	0.229				
Within Group means	11	0.52881	0.03727	0.47441	10	0.47178	0.047178	
Difference between adjusted means					1	0.21815	0.21815	4.62

P<0.1

(b) COMPARISON OF REGRESSIONS

Source of variation	df	Σx^2	Σxy	Σy^2	Deviation from Regression df	ss	ms	F
Control	4	0.09892	0.05328	0.05572	3	0.02702		
Treated	7	0.42989	- 0.01609	0.41869	6	0.41809		
Deviation from individual lines					9	0.44511	0.09891	
Common Regression	11	0.52881	0.03727	0.47441	10	0.47178		
Difference in slopes					1	0.02667	0.02667	0.27 NS

(c) SIGNIFICANCE OF REGRESSION ADJUSTED BY COVARIANCE

Source of variation	df	ss	ms	F
Unadjusted Σy^2	11	0.47411		
Adjusted by Regression	1	0.00263	0.00263	
Adjusted Σy^2	10	0.47178	0.047178	0.06 NS

APPENDIX 2:0 FOR DATA FROM WHOLE EXPERIMENTAL PERIOD. SUMMARY OF ANOVA AND COVARIANCE ANALYSIS OF DATA OF TREATED AND CONTROL QUARTERS IN RESPECT OF S.C.C., MILK YIELD, FAT AND PROTEIN CONCENTRATION (%) IN THE MILK.

APPENDIX 2:1 EFFECT OF TREATMENT ON S.C.C. OF SUBCLINICALLY INFECTED QUARTERS (LOG CELLS/ML).

	Infected but not treated			Infected and treated	
Data:	Preliminary Period	Experimental Period		Preliminary Period	Experimental Period
Quarter	Log cells/ml	Log cells/ml	Quarter	Log cells/ml	Log cells/ml
18 RF	2.57	2.49	18 LF	2.83	2.15
28 RR	2.70	3.29	22 RR	2.49	1.95
114 LF	3.60	3.21	43 RR	4.25	2.05
114 RF	3.21	3.13	72 RF	2.83	1.68
144 LR	2.78	2.77	81 RR	3.04	2.09
			114 RR	3.34	3.12
			134 LR	3.63	3.44
			144 RF	3.20	2.87
	$\Sigma X_1 = 14.86$	$\Sigma X = 14.89$		$\Sigma X_2 = 25.61$	$\Sigma X = 19.35$
	$\bar{X}_1 = 2.972$	$\bar{X} = 2.978$		$\bar{X}_2 = 3.20125$	$\bar{X} = 2.41875$

METHODS OF ANALYSIS: (i) ANOVA

PRELIMINARY ANALYSIS OF VARIANCE IGNORING THE DISPROPORTION

Source of variation	df	ss	ms
Total	25	8.7526885	
Before vs After Trt	1	1.4928038	
Control vs Treated	1	0.1675385	
Individuals within groups	23	7.0923462	0.308362878

DISPROPORTIONATE SUBCLASS NUMBERS: A 2 X 2 DESIGN

	Infected but not treated		Infected and treated		$\frac{n_1 n_2}{n_1 + n_2} = W$	
					$\bar{X}_2 - \bar{X}_1 = D$	
	n_1	$\bar{X}_1$	n_2	$\bar{X}_2$	n	W
Before Trt	5	2.972	8	3.20125	13	3.0769
After Trt	5	2.978	8	2.41875	13	3.0769

	W	$\bar{X} - \bar{X}_1$ (D)	WD	WD^2
Before	3.0769	0.22925	0.705379325	0.16170821
After	3.076	- 0.55925	- 1.720756325	0.962332974
	$\Sigma W = 6.1538$		$\Sigma WD = -1.015377$	$\Sigma WD^2 = 1.124041184$

$$\text{S.S. for interaction} = \Sigma WD^2 - \frac{(\Sigma WD)^2}{\Sigma W}$$

$$= 1.124041184 - \frac{(-1.015377)^2}{6.1538}$$

$$= 1.124041184 - 0.167537204$$

$$= 0.95650398$$

$$F = \frac{0.95650398}{0.308362878}$$

$$= 3.109 \qquad P<0.1$$

(ii) (a) ANALYSIS OF COVARIANCE

Source of variation	df	Σx^2	Σxy	Σy^2	Deviation from Regression df	ss	ms	F
Total	12	2.99568	0.84695	4.23811	11	3.99866		
Between Group means	1	0.16171	- 0.39448	0.96204				
Within Group means	11	2.83397	1.24143	3.27607	10	2.73225	0.273225	
Difference between adjusted means					1	1.26641	1.26641	4.64

$P<0.1$

(b) COMPARISON OF REGRESSIONS

Source of variation	df	Σx^2	Σxy	Σy^2	Deviation from Regression df	ss	ms	F
Control	4	0.72348	0.33312	0.45568	3	0.3023		
Treated	7	2.11049	0.90831	2.82039	6	2.4295		
Deviation from individual lines					9	2.7318	0.30353	
Common Regression	11	2.83397	1.24143	3.27607	10	2.7323		
Difference in slopes					1	0.0005	0.0005	0.0016 NS

(c) SIGNIFICANCE OF REGRESSION ADJUSTED BY COVARIANCE

Source of variation	df	ss	ms	F
Unadjusted Σy^2	11	3.27607		
Adjusted by Regression	1	0.54382	0.54382	
Adjusted Σy^2	10	2.73225	0.273225	1.99 NS

APPENDIX 2:2 EFFECT OF ANTIBIOTIC TREATMENT ON MILK YIELD OF SUBCLINICALLY INFECTED QUARTERS (KG QUARTER^{-1} DAY^{-1}).

