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**ONCE DAILY MILKING IN LATE LACTATION :
EFFECTS ON SOMATIC CELL COUNTS, MILK
YIELD AND COMPOSITION OF DAIRY COWS WITH
HIGH OR LOW SOMATIC CELLS COUNTS**

**A THESIS PRESENTED IN PARTIAL
FULFILMENT OF THE REQUIREMENTS FOR THE
DEGREE OF MASTER OF AGRICULTURAL
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DEDICATED TO MY MOTHER

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CHAPTER 1

CHAPTER ONE

1.1 INTRODUCTION

Somatic cell count (SCC) expresses the estimated total number of 'somatic' (body) cells present in one ml of milk. Mastitis infections are characterised by an elevation in the number of somatic cells in milk, which can be used as an indirect measure of infection (Nickerson and Heald, 1982; Holdaway, 1990). SCC is widely used for monitoring udder health in dairy herds. Also, in many countries, it is used as one criterion for milk payment to producers. Thus the degree of association between SCC and prevalence of mastitis is an important parameter.

Mastitis is inflammation of the udder mainly associated with bacterial infection (Dodd, 1971). On the basis of severity of inflammation of the mammary gland, two broad categories of mastitis are recognised: clinical mastitis where physical examination of the udder or the milk reveal abnormalities, and subclinical mastitis where mammary gland inflammation exists in the absence of visible signs. The subclinical form is detected by tests such as SCC applied to the milk to detect the effects of inflammation.

Research on SCC response to infection has resulted in the recommendation by the International Dairy Federation (IDF) of 500,000 cells/ml as a threshold value for mastitis diagnosis (Tolle, 1975). However, several workers report inaccuracies in diagnosis with the IDF definition, because infections are often associated with SCC below the threshold (Berning and Shook, 1992). Elevation of SCC can also occur in response to other factors related to the cow or management (Ward and Schulz, 1972). Moreover, threshold values for SCC tend to vary depending on whether there has been a low or high incidence of udder infection in the herd (Holdaway, 1990). Nevertheless, it is certain that intramammary infection will increase SCC. For this reason SCC is a useful parameter for the detection of subclinical mastitis.

Indirect methods which determine the SCC of milk samples such as the California Mastitis Test (CMT) and Wisconsin Mastitis Test (WMT) have been available for some time, as has the direct microscopic SCC procedure (Schalm *et al.*, 1971). More recently, automated devices for rapid determination of SCC in milk samples have become available. The two most commonly used are the Coulter Milk Cell Counter which counts particles as they flow through an electric field, and the Fossomatic, which stains cells with fluorescent dye and then counts the number of fluorescing particles. Both devices are capable of rapid, inexpensive determination of SCC in large numbers of samples (Heeschen, 1975). The ability to correctly interpret these SCC data, however, depends on an understanding of various factors which may affect them, plus the interaction between their effects.

1.2 ORGANISATION OF THE THESIS

This thesis consists of five chapters. Chapter One is an introductory chapter presenting highlights of the degree of association between SCC and udder infection.

Chapter Two contains a detailed review of literature relating to the importance of SCC and infection to the dairy industry; SCC interpretation; the origin of somatic cells, mechanism of their movement into the milk, and possible factors affecting their concentration in milk; effects of milking frequency on yield and composition of milk; and physiological explanations for milking frequency response. This chapter ends by presenting the objectives of the present study.

Experimental procedures and results are given in Chapters Three and Four respectively, discussion of the results, a general conclusion and suggestions for further investigation are presented in Chapter Five.

CHAPTER 2

REVIEW OF THE LITERATURE

CHAPTER TWO

REVIEW OF THE LITERATURE

To gain an understanding of the occurrence of somatic cells in normal and mastitic milk and related changes in milk following infection or functional disturbance of the udder, some knowledge of where and how milk is produced is necessary. This chapter will therefore start by reviewing the structure of the mammary gland, a gland which not only produces a secretion (milk) whose composition is markedly different from that of blood plasma or intracellular fluid, but also maintains the difference in composition during storage in its lumen until the milk is removed by milking or suckling.

2.1 THE MAMMARY GLAND

Milk is produced by the mammary gland, the structure of which has been described by several authors (Schmidt, 1971; Cowie, 1977; Anderson, 1978). Mammary glands are skin glands held exterior to the body cavity. A cow carries four mammary glands or quarters which are formed into the udder. Each of the four quarters is drained by its own teat and is made up of a variety of structures and tissue.

The basic components of secretory tissue are the alveoli, small saccular evaginations from alveolar ducts which are composed of a single layer of epithelial cells resting on a basement membrane. The cells are joined together by desmosomes but near the cell apices there are specialised regions of their lateral walls, the "tight junctions", which do not allow molecules to pass between the cells (Linzell, 1975). Surrounding the alveoli and lying between them and basal membrane are highly specialised cells, the myoepithelial cells. When activated by oxytocin, these cells cause milk ejection from the lumina of the alveoli (Grosvenor and Mena, 1974). Each alveolus is surrounded by capillaries that supply blood containing milk precursors to epithelial

cells for the synthesis of milk. Milk from the epithelial cells is secreted into the alveolar lumen and is drained away by a small duct. These ducts join to form several inter-lobular ducts which carry milk to the exterior of the lobule, a cluster of alveoli.

The constituents of milk are produced in two ways. One group of compounds which include milkfat, most of the protein components and the lactose, is synthesised in the epithelial cells from the precursors absorbed from the blood and then secreted into the lumen of the alveolus. Other milk constituents such as minerals, vitamins, serum albumin and immunoglobulins pass from the blood and move across epithelial cells (transcellular route) or between them (paracellular route) into the alveolar lumen without being altered by the epithelial cells.

A model for movement of small molecules and ions across the mammary epithelial cells has been given by Peaker (1978). Milk is always in osmotic equilibrium with the blood flowing through the udder, lactose being the major osmotically active component of the milk. Potassium, sodium and chloride are the major ions in milk, although other ions, for example citrate, phosphate and calcium, contribute to the total ionic concentration. The higher the lactose concentration in milk the lower the concentration of ions with sodium:potassium maintained at approximately 1:3. The maximum possible lactose concentration is about 300 mM or isotonic with body fluids and the maximum cationic or anionic strength is approximately 150 mM. As lactose is synthesised in the lumen of the Golgi apparatus, water diffuses into the cell to keep the contents isotonic with the cytoplasm, and as fluid accumulates in the Golgi vesicle, ions diffuse down their concentration gradient. Further movement of water will be encouraged as the osmotic pressure is increased by the diffusion of the ions into the vesicle. For sodium and potassium movement, the essential features of the scheme are that the intercellular concentration of potassium is kept at a high level and sodium at a low level by a typical sodium pump on the basolateral cell membrane, while these ions are freely distributed between intracellular and milk according to the electrical potential difference across the apical membrane (Peaker, 1978). This size of the potential difference determines the concentration of sodium and potassium ions in the milk and, indirectly, the concentration of lactose.

2.2 SIGNIFICANCE OF SCC

2.2.1 SCC in relation to milk yield

Although SCC has been used to differentiate between infected and non-infected cows, the relationship between SCC and level of milk production is also important to dairy men. Numerous studies have attempted to measure the relationship between milk yield and SCC or infection (Janzen, 1970; Dahoo and Meek, 1982; Hoare, 1982). From these studies, decreases in milk yields by cows with high SCC have been established, although at varying levels depending on severity of infection and the type of pathogen. An increase in bulk milk SCC of 100,000 cells per ml was associated with a decrease of between 0.24 and 0.30 litres per cow per day between herds (Gill and Holmes, 1978). Herds with higher SCC were reported to produce lower milk yield per cow. Daniel and Fielden (1971) found a decrease of 0.065 kg of milk per cow per day in association with an increase in 1 mm in WMT, while an increase in SCC was related to a decrease in production index, particularly in older cows (MacMillan *et al.*, 1980). The role of compensatory production by unaffected quarters in offsetting the milk yield loss due to infection has been the subject of few studies but was dismissed for lack of convincing evidence (Morris, 1973; Hoare, 1982). Nevertheless, convincing evidence from identical twin studies (Woolford *et al.*, 1984; Woolford, 1985) suggest that compensation does take place at least in older animals. Of importance, however, is the demonstration by these studies of the consistently lower production in individual quarters with higher SCC.

Some researchers have studied the possibility of carry-over effects of high SCC on milk production from one lactation to the next. Raubertas and Shook (1982) reported that a unit increase in total lactation average log SCC resulted in small but non significant milk loss in the following lactation. In another study, the carry-over effect of mastitis and high SCC from one lactation to the next was small but significant, amounting to less than half of the effects of high SCC in the current lactation. On the other hand, Woolford *et al.* (1984) reported that intramammary infection occurring in first lactation heifers resulted in significant yield losses (milk and fat per

cow) both in the first and the following lactation, even where the infection was cured over the intervening dry period. In this study, however, quarters cured of infection in mature cows recovered their productive capacity in the following lactation. The carry-over effect of mastitis and high SCC from one lactation to the next therefore needs further investigation.

2.2.2 Relationship between SCC and milk composition

In general, the yield of milk, fat, protein and lactose are all decreased by mastitis, in association with increased SCC. The relationship between mastitis or SCC and milk composition has been reviewed on several occasions (Kitchen, 1981; Hoare, 1982; Munro *et al.*, 1984). Milk fat percentage generally falls as a result of udder infection associated with high SCC, but only by a small amount. Work carried out by King (1978) indicated reductions in the number of fat globules and their diameter at higher SCC. Changes in the composition of fat in the secretion have also been observed, for example, phospholipid concentrations are decreased while lipase and free fatty acid concentrations are increased with increasing SCC (Randolf and Erwin, 1974; Erwin and Randolph, 1975; Salih and Anderson, 1979). Reduction in the phospholipid concentration of the fat globule membrane makes the globule more susceptible to the action of lipase thereby increasing free fatty acids and hence the acid degree values in milk (Hoare, 1982). The increased lipase concentration may be related to changes in the concentration of milk lipase secreted or to the increased leukocytes, which apparently have lipase activity (Schultz, 1977).

The yield of proteins generally decreases because of the decrease in milk yield. However, the concentration of total milk protein does not show large changes with increasing SCC or level of infection in the gland. When each of the individual proteins is examined, some protein components decrease while others increase (Haenlein *et al.*, 1973; Randolph *et al.*, 1974). Those components synthesised in the mammary gland (α -casein, β -casein, α -lactalbumin and β -lactoglobulin) decrease, while those derived directly from the blood (mainly immunoglobulins - IgGs and serum albumin) increase. There is an approximate balance between these changes,

resulting in the total protein concentration remaining at about the same level (Schultz, 1977).

Lactose yield generally decreases due to a decrease in milk yield, and to a decrease in lactose percentage. Lactose biosynthesis is reduced at high SCC as synthetic ability of the enzyme system in damaged secretory cells is decreased. Since α -lactalbumin, a component of an enzyme involved in the final step of lactose synthesis, is also reduced at high SCC, this could be a partial explanation for decreased lactose synthesis (Schultz, 1977).

The changes which occur in the concentrations of ions at high SCC reflect the changes in the permeability of the udder as well as the impaired activity of the epithelial cells. Samples from infected quarters with high SCC contain substantially higher concentrations of sodium, chloride, copper, iron, zinc, magnesium and phosphorus than samples from healthy quarters (Kitchen, 1981). Of these ions, the increase in sodium and chloride are substantial (Munro *et al.*, 1984). In addition, the concentrations of enzymes increases at high SCC, including catalase, lactase dehydrogenase, GOT, alkaline phosphatase, arylasterase, NAGase, beta-glucoronidase, and arylsulphatase (Hoare, 1982). The enzymes can come from serum, mammary epithelium or leukocytes (Kitchen, 1981).

2.2.3 SCC and processing properties of milk

The compositional changes in milk that are observed at high SCC can affect the processing properties and influence the product quality. The flavour quality of pasteurised milk decreases with increasing SCC. Shelf life of pasteurised products is reduced by high SCC raw milk (Janzen, 1972). The combination of lower total solids and higher chlorides which are associated with the increase in SCC can affect the desirable characteristics of milk (Janzen and Northern, 1972). Off flavours due to lipolysis were detected at a SCC concentration of 500,000 cells/ml (Gudding, 1982).

Signs of oxidation in butter made from milk with high SCC were observed after two months of storage (Brus *et al.*, 1966). In a more recent study whipping time and cream stiffness increased as the proportion of high cell count in milk increased (Need *et al.*, 1988). In the process of cheese making, fat and casein are the major milk solids incorporated into the final product. Milk with high SCC shows many effects which are important for cheese manufacturing, including rennet clotting time, curd firmness, whey expulsion and rate of acid development (Munro *et al.*, 1984; Gilles and Lawrence, 1985; Marziali and Ng-Kwai Hang, 1986). The amount of milk components lost in the whey indicates the efficiency with which milk solids are converted into cheese. Elevated SCC were associated with significantly higher protein and fat losses in whey (Ali *et al.*, 1980; Barbano and Sherbon, 1984). Other studies reported production of cheese with high moisture content from milk with elevated SCC (Munro *et al.*, 1984; Mitchel *et al.*, 1986). Barbano *et al.* (1991) conducted an experiment to determine the quantitative relationship between milk SCC and Cheddar cheese yield, cheese yield losses and cheese composition over the range from < 100,000 to approximately 1,300,000 cell/ml of milk. They concluded that any increase in milk SCC above 100,000 cells/ml has a negative impact on cheese yield.

In conclusion, udder infection, as indicated by an elevation in SCC, reduces the yield of milk and its constituents and also affects milk composition. These changes imposes heavy costs on the dairy farmers and dairy industry as a whole. The losses accrue from reduced milk secretion, degrading of milk and milk products due to poor quality and costs related to control measures of mastitis. SCC is therefore important in detecting infection and in mastitis control programmes, and is one of the useful measure of quality of milk and dairy products.

2.3 ORIGIN AND OCCURRENCE OF SOMATIC CELLS IN MILK

The predominant cell type in milk secreted by non-infected mammary glands is the macrophage (Lee *et al.*, 1980). There are very few mammary epithelial cells present

in milk and lymphocytes constitute about 1-2% of the total cell population (Concha *et al.*, 1978). This finding, based on electron microscopy and supported by assessment of cell function *in vitro* (Craven and Williams, 1985), is in contrast to many earlier reports where macrophages had been incorrectly identified to be epithelial in origin. Epithelial cells are present in normal milk as a result of normal breakdown and repair of the mammary gland (Schultz, 1977). The local population of leukocytes including macrophages, neutrophils and lymphocytes serves as an important defence mechanism. All these cell types make up the total somatic cell count.

Macrophages and neutrophils play a role in the recognition, ingestion (phagocytosis) and digestion of foreign particles, such as bacteria (Paape *et al.*, 1979). Lymphocytes regulate the induction and suppression of the immune response by becoming sensitised via exposure to specific antigens, maintaining this sensitised state and eliciting a response to subsequent antigen exposure (Nickerson, 1989). During infection, the SCC increases due to migration of polymorphonuclear neutrophils (PMN) from the blood to the milk (Craven and Williams, 1985). The protective role of these phagocytes in the lactating udder was clearly demonstrated in the experiments of Jain *et al.* (1971) and Schalm *et al.* (1976) where unrestricted bacterial growth occurred in the udders of cows which had been rendered neutropaenic by injection of anti-bovine leukocyte serum. The cow has the potential to mobilise tremendous numbers of PMN into the mammary gland from three sources: a marginal pool located alongside the alveoli, a circulating pool of mature PMN, and in severe inflammation from a storage pool of mature and immature PMN in the bone marrow (Paape *et al.*, 1979).

The subject of inflammatory cellular response has been extensively reviewed (O'Flaherty, 1983; Dale and Foreman, 1984; Wilkinson, 1984). The basic mechanisms of initiation of PMN migration in bovine mammary gland are poorly understood, and the sequence of events by which bacteria activate an inflammatory response is extremely complex (Mattila, 1985). In general, the provoking agent, such as bacteria or toxin, causes the generation of one or more pro-inflammatory mediators

which affect the local micro-circulation leading to increased perfusion, opening of endothelial intercellular junctions and a consequent exudation of blood component into the milk. The mediators may also activate PMN within the blood and these activated PMN move towards increasing concentrations of the proinflammatory mediators, and consequently the provoking agent. The PMN may themselves release further phlogistic agents which amplify the process (Craven and Williams, 1985). Histological studies of bovine mammary tissue have confirmed that PMN migrate in response to a variety of stimuli following the above pattern (Harmon and Heald, 1982; Frost *et al.*, 1984; Nickerson and Pankey, 1984).

The consequent increased permeability of microvasculature vessels and opening up of the tight junctions also results in leakage of blood proteins into the milk. Ionic changes are observed, sodium and chloride increase, potassium decrease, pH and electric conductivity increases (Holdaway, 1990). These reflect changes in permeability of the glandular epithelium and in activity of mechanisms which maintain the concentration of the ions.