	Infected but not treated			Infected and treated	
Data:	Preliminary Period	Experimental Period		Preliminary Period	Experimental Period
Quarter	Yield	Yield	Quarter	Yield	Yield
18 RF	3.11	2.40	18 LF	3.07	2.62
28 RR	3.28	2.13	22 RR	3.98	3.18
114 LF	2.62	2.11	43 RR	3.59	4.49
114 RF	2.39	1.86	72 RF	3.28	2.47
144 LR	6.56	5.46	81 RR	3.82	2.83
			114 RR	3.61	2.59
			134 LR	3.28	2.67
			144 RF	8.22	6.57
	$\Sigma Y_1 = 17.96$	$\Sigma y = 13.96$		$\Sigma y_2 = 32.85$	$\Sigma y = 27.42$
	$\bar{Y}_1 = 3.592$	$\bar{y} = 2.792$		$\bar{y}_2 = 4.10625$	$\bar{y} = 3.4275$

METHODS OF ANALYSIS: (i) ANOVA

PRELIMINARY ANALYSIS OF VARIANCE IGNORING THE DISPROPORTION

Source of variation	df	ss	ms
Total	25	60.2880347	
Before vs After Trt	1	3.4201884	
Control vs Treated	1	2.0337309	
Individuals within groups	23	54.8341154	2.384091973

DISPROPORTIONATE SUBCLASS NUMBERS: A 2 X 2 DESIGN

	Infected but not treated		Infected and treated			
					$\frac{n_1 n_2}{n_1 + n_2} = W$	
					$\bar{y}_2 - \bar{y}_1 = D$	
	n_1	$\bar{Y}_1$	n_2	$\bar{y}_2$	n	W
Before Trt	5	3.592	8	4.10625	13	3.0769
After Trt	5	2.792	8	3.4275	13	3.0769

	W	$\bar{Y}_2 - \bar{Y}_1$ (D)	WD	WD^2
Before	3.0769	0.51425	1.58230	0.813698
After	3.0769	0.6355	1.95537	1.242638
	$\Sigma W = 6.1538$		$\Sigma WD = 3.53767$	$\Sigma WD^2 = 2.056336$

$$\text{S.S. for interaction} = \Sigma WD^2 - \frac{(\Sigma WD)^2}{\Sigma W}$$

$$= 2.056336 - \frac{(3.53767)^2}{6.1538}$$

$$= 2.056336 - 2.033720$$

$$= 0.02262$$

$$F = \frac{0.02262}{2.38409}$$

$$= 0.0095 \quad \text{NS}$$

(ii) (a) ANALYSIS OF COVARIANCE

Source of variation	df	Σx^2	Σxy	Σy^2	Deviation from Regression df	ss	ms	F
Total	12	32.31477	26.35346	24.55308	11	3.06120		
Between Group means	1	0.8137	1.00555	1.24265				
Within Group means	11	31.50107	25.34791	23.31043	10	2.91378	0.291378	
Difference between adjusted means					1	0.14742	0.14742	0.51 NS

(b) COMPARISON OF REGRESSIONS

Source of variation	df	Σx^2	Σxy	Σy^2	Deviation from Regression df	ss	ms	F
Control	4	11.52828	10.09728	9.04388	3	0.19997		
Treated	7	19.97279	15.15063	14.26655	6	2.62163		
Deviation from individual lines					9	2.8216	0.31351	
Common Regression	11	31.50107	25.34791	23.31043	10	2.91378		
Difference in slopes					1	0.09218	0.09218	0.29 NS

(c) SIGNIFICANCE OF REGRESSION ADJUSTED BY COVARIANCE

Source of variation	df	ss	ms	F
Unadjusted Σy^2	11	23.31043		
Adjusted by Regression	1	20.39665	20.39665	
Adjusted Σy^2	10	2.91378	0.291378	70**

** $P<0.01$

APPENDIX 2:3 EFFECT OF ANTIBIOTIC TREATMENT ON MILKFAT CONCENTRATION (% FAT) OF SUBCLINICALLY INFECTED QUARTERS.