2.4 INTERPRETATION OF SCC

SCC can be measured in samples which come from a quarter, the whole composite udder or cow, or from bulk milk, and the interpretation differs depending on the origin of the sample.

2.4.1 Quarter sample

Anatomically, the quarter is a secreting unit since its secretions are collected by a duct system with only one external connection, the teat duct. Secretory disturbances or infection in a quarter appear in the cell count range of 100,000 to 150,000 cells/ml (Reichmuth, 1975; Holdaway, 1990). In practice, foremilk samples are usually used instead of a representative sample from the whole quarter, because they are more convenient to collect. This imposes restrictions on the interpretation of cell count

since SCC in foremilk is higher than in a whole quarter sample, but is justified by the fact that the correlation between total and foremilk counts for quarter milk samples is 0.86 ($P < 0.01$) (Reichmuth, 1975).

2.4.2 Composite udder or cow samples

These represent pooled samples from the four independent quarters of a cow's udder. It is unlikely that all the four quarters in a normal or mastitic cow will show identical values for SCC. Generally, mastitic quarters show higher values and therefore dilution from uninfected quarters should be considered (Holdaway, 1990). From an individual cow SCC, the best discrimination between cows either infected or not infected by a major pathogen was obtained at a threshold between 200,000 and 300,000 cells/ml (Series, 1985; Holdaway, 1990). Individual cow diagnosis appear to be mainly of value in detection of cows which are reservoirs of infection. It is then possible to apply specific recommendations to prevent contagion by various management methods (for example, regulating the milking order so that cows with high SCC are milked last, use of dry cow therapy and culling of perpetually infected cows).

2.4.3 Bulk, herd, milk samples

The bulk milk of the herd is the economic output delivered to the dairy plant. SCC of bulk milk samples have been widely adopted as a convenient measure for monitoring the incidence of mastitis, and progress of mastitis control measures in a herd. Although the relationship between bulk milk cell count and the percentage quarters infected is well established, the estimates for the correlation coefficient vary considerably, ranging from 0.46 to 0.96 (Emanuelson and Funke, 1991). While SCC of less than 250,000 cells/ml indicates a low level of subclinical mastitis in herd bulk milk, the same SCC for an individual cow might indicate a healthy udder (Duirs, 1980).

Setting thresholds is a simplification necessary in order to facilitate analysis and interpretation of results. However, non bacterial factors discussed in section 2.5 may interfere with the diagnostic value of SCC. When factors such as age of the cows, stage of lactation and infection status of the herd are taken into account the interpretation of the SCC is improved (Brolund, 1985). Thus threshold values should be lower for young cows than older cows and for cows in mid-lactation than in late lactation, and higher for herds with higher incidences of infection (Holdaway, 1990).

2.5 FACTORS AFFECTING NUMBER OF SOMATIC CELLS IN MILK

2.5.1 Intramammary infection

The role of intramammary infection in affecting the SCC of the milk from individual quarters and consequently from the cow and the herd has been discussed elsewhere in this thesis. Bacterial infection increases SCC of the cow's milk consistently and significantly (Holdaway, 1990). SCC in foremilk from healthy bovine quarters is commonly less than 100,000 per ml whereas SCC in composite samples taken from cows with all quarters free of infection have been reported to average from 113,000 to 251,000 cells/ml (Dahoo and Meek, 1982). In his review, Holmes (1981) concluded that all cows with SCC which are consistently higher than 200,000 to 300,000/ml but without clinical signs, have subclinical mastitis. Series (1985) reported that 93% of individual cow SCC were below 300,000 cells per ml in the absence of infection. Holdaway (1990) reported a range of critical thresholds for individual herds of 73,000 to 344,000 cells/ml in an effort to distinguish between infected and uninfected cows.

The presence of pathogens causes the greatest cell response but there is variation in response to different pathogens, and previous mastitis infections can cause cell counts to be maintained at high levels (Holdaway, 1990).

2.5.2 Stage of lactation

The SCC is generally high during the first week of lactation, it decreases in mid lactation and rises again at the end (Schalm *et al.*, 1971). The increases in SCC with advancing lactation may be due to an increase in new infection rate through lactation (Reichmuth, 1975; Schultz, 1977). However, in studies where observations were restricted to uninfected cows, an increase in SCC as lactation advances were also recorded (Sheldrake *et al.*, 1983; Brolund, 1985; Emanuelson *et al.*, 1988). Since involution is associated with an increase in SCC (Lee *et al.*, 1969), this has also been considered as a factor which might contribute to the increase in SCC with advancing lactation (Holdaway, 1990). The rise in SCC in the absence of infection might also be an effect of increased concentration as milk yield decreases (Jaartsveld *et al.*, 1983; Ng-Kwai Hang *et al.*, 1984).

2.5.3 Age or lactational number

Some researchers have reported an increase in SCC with increasing lactation age (Weihe, 1969; Systad and Roy, 1970; Kennedy *et al.*, 1982; Ng-Kwai Hang *et al.*, 1984). This is probably a reflection of the past infection history of the udder since in some studies (Natzke *et al.*, 1972; Wanasinghe and Frost, 1979) uninfected animals showed no significant increase in SCC with age. Similarly, Sheldrake (1982) reported that the effect of stage of lactation and number of lactations upon SCC in quarters free from infection were small when compared to elevation in SCC caused by infection. It has also been found that older cows have a greater cellular response to both minor and major pathogens (Marshall and Edmondson, 1962; Eberhart *et al.*, 1979). It appears therefore that an increase in SCC with age is attributed to a number of factors including more quarters being infected, more extensive tissue damage in long standing infections and a greater cellular response in quarters that have been previously infected (Ward and Schultz, 1972).

2.5.4 Diurnal variation

Diurnal fluctuation of SCC has been reported by many authors (eg Cullen, 1967; Dijkman, 1975). Considerable variations are seen between morning and evening samples, especially with irregular milking intervals and also among morning and evening samples collected on consecutive days (Duitschaever and Ashton, 1972). Comparison of SCC in foremilk samples have generally shown higher counts after shorter intervals (Schalm *et al.*, 1971). Moreover, total SCC in foremilk are usually lower than those in stripping milk, while SCC in the middle milk is the lowest (Smith and Schultze, 1967). Schalm *et al.* (1971) were of the opinion that this variation in cell numbers can be best explained on the basis of total volume of milk secreted leading to greater dilution of somatic cells during longer milking interval. Hoare (1977), cited by Gill (1977), showed that the number of cells shed per milking tend to be constant and the higher milk production generally obtained at the morning milking results in lower cell concentration.

Day to day variation in SCC has also been investigated in samples from quarters, cows and herds (Cullen, 1967; Duitschaever and Ashton, 1972; Clarkson, 1975; Gill and Holmes, 1978). Fluctuation in individual quarter samples suggest physiological factors acting at the cow level (Cullen, 1967). In other situations where composite samples were taken repeatedly over the whole lactation, large periodic increases in SCC were detected in the absence of mammary pathogens (Duitschaever and Ashton, 1972). These increases in SCC were suggested to have been due to the infections which were eliminated before they were isolated, or to stress or trauma. For individual herds, the coefficient of variation in monthly bulk tank SCC has been reported to range from 4 and 46% (Gill and Holmes, 1978). These results suggest that it is advantageous to sample cows or quarters several times throughout a lactation. In this connection, sampling at least five times during a single lactation has been recommended (Clarkson, 1975).

2.5.5 Stress related factors

The effect of naturally occurring stress factors and artificially induced stress caused by injecting corticosteroids (CTS) have been measured but the results are inconsistent. Stressful situations such as isolation from the herd, chasing by a dog and sudden thunderstorms can result in an increase in SCC (Whittlestone *et al.*, 1970). Similarly, movement of cows between groups increased SCC from 175,000 to a peak of 420,000 cells/ml four days after mixing (Kay *et al.*, 1977). However, these studies did not measure the infection status of the cow. Injection of CTS caused a rise in leukocytes in the blood but not in milk (Paape *et al.*, 1971, 1972). In another study, there were no changes in SCC of cows under thermal stress (Bandaranyake, 1971). Contrary to these findings, Wegner *et al.* (1976) recorded an increase in SCC of milk in mastitis free cows in response to a heat stress, and in cows injected with corticotropin. The effect of stress on SCC therefore remains controversial. However, it appears that udders which are already inflamed can show an exacerbation of the inflammatory response under stress condition (Whittlestone *et al.*, 1970).