	Infected but not treated			Infected and treated	
Data:	Preliminary Period	Experimental Period		Preliminary Period	Experimental Period
Quarter	% Fat	% Fat	Quarter	% Fat	% Fat
18 RF	6.18	6.20	18 LF	5.50	6.23
28 RR	5.49	6.21	22 RR	4.85	5.68
114 LF	4.43	4.91	43 RR	4.21	4.85
114 RF	4.23	4.93	72 RF	5.68	5.88
144 LR	4.97	4.97	81 RR	5.04	5.13
			114 RR	4.20	5.11
			134 LR	4.33	4.31
			144 RF	4.89	4.93
	$\Sigma X_1 = 25.3$	$\Sigma X = 27.22$		$\Sigma X_2 = 38.7$	$\Sigma X = 42.12$
	$\bar{X}_1 = 5.06$	$\bar{X} = 5.444$		$\bar{X}_2 = 4.8375$	$\bar{X} = 5.265$

METHODS OF ANALYSIS: (i) ANOVA

PRELIMINARY ANALYSIS OF VARIANCE IGNORING THE DISPROPORTION

Source of variation	df	ss	ms
Total	25	10.7839385	
Before vs After Trt	1	1.0967538	
Control vs Treated	1	0.2480035	
Individuals within groups	23	9.4391812	0.41039182

DISPROPORTIONATE SUBCLASS NUMBERS: A 2 X 2 DESIGN

	Infected but not treated		Infected and treated			
					$\frac{n_1 n_2}{n_1 + n_2} = W$	
					$\bar{X}_2 - \bar{X}_2 = D$	
	n_1	$\bar{X}_1$	n_2	$\bar{X}_2$	n	W
Before Trt	5	5.06	8	4.8375	13	3.0769
After Trt	5	5.444	8	5.265	13	3.0769

	W	$\bar{X}_2 - \bar{X}_1$ (D)	WD	WD^2
Before	3.0769	- 0.2225	- 0.68461	0.15233
After	3.0769	- 0.179	- 0.55077	0.09859
	$\Sigma W = 6.1538$		$\Sigma WD = - 1.123538$	$\Sigma WD^2 = 0.25092$

$$\text{S.S. for interaction} = \Sigma WD^2 - \frac{(\Sigma WD)^2}{\Sigma W}$$

$$= 0.25092 - \frac{(- 1.123538)^2}{6.1538}$$

$$= 0.25092 - 0.248003468$$

$$= 0.0029165316$$

$$F = \frac{0.00292}{0.41039}$$

$$= 0.0071 \quad NS$$

(ii) (a) ANALYSIS OF COVARIANCE

Source of variation	df	Σx^2	Σxy	Σy^2	Deviation from Regression df	ss	ms	F
Total	12	4.93588	4.06595	4.75131	11	1.40197		
Between Group means	1	0.15233	0.12255	0.09859				
Within Group means	11	4.78355	3.9434	4.65272	10	1.40191	0.140191	
Difference between adjusted means					1	0.00006	0.00006	0.0004 NS

(b) COMPARISON OF REGRESSIONS

Source of variation	df	Σx^2	Σxy	Σy^2	Deviation from Regression df	ss	ms	F
Control	4	2.5332	1.9818	1.93232	3	0.38190		
Treated	7	2.25035	1.9616	2.7204	6	1.01050		
Deviation from individual lines					9	1.3924	0.15471	
Common Regression	11	4.78355	3.9434	4.65272	10	1.40191		
Difference in slopes					1	0.00951	0.00951	0.062 NS

(c) SIGNIFICANCE OF REGRESSION ADJUSTED BY COVARIANCE

Source of variation	df	ss	ms	F
Unadjusted Σy^2	11	4.65272		
Adjusted by Regression	1	3.25081	3.25081	23.188**
Adjusted Σy^2	10	1.40191	0.140191	

**P<0.01

APPENDIX 2:4 EFFECT OF ANTIBIOTIC TREATMENT ON MILK PROTEIN CONCENTRATION (% PROTEIN) OF SUBCLINICALLY INFECTED QUARTERS.

	Infected but not treated			Infected and treated	
Data:	Preliminary Period	Experimental Period		Preliminary Period	Experimental Period
Quarter	% Protein	% Protein	Quarter	% Protein	% Protein
18 RF	4.27	4.09	18 LF	4.20	4.06
28 RR	3.95	4.10	22 RR	3.77	3.58
114 LF	4.22	4.08	43 RR	4.48	3.27
114 RF	4.23	4.10	72 RF	3.96	3.97
144 LR	3.96	3.71	81 RR	3.84	3.75
			114 RR	4.34	4.12
			134 LR	4.00	3.76
			144 RF	3.96	3.75
	$\Sigma y_1 = 20.63$	$\Sigma y = 20.08$		$\Sigma y_2 = 32.55$	$\Sigma y = 30.26$
	$\bar{y}_1 = 4.126$	$\bar{y} = 4.016$		$\bar{y}_2 = 4.06875$	$\bar{y} = 3.7825$

METHODS OF ANALYSIS: (i) ANOVA

PRELIMINARY ANALYSIS OF VARIANCE IGNORING THE DISPROPORTION

Source of variation	df	ss	ms
Total	25	1.665385	
Before vs After Trt	1	0.3102153	
Control vs Treated	1	0.1300547	
Individuals within groups	23	1.225115	0.053265869

DISPROPORTIONATE SUBCLASS NUMBERS: A 2 X 2 DESIGN

	Infected but not treated		Infected and treated		$\frac{n_1 n_2}{n_1 + n_2} = W$	
					$\bar{y}_2 - \bar{y}_1 = D$	
	n_1	$\bar{y}_1$	n_2	$\bar{y}_2$	n	W
Before Trt	5	4.126	8	4.06875	13	3.0769
After Trt	5	4.016	8	3.7825	13	3.0769

	W	$\bar{y}_2 - \bar{y}_1$ (D)	WD	WD^2
Before	3.0769	- 0.05725	- 0.176152525	0.010084732
After	3.0769	- 0.2335	- 0.71845615	0.167759511
	$\Sigma W = 6.1538$		$\Sigma WD = - 0.894608675$	$\Sigma WD^2 = 0.177844243$

$$\text{S.S. for interaction} = \Sigma WD^2 - \frac{(\Sigma WD)^2}{\Sigma W}$$

$$= 0.177844243 - \frac{(- 0.894608675)^2}{6.1538}$$

$$= 0.177844243 - 0.130054121$$

$$= 0.047790121$$

$$F = \frac{0.047790121}{0.053265869}$$

$$= 0.897 \quad \text{NS}$$

(ii) (a) ANALYSIS OF COVARIANCE

					Deviation from Regression			
Source of variation	df	Σx^2	Σxy	Σy^2	df	ss	ms	F
Total	12	0.53889	0.07238	0.81743	11	0.8077		
Between Group means	1	0.01008	0.04113	0.16776				
Within Group means	11	0.52881	0.03125	0.64967	10	0.6478	0.06478	
Difference between adjusted means					1	0.1599	0.1599	2.468 NS

(b) COMPARISON OF REGRESSIONS

Source of variation	df	Σx^2	Σxy	Σy^2	Deviation from Regression df	ss	ms	F
Control	4	0.09892	0.06142	0.11732	3	0.0792		
Treated	7	0.42989	-0.03018	0.53235	6	0.5302		
Deviation from individual lines					9	0.6094	0.0677	
Common Regression	11	0.52881	0.03125	0.64967	10	0.6478		
Difference in slopes					1	0.0384	0.0384	0.567 NS

(c) SIGNIFICANCE OF REGRESSION ADJUSTED BY COVARIANCE

Source of variation	df	ss	ms	F
Unadjusted Σy^2	11	0.64967		
Adjusted by Regression	1	0.00187	0.00187	0.029 NS
Adjusted Σy^2	10	0.6478	0.06478	

APPENDIX 2:5 FOR DATA COVERING THE FIRST MONTH OF EXPERIMENTAL PERIOD. SUMMARY OF COVARIANCE ANALYSES OF DATA OF SUCCESSFULLY TREATED AND UNTREATED QUARTERS IN RESPECT OF THEIR SOMATIC CELL COUNT, MILK YIELD, FAT AND PROTEIN PERCENTAGES.

(a) SOMATIC CELL COUNT

Source of variation	df	Σx^2	Σxy	Σy^2	Deviation from Regression df	ss	ms	F
Total	9	2.6004	0.0044	3.3166	8	3.31659		
Common Regression	8	2.56676	0.31412	0.46504	7	0.42660	0.060943	
Difference between adjusted means					1	2.88999	2.88999	47.42**

** $P<0.01$

(b) MILK YIELD

Source of variation	df	Σx^2	Σxy	Σy^2	Deviation from Regression df	ss	ms	F
Total	9	12.0958	11.4083	15.19529	8	4.43541		
Common Regression	8	12.09096	11.45604	14.7244	7	3.86994	0.55285	
Difference between adjusted means					1	0.56547	0.56547	1.02 NS

(c) MILK FAT CONCENTRATION (% FAT)

Source of variation	df	Σx^2	Σxy	Σy^2	Deviation from Regression df	ss	ms	F
Total	9	3.87816	4.2433	5.71165	8	1.06883		
Common Regression	8	3.87812	4.2460	5.5294	7	0.88062	0.12580	
Difference between adjusted means					1	0.18821	0.18821	1.50 NS

(d) MILK PROTEIN CONCENTRATION (% PROTEIN)

Source of variation	df	Σx^2	Σxy	Σy^2	Deviation from Regression df	ss	ms	F
Total	9	0.45136	0.01994	0.65396	8	0.65308		
Common Regression	8	0.43692	-0.04542	0.35812	7	0.35340	0.05048	
Difference between adjusted means					1	0.29968	0.29968	5.94*

*P<0.05

APPENDIX 2:6 FOR DATA COVERING THE WHOLE EXPERIMENTAL PERIOD. SUMMARY OF COVARIANCE ANALYSES OF DATA OF SUCCESSFULLY TREATED AND UNTREATED QUARTERS IN RESPECT OF THEIR SOMATIC CELL COUNT, MILK YIELD, FAT AND PROTEIN PERCENTAGES.