2.5.6 Management factors

Management factors influencing the incidence of mastitis and hence SCC have been widely studied. Many factors are involved in influencing SCC either through an infection rate or other conditions of stress, and thus make it difficult to establish the specific management factors which can affect the cell count (Schultz, 1977). It appears, however, that regular teat disinfection has consistently been associated with lower SCC (Bodoh *et al.*, 1976; Hayward and Webster, 1977; Mein *et al.*, 1977; Goodhope and Meek, 1980). Dry cow therapy has reduced cell levels at the start of subsequent lactation but teat disinfection was required to maintain the advantage (Hayward and Webster, 1977). Trauma, due to mechanical injury will elevate SCC (Dahoo and Meek, 1982), but this effect is less common in grazing cows.

2.5.7 Milking frequency

It is believed that increasing milking frequency has beneficial effects on the incidence of mastitis (Jarret, 1977) and some experimental results support this view. Pearson *et al.* (1979) reported a reduction in Wisconsin Mastitis Test (WMT) scores and a reduction in the amount of discarded milk when cows were milked three times per day as opposed to cows milked twice per day. In another study, there was no significant difference in the incidence of infection although cows in the thrice daily milking group exhibited a smaller number of new infections (Waterman *et al.*, 1983).

The effects of reducing milking frequency on udder health and SCC has also been studied. Maroske *et al.* (1984) observed an increase in WMT scores, in cows which missed one milking per week. Similarly, once daily milking consistently increased SCC in a short term trial and a full lactation study (Mackenzie *et al.*, 1990; Holmes *et al.*, 1992). Clinical mastitis was low in both studies and incidence of infection measured bacteriologically in the later study revealed no difference between the once and twice daily milked groups. This suggests that the increases in SCC per ml of milk observed were due to physical and/or physiological changes in the secretory tissue brought about by milking once a day (Holmes *et al.*, 1992). These studies, however, did not report the total number of somatic cells in milk secreted over 24 hours. It is unclear, therefore, whether infiltration of somatic cells into the milk was increased by extended milking intervals or if the increase in SCC per ml of milk observed is due to decreased milk yield per day.

Several factors affecting SCC have been identified in the preceding discussion. Nevertheless, substantial experimental evidence demonstrates a consistently higher SCC as milk yield decreases regardless of the cause (Emanuelson and Funke, 1991; Miller *et al.*, 1993). This, together with changes in milk composition, reflects either true biological effects of udder inflammation or associated effects due to changes in somatic cell infiltration into the milk, or both. SCC should therefore be considered as a measure of the functional status of secretory tissue. However, for secretory

activity, intramammary infection is probably the most important factor causing functional disturbance in the secretory tissue and affect SCC to a higher degree.

In the following section, the effects of milking frequency on factors other than SCC will be discussed.

2.6 EFFECT OF MILKING FREQUENCY ON YIELD AND COMPOSITION OF MILK

Effects of milking frequency on performance have been studied by a variety of techniques, including half udder studies, use of monozygous twins, and use of cows and heifers, both for short period and full lactation trials.

2.6.1 Three times milking

Milking three times daily is widely practiced in North America, Israel and Western Europe, particularly where herds are large, milk yield per cow is high and modern milking facilities and equipment are used on the dairy farm. Half udder studies have reported increases of 11 to 32% in milk yield for thrice daily milked cows compared with those milked twice daily (Ludwick *et al.*, 1941; Cash and Yapp, 1950; Morag, 1973).

Analyses of field data with cows paired on the basis of parity, season of calving and milk and fat production in their previous lactation have shown an 18% increase in milk yield for three times daily milking (Pelissier *et al.*, 1978). A similar increase was observed by Lush and Shrode (1950). Elliot (1959) reported a 20% increase of milk yield from three times compared with twice daily milking during a portion of lactation. Short trials with cows that were switched from twice to thrice daily milking during that lactation resulted in a 3 to 10% improvement of milk yield. British work evaluating three times milking during the first 20 weeks of lactation, showed increases in milk yield of 19 and 13% for older cows and first lactation

heifers, respectively (Poole, 1982). Cows milked three times daily during the first part of lactation were reported to have shown a 20% increase in milk yield (Pearson *et al.*, 1979).

Complete lactation studies have reported increases in milk yield of 15 and 6% and 19 and 25% for older cows and heifers, respectively, with thrice daily milking (DePeters *et al.*, 1985; Amos *et al.*, 1984). Larger differences in production with advancing stage of lactation were also recorded (Cash and Yapp, 1950; Pellisier *et al.*, 1978; Pearson *et al.*, 1979). In most of these studies, there were increases in the yield of milk constituents such as fat, protein and lactose. These increases in milk constituents were related to increases in milk output since milk composition was not significantly affected by milking three times a day (Henderson *et al.*, 1983; DePeters *et al.*, 1985; Amos *et al.*, 1984; Gisi *et al.*, 1986; Van der Iest and Hillerton, 1989). Moreover, the response of milk yield to three times milking was rapid, occurring within hours and was maintained as long as more frequent milking was applied (Pearson *et al.*, 1979). However, variations in percentage increase in milk yield from different studies and the suggestion that cows of higher genetic merit are less responsive to three times daily milking than cow of low genetic merit (Barnes *et al.*, 1990), makes the prediction of exact increases as a result of three times daily milking impossible.

A recent economic evaluation of three times milking in USA showed that with current milk and feed prices, the regime would be profitable only if the increased cost of labour was very low compared to milk yield (Culotta and Schmidt, 1988). Consequently, adoption of three times daily milking in countries like New Zealand seems to be unlikely on economic grounds since dairy production is based on grazing pastures throughout the year with relatively low per cow milk production, minimal mechanisation, and relatively low prices received for milk (approximately 0.3 to 0.4 times the price in USA and Western Europe).

2.6.2 Twice daily milking

Even though thrice daily milking results in greater production, farmers in New Zealand milk their cows daily for most parts of lactation. For practical reasons, the milking intervals with twice daily milking are usually unequal, with a long interval night and a short interval during the day. Until recently, it was believed that cows would produce more milk when milked at 12-12 hour intervals than when milked at unequal intervals (McMeekan and Brumby, 1956). However, milk secretion experiments with mature cows over the whole lactation have shown that milking intervals less than 16 hours do not significantly affect milk yield and its constituents (Schmidt, 1971; Dodd and Griffin, 1977), but this may not be true for very high yielders.

2.6.3 Once daily milking

Limited experiments have considered the effect of reducing the frequency of milking from twice to once per day. In a Swedish experiment (Claesson *et al.*, 1959) which used identical twins, once daily milking for the whole lactation gave 50% less milk in first lactation heifers and 40% less in second lactation cows. The percentage reduction, where the loss was expressed as a percentage of the yield of the cows milked twice daily, was greater earlier in lactation than late in lactation. In this study the average composition of the milk was influenced by the once daily milking and larger depressions in lactose and increase in concentrations of whey proteins and chloride were observed. In another full lactation study (Holmes *et al.*, 1992), once daily milked cows produced 35, 31, 33 and 37% less milk, fat, protein and lactose, respectively, than twice daily milked control group. Lactose concentrations were significantly reduced for the once daily milked cows.

The reduction of milk and milkfat caused by once daily milking for short periods of lactation is relatively small compared to the effects reported from full lactation studies, ranging between 15-37% (Parker, 1965; Wilson, 1965; Bryant, 1978; Njaritta, 1989; Carruthers and Copeman, 1990; Mackenzie *et al.*, 1990). Daily

production losses were higher in early lactation compared to those observed in late lactation (Carruthers and Copeman, 1990).

Although once daily milking results in production losses and other compositional changes, the responses are quite variable. This variability is probably brought about by the difference in age of the cows, stage of lactation and many other undetermined characteristics of the cows used which can be genetical, environmental or both.

2.7 PHYSIOLOGICAL EXPLANATIONS FOR DIFFERENCES IN THE EFFECTS OF MILKING FREQUENCY

Many investigations have sought to explain the reasons for the responses observed with various milking frequencies and various physiological factors have been identified. It is most likely that several factors may be involved but the contribution of each to the observed response probably varies depending on the interval between milkings and the period for which a certain milking frequency regimen is applied.