(a) SOMATIC CELL COUNT

						Deviation from Regression		
Source of variation	df	Σx^2	Σxy	Σy^2	df	ss	ms	F
Total	9	2.6004	0.1724	3.06249	8	3.05106		
Common Regression	8	2.56676	0.46066	0.59240	7	0.50972	0.0728	
Difference between adjusted means					1	2.54134	2.54134	34.91**

$P<0.01$

(b) MILK YIELD

						Deviation from Regression		
Source of variation	df	Σx^2	Σxy	Σy^2	df	ss	ms	F
Total	9	12.0958	10.4792	11.94665	8	2.86799		
Common Regression	8	12.09096	10.51506	11.68096	7	2.53640	0.36234	
Difference between adjusted means					1	0.33159	0.33159	0.92 NS

(c) MILK FAT CONCENTRATION (% FAT)

						Deviation from Regression		
Source of variation	df	Σx^2	Σxy	Σy^2	df	ss	ms	F
Total	9	3.87816	3.06068	3.21709	8	0.80157		
Common Regression	8	3.87812	3.06178	3.18684	7	0.76956	0.1099	
Difference between adjusted means					1	0.03201	0.03201	0.29 NS

(d) MILK PROTEIN CONCENTRATION (% PROTEIN)

Source of variation	df	Σx^2	Σxy	Σy^2	Deviation from Regression df	ss	ms	F
Total	9	0.45136	0.37642	0.42649	8	0.11257		
Common Regression	8	0.42692	0.35932	0.40624	7	0.10382	0.01483	
Difference between adjusted means					1	0.00875	0.00875	0.59 NS

APPENDIX 3:0 FOR DATA COVERING ONE MONTH OF EXPERIMENTAL PERIOD. TESTS OF SIGNIFICANCE OF MEAN DIFFERENCES OF TREATED AND UNTREATED QUARTERS IN RESPECT OF S.C.C., MILK YIELD, FAT AND PROTEIN CONCENTRATION IN THE MILK.

(i) SOMATIC CELL COUNT (log cells/ml)

METHODS OF TESTING: (a) ANALYSIS OF VARIANCE (ANOVA)

PROCEDURE: THE FOLLOWING DATA WAS CODED BY ADDING 1.0 TO EACH FIGURE OF THE MEAN DIFFERENCE.

	Untreated mean difference	Treated mean difference	
	0.54	1.47	
	0.26	1.35	
	1.2	3.05	
	1.01	2.1	
	1.07	1.89	
		1.35	
		1.6	
		1.39	
Total	4.08	14.2	GT = 18.28

RSS = 31.5468

CT = 25.7044923

TSS = 5.8423077

$$\text{Between Groups SS} = \frac{(4.08)^2}{5} + \frac{(14.2)^2}{8} - CT$$

$$= 28.53428 - 25.7044923$$

$$= 2.8297877$$

ANOVA

Source of variation	df	ss	ms	F
Total	12	5.8423077		
Between Group means	1	2.8297877		10.333**
Within Group means	11	3.01252	0.273865454	

**P<0.01

(b) USING STUDENT'S t-TEST

$$\text{Variance of difference} = \frac{s^2}{n_1} + \frac{s^2}{n_2}$$

$$= s^2 \left[\frac{1}{n_1} + \frac{1}{n_2}\right]$$

But from the above analysis, $s^2 = 0.273865454$

Hence, variance of difference $= 0.273865454 \left[\frac{1}{5} + \frac{1}{8}\right]$

$$\text{Standard deviation} = s\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}$$

$$= 0.52321558\sqrt{\frac{1}{5} + \frac{1}{8}}$$

$$= 0.52321558\sqrt{0.325}$$

$$= 0.52321558(0.570087712)$$

$$= 0.298339189$$

$$\text{Thus } t = \frac{\text{Difference}}{\text{Standard deviation}}$$

$$= \frac{2.8298}{0.2983} \quad \text{with 1, 11 df}$$

$$= 9.486423064$$

$$= 9.486***$$

***P<0.001

(ii) MILK YIELD (kg/day)

METHODS USED: (a) ANALYSIS OF VARIANCE (ANOVA)

PROCEDURE: CODING OF THE FOLLOWING DATA WAS DONE BY ADDING 2.0 TO EACH FIGURE OF THE MEAN DIFFERENCE.