2.7.1 Hormonal effect

Prolactin is usually considered to be the pituitary hormone of most importance in the mammary gland differentiation, initiating milk synthesis and in many species (excluding ruminants) maintaining established lactation. In ruminants, growth hormone (GH) is of major importance for maintenance of milk secretion but its mechanism of action is not known (Topper and Freeman, 1980). GH added with prolactin to culture medium of goat mammary explants stimulated casein synthesis (Scarda *et al.*, 1982). Injection of GH into dairy cows stimulates milk production by 10-40% (Hart, 1983; Collier *et al.*, 1984; Davis and Bass, 1984; Bauman and McCutcheon, 1985; Davis *et al.*, 1988). This enhancement of milk yield is associated with preferential partitioning of nutrients for milk synthesis since the proportion of cardiac output delivered to the udder also increased following treatment of the cows with GH (Peel *et al.*, 1981; Davis *et al.*, 1983). Milking has also been

shown to increase the mammary blood flow of cows and this is expected to increase the delivery of galactopoietic hormones to the secretory epithelial cells (Davis and Collier, 1985). However, the evidence that galactopoietic effect of GH is largely due to enhanced nutrient supply to the mammary gland is not convincing (see review by Gluckman *et al.*, 1987). Effects of growth hormone are indirect and may be mediated by the insulin-like growth factors (Gluckman *et al.*, 1987).

Milking stimulates the release of GH in goats, prolactin in cows and goats and also glucocorticoid in cows (Hart and Flux, 1973; Fell *et al.*, 1971; Koprowski and Tucker, 1973). However, these increases in the circulating concentration of galactopoietic hormones at the time of extra milkings does not appear to be primarily responsible for the stimulation of milk synthesis. There was no difference in GH and prolactin concentration between cows milked three times and two times daily (Kazmer *et al.*, 1986).

2.7.2 Intramammary pressure

The changes in intramammary pressure and secretion rates which occur in the udder during milking and between milkings have been measured by Dodd and Griffin (1977). At the end of milking when all available milk has been removed, the pressure within the teat sinus is at atmospheric pressure but within an hour it rises by 1.1-1.5 kPa as milk residues drain and fill the collapsed teat and udder cisterns. Thereafter, pressure increases slowly for 5 or 6 hours as the hydrostatic head of milk stored in the udder increases. Once the main ducts and cisterns are full, the capacity is increased by the udder becoming distended with milk. In the later phase of storage the milk pressure increases more rapidly until at the end of a normal milking interval pressure of 2-4 kPa above atmospheric pressure are reached. When storage capacity has been reached, milk secretion ceases or the rate of secretion equals the rate of absorption.

The increase in milk production with more frequent milking was therefore associated with the relief of intramammary pressure following short milking intervals. The

opposite is also true for less frequent milking. However, the trends in intramammary pressure during the interval between milkings are not closely related to the trends in the rate of milk secretion (Schmidt, 1971). Moreover, the time needed for increased intramammary pressure to inhibit milk secretion varies between cows depending on age, stage of lactation, udder volume, level of milk yield and the amount of residual milk (Turner, 1955; Johanson and Malven, 1966; Tucker *et al.*, 1961). Nevertheless, alteration of milk composition with less milking frequency can partly be explained by the changes in intramammary pressure. The increased pressure in the alveoli following longer milking intervals, loosens the tight junctions and the secretory cells are forced apart. As a result exchange occurs more freely between secreted milk in the alveoli and the fluids in surrounding blood vessels and interstitial tissue (Wheelock *et al.*, 1965; Mackenzie *et al.*, 1990). The concentration of lactose and potassium in milk decreases as they leak out while that of sodium and blood proteins increase because they can leak into the milk. Lactose, and later protein, were observed in the urine following longer milking intervals of more than 18 hours (Lyster and Wheelock, 1967). Moreover, the increase in SCC of cows with extended milking interval also indicates the flow of blood serum components into the milk. Reduction in blood flow to the udder may occur when milk accumulation in the udder causes mammary distension (Fleet and Peaker, 1978). This is because stretching of the secretory cells reduces their metabolic activities and so they produce less CO₂ and other vasodilatory metabolites resulting in constriction of blood vessels and reduced blood flow (Peaker, 1980). The fall in mammary blood flow associated with mammary distension may reduce the availability of milk precursors to the secretory cells, thus reducing milk yield.

2.7.3 Udder storage capacity

Udder capacity as defined by Davis and Hughson (1988) is the quantity of milk contained in the udder when, following a period of milk accumulation, secretion rate decreases to zero. Udder capacity can also be expressed as "hours worth of secretion" and is correlated to empty udder volume (Peaker, 1980; Davis *et al.*, 1983). The latter can be estimated from linear measurements of the udder (Davis and

Hughson, 1988). A knowledge of udder capacity is essential in understanding the physiology underlying the response observed with different milking intervals.

One of the constraints to extending the milking interval without loss of production is the ability of the udder to store the milk produced over the extended period. Milk yield and udder volume were correlated in cows at all stages of lactation (Davis *et al.*, 1985). Moreover, udder capacity is greater in cows than in heifers (Davis and Hughson, 1988) and declines as lactation advances (Turner, 1955). Investigations have also shown the tendency for udder capacity to be greater in cows with high milk solid contents and that these cows are more tolerant to once daily milking (Carruthers *et al.*, 1989). Thus, estimates of udder capacity in Jersey cows (with high milk solid contents) suggest that once daily milking without production loss can be an attainable objective (Carruthers *et al.*, 1989). However, this is only possible if udder capacity is the sole constraint to production yields with extended milking intervals. Recent studies have shown increased yields in cows treated with bovine somatotropin and milked once daily, suggesting that udder capacity may not be the only constraint to production with extended milking intervals (Carruthers *et al.*, 1991).

2.7.4 Chemical inhibitor

The mechanism of response to frequent milking has been studied extensively in lactating goats. When the frequency of milking was increased from twice a day to either hourly or three times a day in one gland, the rate of milk secretion increased only in that gland and the response was maintained as long as more frequent milking was continued (Linzell and Peaker, 1971; Henderson *et al.*, 1983). This unilateral effect therefore, could not be explained by systemic control, such as the additional release of galactopoietic hormones. Milk removal was essential for the response to more frequent milking and an hourly massage of the gland without milk removal showed no effect (Linzell and Peaker, 1971). Furthermore, the response could not be ascribed simply to a reduction of physical distension of the gland during a long milking interval because replacement of milk removed from thrice daily milked glands at the extra milking by an equal volume of iso-osmotic sucrose did not impair the

increase in milk yield from that gland (Henderson and Peaker, 1984). It was therefore proposed by Linzell and Peaker (1971) that the response to more frequent milking and the reduction in milk secretion that take place between longer milking intervals can be explained by the presence in milk of a locally active chemical inhibitor, which limits milk secretion by negative feedback, that is, by an autocrine mechanism. The presence of the chemical inhibitor was confirmed using cultures of mammary explant from mid-pregnant rabbits as a bioassay to test fractions prepared from goat's milk (Wilde *et al.*, 1987a). The inhibitor was shown to be a heat labile constituent of whey protein, with a molecular mass of 10^4 to 3×10^4 daltons. The presence of the inhibitor reduces the binding and thus effectiveness of prolactin (Wilde and Peaker, 1990).

Experiments in dairy cows have shown that residual milk left in the gland at milking has an inhibitory effect on milk secretion (Elliot, 1961; Woolford *et al.*, 1985; Peaker and Blatchford, 1988). The importance of residual milk in the control of milk secretion was further demonstrated by studies in which only cisternal milk of the goat's mammary glands was removed by catheter (Henderson and Peaker, 1987). When alveolar milk was left in the gland, frequent milking did not stimulate milk yield. This showed that the site of feedback inhibition is the secretory alveoli. Inhibition by an autocrine factor appeared to be a concentration dependent process (Wilde *et al.*, 1987a; Peaker and Blatchford, 1988). It has also been shown that milk synthesis is susceptible to control by autocrine mechanism at all stages of lactation (Blatchford and Peaker, 1982). Further research is required on whether residual milk is a function of udder characteristics, such as the ratio of cisternal:alveolar volume, or of the cows responsiveness to oxytocin (Wilde and Peaker, 1990).

2.7.5 Differentiation and growth of secretory cells

Mammary growth is stimulated by frequent milking over a prolonged period. In goats after 37 weeks of unilateral thrice daily milking, the gland receiving the extra milking was larger than the other (by 34%) and contained 22% more secretory cells (Wilde *et al.*, 1987b). Also, there were significant increases in the activities of two

key lipogenic enzymes, acetyl-CoA carboxylase and fatty acid synthetase and in galactosyltransferase activity in the gland receiving the extra milking. A selective accumulation of key goat mammary enzymes, including acetyl CoA carboxylase, fatty acid synthetase and galactosyltransferase was also observed during normal differentiation in pregnancy and lactation (Wilde *et al.*, 1986). It appears, therefore, that an early response to thrice daily milking is an increased rate of secretory cell differentiation. In cows, similar increases in mammary growth to more frequent milking were also observed. The higher rate of DNA synthesis plus the quantitative histology suggested that glands milked four times daily had more epithelial cells per secretory alveolus in heifers and cows (Hillerton *et al.*, 1990).

Circumstantial evidence suggests that the size of the mammary cell population may be subject to control by a local growth inhibitor (Knight and Peaker, 1982). In this connection, an inhibitor of mammary cell proliferation *in vitro* has been isolated from bovine mammary tissue and has been shown to be secreted in association with the milkfat globule membrane (Bohmer *et al.*, 1987; Brandt *et al.*, 1988).

2.7.6 Extended milking intervals as an initial stage of involution

Extended milking intervals may be considered to be an initial stage of involution since the process of involution in dairy cows is initiated between 12 and 24 hours after cessation of milking (Hurley, 1989). An early event seems to be the breakdown of intracellular mechanisms involved in secretion of milk products (Wheelock *et al.*, 1967). Light and electron microscopic mammary tissue examination in dairy cows during the first two weeks after cessation of milking, revealed a progressive increase in stroma and non-active secretory epithelium with concomitant decreases in epithelial lumen, presence of large vacuoles in the cells and a decrease in the number of organelles associated with milk synthesis and secretion (Holst *et al.*, 1987; Sordillo *et al.*, 1987; Sordillo and Nickerson, 1988). Lactose yield declines rapidly which is consistent with decreased enzyme lactose synthetase activity observed in mammary tissue during involution (Bauman *et al.*, 1974). Milk fat concentration also declines as does the proportion of short and medium-chain fatty acids (Smith *et al.*, 1967;

Wheelock *et al.*, 1967; Hurley, 1987). Despite the declining concentration of the milk specific proteins, α -lactalbumin, β -lactoglobulin and casein (Hurley and Rejmain, 1986), total protein concentration increases (Hurley, 1987). This increase is primarily the result of increased concentration of blood derived proteins such as serum albumin, immunoglobulins and lactoferrin (Watson *et al.*, 1972; Welty *et al.*, 1976). All these changes are influenced by the action of oxytocin and anterior pituitary hormones, prolactin, growth hormone and adrenal corticosteroid hormone (Lascelles and Lee, 1978).

Changes in milk composition following longer milking intervals (for example in once daily milking) have been taken to imply a breakdown in the tight junctional complexes between epithelial cells associated with increased glandular distension and intramammary pressure (Mackenzie *et al.*, 1990). However, histological studies of involuting bovine mammary glands (Holst *et al.*, 1987) have shown the presence of tight junctions throughout involution. Nevertheless, they stained less densely, as observed by electron microscopy, perhaps indicating a loss of integrity of the tight junction upon cessation of milking.

An observed increase in the concentration of somatic cells in milk with longer milking intervals is similar to that shown by the involuting gland. When milking ceased, leukocyte infiltration into mammary tissue increased and these macrophages and neutrophils contained milk components and cellular debris within their cytoplasm (Sordillo *et al.*, 1989). In another similar study, infected quarters had more leukocyte in epithelium, lumen and stroma compared with uninfected involuting quarters.

The changes in milk yield and composition observed following extended milking intervals may therefore be explained in terms of physiological, biochemical and the structural integrity of the glandular epithelium.

From the foregoing discussion, it seems that mastitis infection and reduced milking frequency have some related effects on secretory activities of the mammary gland. In both cases, production loss of milk and its constituents and changes in milk

composition have been observed. The degree of production loss depends on the intensity of infection, and in the case of extended milking intervals, various factors related to cow and/or management might be involved. The extent of this production loss and infection can be measured by SCC, which also tends to increase when milking frequency is reduced.

2.8 RATIONALE FOR THE PRESENT STUDY

Several studies have demonstrated an increase in SCC during the later stages of lactation (Brolund, 1985; Emanuelson *et al.*, 1988; Holdaway, 1990). For various reasons some New Zealand dairy farmers milk their cows once a day towards the end of lactation. However, once daily milking has also been shown to cause an increase in SCC (Mackenzie *et al.*, 1990; Lynch *et al.*, 1991; Holmes *et al.*, 1992). This increase in SCC with once daily milking is hypothesised to be partially due to a "concentration effect" caused by a decline in milk yield. According to this hypothesis, the total number of somatic cells secreted into the milk is normally constant in the absence of udder infection. However, there is no information about the magnitude of SCC increase in infected cows as compared to those which are not infected when milked once a day. Additional information on the effect of once daily milking on SCC in late lactation is also desirable. Moreover, the response to once daily milking in later lactation on milk yield and composition for infected as opposed to uninfected cows would be of interest. This trial was therefore undertaken in late lactation to study the effects of once daily milking and to measure the interaction between the effects of mastitis and once daily milking on SCC, milk yield and composition. The results were intended to improve the accuracy with which SCC data for all cows can be interpreted, regardless of milking frequency.

CHAPTER 3

MATERIALS AND METHODS

CHAPTER 3

MATERIALS AND METHODS

CHAPTER THREE

3.1 ANIMALS

Thirty six cows at the Dairy Cattle Research Unit, Massey University, were used in the experiment. These cows were a mixture of Jersey and Friesian in their later lactation with ages ranging from 2 to 10 years (average 5 years). They included 18 IHSCC cows ($> 250,000$ cells/ml) and 18 ILSCC cows ($< 250,000$ cells/ml). Half of the cows from each group were milked either once or twice per day. All cows were grazed and milked with the rest of the herd for the whole of the experimental period.

3.2 EXPERIMENTAL DESIGN AND PROCEDURE

The experiment was a 2 x 2 factorial design. The treatment imposed were milking frequency (twice and once daily milking) and initial SCC level (high or low) to give four treatment combinations; IHSCC x 1, IHSCC x 2, ILSCC x 1 and ILSCC x 2. The experiment consisted of two weeks preliminary, 4 weeks treatment and 2 weeks post treatment periods (Table 3.1).

Table 3.1 A simple chronological plan of the experiment.

Period	Week of experiment	Measurements
Pre-treatment (2 weeks) (All cows milked twice daily)	1	- Identification of cows with high and low SCC - Bacteriology of 36 selected cows, 2 consecutive days
	2	- Milk yield and composition, SCC
Treatment (4 weeks) (Milked twice or once daily)	3-6	- Milk yield and composition, SCC
Post-treatment (1 week) (All cows milked twice daily)	7	- Bacteriology repeated

On two consecutive days of the first week of the preliminary period, foremilk samples were taken aseptically for bacteriological tests from the individual quarters of 48 cows selected on the basis of their previous SCC records. The analyses were repeated on the third day for samples which had contrasting results on the first and second day. Thirty six experimental cows were identified and grouping of cows for treatments were balanced as much as possible for age and initial SCC. Bacteriological tests were repeated again at the end of the experiment to determine any change in the incidence of infection between and within the treatment groups. All cows were milked twice daily during the preliminary and post-treatment period. Composite milk samples for yield, composition and SCC were collected on two complete milking days of the week (from Tuesday evening to Thursday morning).

During the treatment period, the twice daily milked cows were milked at 0600 hours and 1500 hours while once daily milked cows were milked at 0600 hours. Milk samples were taken using milk meters which give a proportionately representative sample of milk from all four quarters of the udder of the cow.

3.2.1 Determination of milk yield and composition

Milk yield produced by each cow was measured using Metatron Milk Meters (Westfalia). Milk samples collected at consecutive afternoon and morning milkings were analysed separately for milk composition using a Milkoscan 140 A/B (A/S N Foss Electric, Denmark). Basic operation of this semi-automatic instrument is similar to that of an infrared spectrophotometer, using a single infrared beam which is focused to pass through the sample and strike a detector. The energy is detected, amplified and converted to digital form which can then be printed out. The analyser was operated in accordance with the manufacturers instructions by staff in the Department of Animal Science, Massey University. Daily yields of milk, fat, protein and lactose were obtained by adding together the yields obtained at consecutive evening and morning milkings. Milk composition values were obtained by multiplying the morning yield and concentration values and those of the evening; and

dividing the sum with the total milk yield obtained for the 24 hour period to give weighted average, for example:

$$\text{fat concentration (kg/l)} = \frac{\{\text{Fat}^1 \text{ (kg/l)} \times \text{Milk}^1 \text{ (l)}\} + \{\text{Fat}^2 \text{ (kg/l)} \times \text{Milk}^2 \text{ (l)}\}}{\{\text{Milk}^1 \text{ (l)} + \text{Milk}^2 \text{ (l)}\}}$$

where ¹ and ² are morning and evening values, respectively.