	Untreated mean difference	Treated mean difference	
	2.62	2.31	
	2.65	2.31	
	2.18	0.25	
	2.2	2.46	
	2.55	2.48	
		2.38	
		2.14	
		2.84	
Total	12.2	17.17	GT = 29.37

RSS = 71.2281

CT = 66.35360769

TSS = 4.87449231

$$\text{Between Groups SS} = \frac{(12.2)^2}{5} + \frac{(17.17)^2}{8} - CT$$

$$= 66.6191125 - 66.35360769$$

$$= 0.26550481$$

ANOVA

Source of variation	df	ss	ms	F
Total	12	4.87449231		
Between Group means	1	0.26550481	0.26550481	0.634 NS
Within Group means	11	4.6089875	0.41898863	

(b) USING STUDENT'S t-TEST

$$\text{Variance of difference} = \frac{s^2}{n_1} + \frac{s^2}{n_2}$$

$$= s^2 \left[\frac{1}{n_1} + \frac{1}{n_2}\right]$$

But from the above analysis, $s^2 = 0.41898863$

Hence, variance of difference $= 0.41898863 \left[\frac{1}{5} + \frac{1}{8}\right]$

$$\text{Standard deviation} = s\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}$$

$$= 0.64729331\sqrt{\frac{1}{5} + \frac{1}{8}}$$

$$= 0.64729331\sqrt{0.325}$$

$$= 0.64729331(0.570087712)$$

$$= 0.369013962$$

$$\text{Thus } t = \frac{\text{Difference}}{\text{Standard deviation}}$$

$$= \frac{0.2655}{0.3690}$$

$$= 0.719512195$$

$$= 0.720 \text{ NS}$$

(iii) FAT CONCENTRATION (% Fat)

METHODS USED: (a) ANALYSIS OF VARIANCE (ANOVA)

PROCEDURE: THE DATA USED WAS CODED BY ADDING 1.0 TO EACH FIGURE OF THE MEAN DIFFERENCE.

	Untreated mean difference	Treated mean difference	
	0.87	0.29	
	0.32	0.14	
	1.15	0.6	
	0.95	0.87	
	0.96	0.98	
		0.19	
		1.05	
		1.01	
Total	4.25	5.13	GT = 9.38

RSS = 8.3456

CT = 6.768030769

TSS = 1.577569231

$$\text{Between Groups SS} = \frac{(4.25)^2}{5} + \frac{(5.13)^2}{8} - CT$$

$$= 6.9021125 - 6.768030769$$

$$= 0.134081731$$

ANOVA

Source of variation	df	ss	ms	F
Total	12	1.577569231		
Between Group means	1	0.134081731	0.134081731	1.022 NS
Within Group means	11	1.4434875	0.131226136	

(b) USING STUDENT'S t-TEST

$$\text{Variance of difference} = \frac{s^2}{n_1} + \frac{s^2}{n_2}$$

$$= s^2 \left[\frac{1}{n_1} + \frac{1}{n_2}\right]$$

But from the above analysis, $s^2 = 0.131226136$

$$\text{Standard deviation} = s\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}$$

$$= 0.362251481\sqrt{\frac{1}{5} + \frac{1}{8}}$$

$$= 0.362251481\sqrt{0.325}$$

$$= 0.362251481(0.570087712)$$

$$= 0.206515117$$

$$\text{Thus } t = \frac{\text{Difference}}{\text{Standard deviation}}$$

$$= \frac{0.1341}{0.2065}$$

$$= 0.649394676$$

$$= 0.649 \text{ NS}$$

(b) USING STUDENT'S t-TEST

$$\text{Variance of difference} = \frac{s^2}{n_1} + \frac{s^2}{n_2}$$

$$= s^2 \left[\frac{1}{n_1} + \frac{1}{n_2}\right]$$

But from the above analysis, $s^2 = 0.131226136$

$$\text{Standard deviation} = s\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}$$

$$= 0.362251481\sqrt{\frac{1}{5} + \frac{1}{8}}$$

$$= 0.362251481\sqrt{0.325}$$

$$= 0.362251481(0.570087712)$$

$$= 0.206515117$$

$$\text{Thus } t = \frac{\text{Difference}}{\text{Standard deviation}}$$

$$= \frac{0.1341}{0.2065}$$

$$= 0.649394676$$

$$= 0.649 \text{ NS}$$

(iv) PROTEIN CONCENTRATION (% Protein)

METHODS USED: (a) ANALYSIS OF VARIANCE (ANOVA)

PROCEDURE: CODING OF THE DATA WAS DONE BY ADDING 0.2 TO EACH FIGURE OF MEAN DIFFERENCE.

	Untreated mean difference	Treated mean difference	
	0.32	0.41	
	0.07	0.44	
	0.28	1.33	
	0.34	0.21	
	0.30	0.26	
		0.45	
		0.38	
		0.34	
Total	1.31	3.82	GT = 5.13

RSS = 3.0961

CT = 2.024376923

TSS = 1.071723077

$$\text{Between Groups SS} = \frac{(1.31)^2}{5} + \frac{(3.82)^2}{8} - CT$$

$$= 2.16727 - 2.024376923$$

$$= 0.142893077$$

ANOVA

Source of variation	df	ss	ms	F
Total	12	1.071723077		
Between Group means	1	0.142893077	0.142893077	1.692 NS
Within Group means	11	0.92883	0.08443909	