3.2.2 Somatic cell counts

Samples collected by milk meters were mixed thoroughly and approximately 20-25 mls was transferred into special plastic bottles containing potassium dichromate as a preservative, stored at 4°C and transported to the Livestock Improvement Corporation, Hamilton on Thursday morning.

SCC given to the closest thousand cells per ml were determined using a Fossomatic Fluoro-optical Counter (A/S N Foss Electric, Denmark). The instrument is fully automatic and prewarming of the samples to 40°C and subsequent shaking are required (Schmidt Madsen, 1975). The testing procedure involves automated mixing of milk samples with a buffer solution to which the fluorescent dye, ethidium bromide has been added. Short wavelength radiation from a xenon lamp makes the coloured cells of thin smears of this milk preparation fluoresce. The fluorescing particles are counted by a photomultiplier as they pass through the slit. The instrument has a capacity of analysing 180 samples per hour.

3.2.3 Bacteriology analyses

Individual quarter foremilk sampling was carried out aseptically at the afternoon milkings between 1500 hours and 1630 hours. Sampling procedure involved washing the teats by hand, using running water to remove gross contamination. The teats were then dried with an individual disposable paper towel, and thoroughly wiped with

cotton wool moistened with 70% alcohol. About 20 mls of milk was drawn directly into labelled sterile glass screw-capped universal bottles, after discarding the first three squirts of milk, and the sample bottles were capped immediately. These samples were taken to the Microbiology Laboratory at the Faculty of Veterinary Science, Massey University and processed within two hours of sampling.

Bacteriological analyses were done according to the methods described by Holdaway (1990). Staphylococci were recorded as being either coagulase positive or coagulase negative on the basis of the tube coagulase test. Streptococci were differentiated by two biochemical tests including the ability to ferment a number of sugars and the Camp test (for details refer to Holdaway, 1990). The colonies of Corynebacteria were identified from their appearance.

3.3 STATISTICAL ANALYSES

Statistical analyses for milk yield, composition and SCC data were carried out by means of Analysis of Variance (ANOVA), and Chi² analysis for bacteriology data, using the Statistical Analysis System (SAS) computing package (SAS Institute, 1987). In order to adjust for sources of bias brought about by differences in cow's milk yield and composition at the beginning of the experiment, the data were adjusted by covariance using the values measured before the treatment period as the covariate. Because of the marked skewness of the frequency distribution of somatic cell concentrations in milk (Ali and Shook, 1980), the SCC data were transformed (Log₁₀ transformation) prior to analysis. The model used to define the data was:

$$Y_{ijk} = \mu + A_i + B_j + AB_{ij} + e_{ijk}$$

where:

Y_{ijk} = an observation of the yield of milk, fat, protein, lactose, total somatic cells; and composition of fat, protein, lactose and SCC

in milk for the k th cow in the i th infection status class and the j th milking frequency.

- μ = General population mean.
- A_i = The fixed effect of the i th infection status class $\{i = 1$ -uninfected, or 2-infected $\}$
- B_j = The fixed effect of the j th milking frequency $\{j = 1$ -once daily milking, or 2-twice daily milking $\}$
- AB_{ij} = The interaction between the effect of the i th infection status and the effect of the j th milking frequency.
- e_{ijk} = The random error associated with the Y_{ijk} observation which is assumed to be normally distributed with mean zero and variance σ^2 .

CHAPTER 4

RESULTS

CHAPTER FOUR

4.1 INCIDENCE OF INFECTION

The incidence of infection in the two treatment groups measured at the beginning and the end of the experiment for ILSCC and IHSCC cows are shown in Table 4.1. The ILSCC quarters milked once or twice daily showed no significant difference in the incidence of infection between the start and the end of the treatment period. For the IHSCC group, however, once daily milking slightly increased the incidence of quarters infected ($P < 0.1$), whereas a significant decrease in the incidence of quarters infected ($P < 0.05$) was recorded in the twice daily milked cows. There was no change in the incidence of infection for cows milked once or twice daily both in ILSCC and IHSCC groups. Two quarters of cows in the IHSCC group milked once daily developed clinical mastitis during the experimental period.

4.2 TREATMENT PERIOD

The mean values for the yields of milk and its main constituents, and milk composition measured during the 4 week treatment period are given in Table 4.2. As expected, the IHSCC group yielded less milk, protein ($P < 0.01$), fat and lactose ($P < 0.05$). The effects of infection on yields are further illustrated in Figures 4.1 (a, b, c and d), which clearly show higher yields obtained from ILSCC cows.

Table 4.1 Prevalence (%) of infection within quarters and cows in two treatment groups at the beginning and end of the experiment (number of cows and quarters in each category = 9 and 36, respectively).

	Twice daily			Once daily		
	Start (%)	End (%)	Sign.	Start (%)	End (%)	Sign.
<u>ILSCC</u>						
<u>Quarters</u>						
Major pathogens ^{1,3}	0	0		0	0	
Minor pathogens ²	56	44		47	53	
Total infection	56	44	NS	47	53	NS
<u>Cows</u>						
Major pathogens ^{1,3}	0	0		0	0	
Minor pathogens ²	67	67		78	78	
Total infections	67	67	NS	78	78	NS
<u>IHSCC cows</u>						
<u>Quarters</u>						
Major pathogens ^{1,3}	25	11		22	28	
Minor pathogens ²	58	42		61	67	
Total infection	83	53	**	83	95	(10%)
<u>Cows</u>						
Major pathogens ^{1,3}	67	67		67	44	
Minor pathogens ²	33	33		22	44	
Total infection	100	100	NS	89	89	NS

¹ *Staphylococcus aureus*

Streptococcus uberis

Streptococcus dysgalactiae

² *Corynebacterium bovis*

Coagulase negative *staphylococcus*

³ Quarters may also have harboured minor pathogens

NS = Not significant

** = P < 0.01

Table 4.2 Daily means for milk yield and composition (\pm SEM; covariance adjusted) and somatic cells in cows with high SCC or low SCC over a 4 week treatment period during which half of the cows of each group were milked once daily and the other half twice daily).

	ILSCC		IHSCC		Pooled ± SEM	Significance		
	Twice	Once	Twice	Once		Inf	Freq	InfxFreq
<u>Yield/cow</u>								
Milk (l)	13.9 ^a	11.9 ^b	14.0 ^a	10.4 ^c	0.30	**	***	**
Fat (kg)	0.70 ^a	0.64 ^b	0.71 ^a	0.54 ^c	0.02	*	***	**
Protein (kg)	0.52 ^a	0.47 ^b	0.52 ^a	0.41 ^c	0.01	**	***	**
Lactose (kg)	0.67 ^a	0.57 ^b	0.68 ^a	0.48 ^c	0.01	*	***	**
Total somatic cells (x10 ⁹)	2.80 ^a	3.20 ^a	11.7 ^b	10.4 ^b	1.30	***	NS	NS
<u>Composition</u>								
Fat (%)	5.04 ^a	5.39 ^b	5.15 ^a	5.22 ^b	0.08	NS	**	NS
Protein (%)	3.78 ^a	3.97 ^b	3.79 ^a	3.96 ^b	0.03	NS	***	NS
Lactose (%)	4.83 ^a	4.75 ^b	4.86 ^a	4.61 ^c	0.02	**	***	***
SCC (Log ₁₀ /ml)	5.19 ^a	5.35 ^a	5.92 ^b	6.35 ^c	0.11	***	***	+