(b) USING STUDENT'S t-TEST

$$\text{Variance of difference} = \frac{s^2}{n_1} + \frac{s^2}{n_2}$$

$$= s^2 \left[\frac{1}{n_1} + \frac{1}{n_2}\right]$$

But from the above analysis, $s^2 = 0.08443909$

$$\text{Standard deviation} = s\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}$$

$$= 0.290584049\sqrt{\frac{1}{5} + \frac{1}{8}}$$

$$= 0.290584049\sqrt{0.325}$$

$$= 0.290584049(0.570087712)$$

$$= 0.165658396$$

$$= 0.1657$$

$$\text{Thus } t = \frac{\text{Difference}}{\text{Standard deviation}}$$

$$= \frac{0.1429}{0.1657}$$

$$= 0.862401931$$

$$= 0.862 \text{ NS}$$

APPENDIX 4:0 FOR DATA COVERING THE WHOLE EXPERIMENTAL PERIOD. TESTS OF SIGNIFICANCE OF MEAN DIFFERENCES OF TREATED AND UNTREATED QUARTERS IN RESPECT OF S.C.C., MILK YIELD, FAT AND PROTEIN CONCENTRATION IN THE MILK.

(i) SOMATIC CELL COUNT (log cells/ml)

METHODS OF TESTING: (a) ANALYSIS OF VARIANCE (ANOVA)

PROCEDURE: THE FOLLOWING DATA WAS CODED BY ADDING 0.6 TO EACH FIGURE OF THE MEAN DIFFERENCE.

	Untreated mean difference	Treated mean difference	
	0.68	1.28	
	0.01	1.14	
	0.99	2.80	
	0.68	1.75	
	0.61	1.55	
		0.82	
		0.79	
		0.93	
Total	2.97	11.06	GT = 14.03

RSS = 20.6815

CT = 15.14160769

TSS = 5.53989231

$$\text{Between Groups SS} = \frac{(2.97)^2}{5} + \frac{(11.06)^2}{8} - CT$$

$$= 1.76418 + 15.29045 - CT$$

$$= 17.05463 - 15.14160769$$

$$= 1.91302231$$

ANOVA

Source of variation	df	ss	ms	F
Total	12	5.53989231		
Between Group means	1	1.91302231	1.91302231	5.802*
Within Group means	11	3.62687	0.329715454	

*P<0.05

(b) USING STUDENT'S t-TEST

$$\text{Variance of difference} = \frac{s^2}{n_1} + \frac{s^2}{n_2}$$

$$= s^2 \left[\frac{1}{n_1} + \frac{1}{n_2}\right]$$

But from the above analysis, $s^2 = 0.329715454$

Hence, variance of difference $= 0.329715454 \left[\frac{1}{5} + \frac{1}{8}\right]$

$$\text{Standard deviation} = s\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}$$

$$= 0.574208545\sqrt{\frac{1}{5} + \frac{1}{8}}$$

$$= 0.574208545\sqrt{0.325}$$

$$= 0.574208545(0.570087712)$$

$$= 0.327349235$$

$$\text{Thus } t = \frac{\text{Difference}}{\text{Standard deviation}}$$

$$= \frac{1.9130231}{0.327349235} \quad \text{with 1, 11 df}$$

$$= 5.843982181$$

$$= 5.844***$$

***P<0.001

(ii) MILK YIELD (kg/day)

METHODS USED: (a) ANALYSIS OF VARIANCE (ANOVA)

PROCEDURE: CODING OF THE FOLLOWING DATA WAS DONE BY ADDING 1.0 TO EACH FIGURE OF THE MEAN DIFFERENCE.

	Untreated mean difference	Treated mean difference	
	1.71	1.45	
	2.15	1.8	
	1.51	0.1	
	1.53	1.81	
	2.1	1.99	
		2.02	
		0.61	
		2.65	
Total	9.0	12.43	GT = 21.43

RSS = 40.6413

CT = 35.32653076

TSS = 5.31476924

$$\text{Between Groups SS} = \frac{(9)^2}{5} + \frac{(12.43)^2}{8} - CT$$

$$= 16.2 + 19.3131125 - CT$$

$$= 35.5131125 - 35.32653076$$

$$= 0.18658174$$

ANOVA

Source of variation	df	ss	ms	F
Total	12	5.31476924		
Between Group means	1	0.18658174	0.18658174	0.4002 NS
Within Group means	11	5.1281875	0.466198863	

(b) USING STUDENT'S t-TEST

$$\text{Variance of difference} = \frac{s^2}{n_1} + \frac{s^2}{n_2}$$

$$= s^2 \left[\frac{1}{n_1} + \frac{1}{n_2}\right]$$

But from the above analysis, $s^2 = 0.466198863$

Hence, variance of difference $= 0.466198863 \left[\frac{1}{5} + \frac{1}{8}\right]$

$$\text{Standard deviation} = s\sqrt{\frac{1}{5} + \frac{1}{8}}$$

$$= 0.682787567\sqrt{0.325}$$

$$= 0.682787567(0.570087712)$$

$$= 0.38924880$$

$$\text{Thus } t = \frac{\text{Difference}}{\text{Standard deviation}}$$

$$= \frac{0.18658174}{0.3892488}$$

$$= 0.479337994$$

$$= 0.479 \text{ NS}$$

(iii) FAT CONCENTRATION (% Fat)

METHODS USED: (a) ANALYSIS OF VARIANCE (ANOVA)

PROCEDURE: THE FOLLOWING DATA WAS CODED BY ADDING 1.0 TO EACH FIGURE OF THE MEAN DIFFERENCE.

	Untreated mean difference	Treated mean difference
	0.98	0.27
	0.28	0.17
	0.52	0.36
	0.30	0.8
	1.0	0.91
		0.09
		1.02
		0.96
Total	3.08	4.58

RSS = 6.0688

CT = 4.513507692

TSS = 1.555292308

$$\text{Between Groups SS} = \frac{(3.08)^2}{5} + \frac{(4.58)^2}{8} - CT$$

$$= 4.51933 - 4.513507692$$

$$= 0.005822308$$

ANOVA

Source of variation	df	ss	ms	F
Total	12	1.555292308		
Between Group means	1	0.005822308	0.005822308	0.041 NS
Within Group means	11	1.54947	0.140860909	

(b) USING STUDENT'S t-TEST

$$\text{Variance of difference} = \frac{s^2}{n_1} + \frac{s^2}{n_2}$$

$$= s^2 \left[\frac{1}{n_1} + \frac{1}{n_2}\right]$$

But from the above analysis, $s^2 = 0.140860909$

Hence, variance of difference $= 0.140860909 \left[\frac{1}{5} + \frac{1}{8}\right]$

$$\text{Standard deviation} = s\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}$$

$$= 0.375314413\sqrt{\frac{1}{5} + \frac{1}{8}}$$

$$= 0.375314413\sqrt{0.325}$$

$$= 0.375314413(0.570087712)$$

$$= 0.213962134$$

$$\text{Thus } t = \frac{\text{Difference}}{\text{Standard deviation}}$$

$$= \frac{0.00582}{0.21396}$$

$$= 0.027201346$$

$$= 0.030 \text{ NS}$$

(iv) PROTEIN CONCENTRATION (% Protein)

METHODS USED: (a) ANALYSIS OF VARIANCE (ANOVA)

PROCEDURE: CODING OF THIS DATA WAS DONE BY ADDING 0.2 TO EACH FIGURE OF THE MEAN DIFFERENCE.

	Untreated mean difference	Treated mean difference	
	0.38	0.34	
	0.05	0.39	
	0.34	1.41	
	0.33	0.19	
	0.45	0.29	
		0.42	
		0.44	
		0.41	
Total	1.55	3.89	GT = 5.44

RSS = 3.488

CT = 2.276430769

TSS = 1.211569231

$$\text{Between Groups SS} = \frac{(1.55)^2}{5} + \frac{(3.89)^2}{8} - CT$$

$$= 2.3720125 - 2.276430769$$

$$= 0.095581731$$

ANOVA

Source of variation	df	ss	ms	F
Total	12	1.211569231		
Between Group means	1	0.095581731	0.095581731	0.942 NS
Within Group means	11	1.1159875	0.101453409	

(b) USING STUDENT'S t-TEST

$$\text{Variance of difference} = \frac{s^2}{n_1} + \frac{s^2}{n_2}$$

$$= s^2 \left[\frac{1}{n_1} + \frac{1}{n_2}\right]$$

But from the above analysis, $s^2 = 0.101453409$

Hence, variance of difference $= 0.101453409 \left[\frac{1}{5} + \frac{1}{8}\right]$

$$\text{Standard deviation} = s\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}$$

$$= 0.318517517\sqrt{\frac{1}{5} + \frac{1}{8}}$$

$$= 0.318517517\sqrt{0.325}$$

$$= 0.318517517(0.570087712)$$

$$= 0.181582922$$

$$\text{Thus } t = \frac{\text{Difference}}{\text{Standard deviation}}$$

$$= \frac{0.0956}{0.1816}$$

$$= 0.526431718$$

$$= 0.526 \text{ NS}$$

APPENDIX 5:0 COMPOSITE* MILK S.C.C. OF EACH COW TOGETHER WITH HER S.C.C. FOR INDIVIDUAL** QUARTERS.

Cow No.	S.C.C. in composite cow's milk (thousand/ml)	S.C.C. in individual quarters (thousand/ml)			
		LF	RF	LR	RR
18	3011	690.7	417.7	991.0	383.0
22	517	30.7	150.0	120.7	590.7
28	309	46.3	550.0	32.7	933.3
36	557	207.0	35.3	53.3	49.3
43	1984	116.3	270.7	72.0	17835.3
46	388	775.7	444.0	210.0	258.3
72	299	61.7	775.0	67.0	147.3
81	470	179.7	52.0	49.3	1142.7
112	792	28-0	26.3	18.3	5387.0
114	6372	6537.7	1732.0	2371.0	2429.3
134	764	110.7	153.3	7287.3	230.3
144	714	28.7	2257.0	687.3	1320.7

* Individual cow S.C.C. values used for the final selection of the 12 cows.

** Average of 3 measurements of S.C.C. for each quarter taken during the preliminary period.

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