*** Significant difference at probability < 0.001

** Significant difference at probability < 0.01

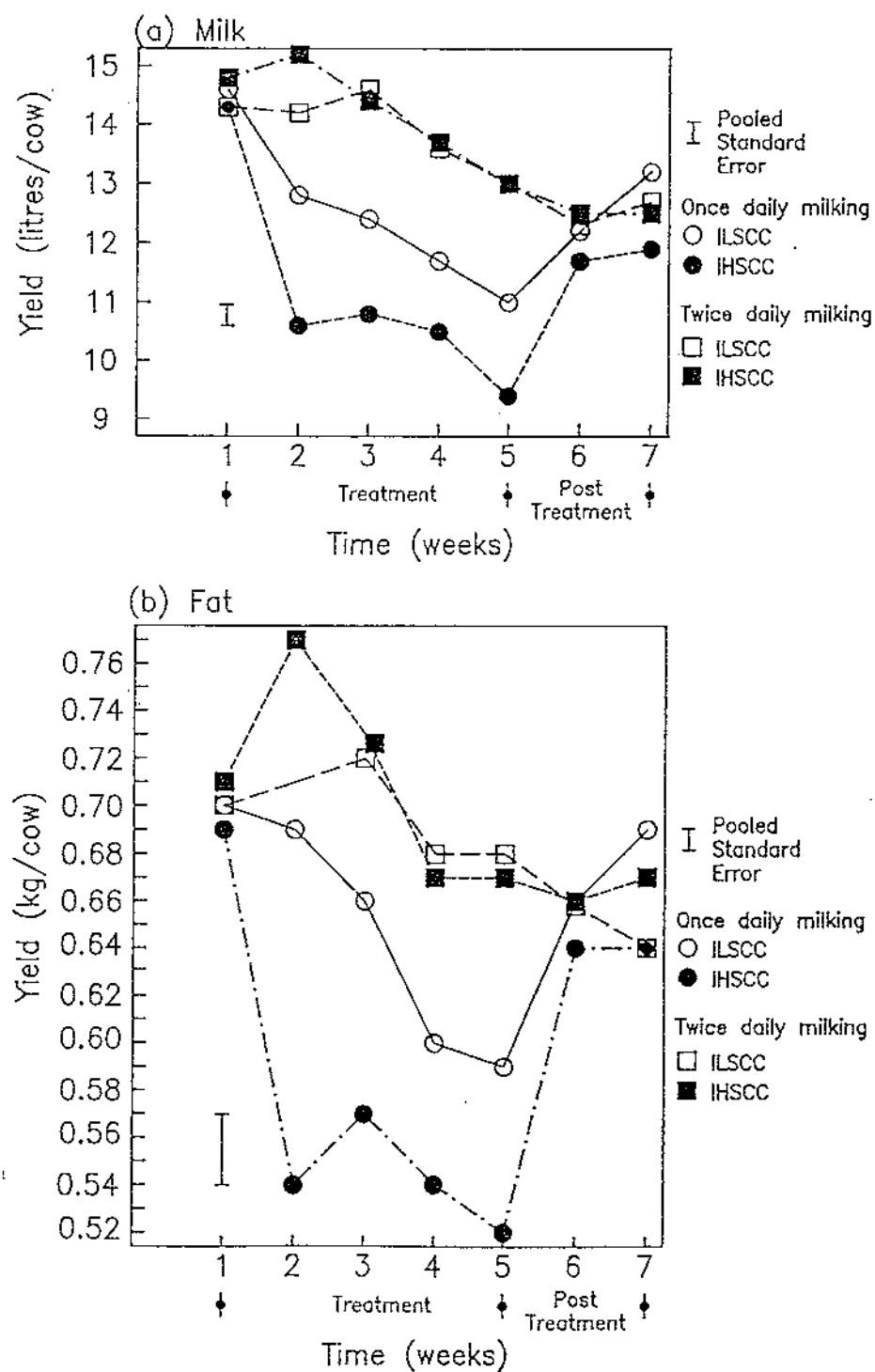
* Significant difference at probability < 0.05

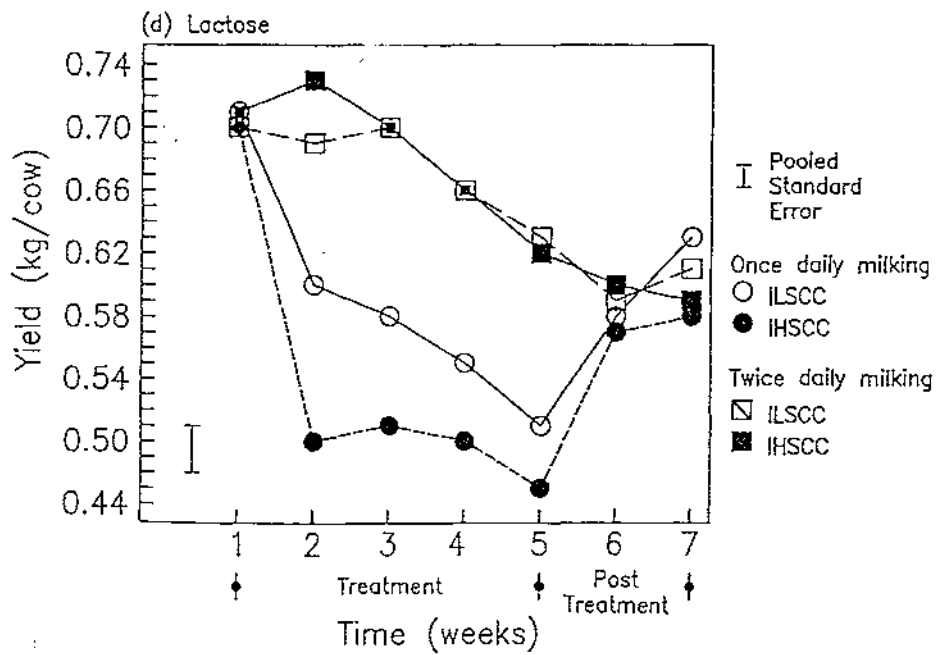
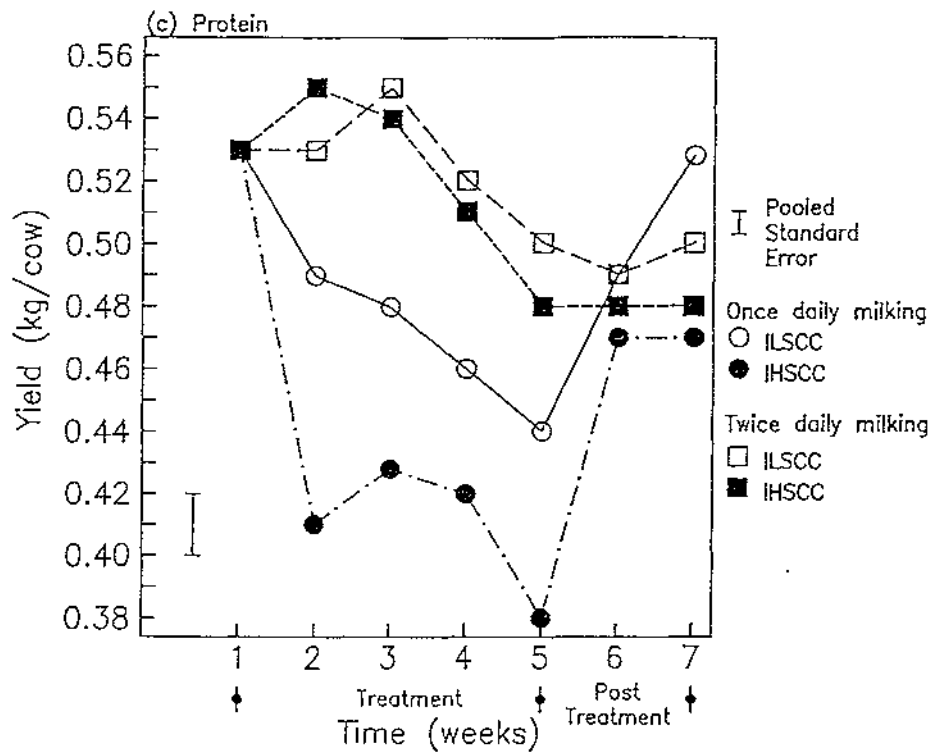
+ Significant difference at probability < 0.1

NS No significance

Note: Means within the same line but with different superscripts are significantly different at probability < 0.05

Figure 4.1 The yield of cows with a high or low initial cell count during a four week period during which half the cows from each group were milked twice daily and the other half once daily, and during a 2 week post-treatment period when all cows were milked twice daily.





Milking once daily significantly reduced the yields of milk, fat, protein and lactose ($P < 0.001$). Figure 4.1 (a, b, c and d) show that the effects of once daily milking on yields was immediate with a sharp fall in yield on the first week of the treatment period. The average fall in the first week of once daily milking was 19% for milk, 13% for fat, 15% for protein and 21% for lactose. Thereafter, there was a gradual decrease in yield of milk and its constituents, a tendency which was also observed in the control, twice daily milked group. The average rate of decrease per day from the second week to the fourth week of treatment for once daily milked cows was 0.5 litres milk, 0.02 kg fat, 0.01 kg protein and 0.04 kg lactose. For the twice daily milked group the rate of decrease per day during the same period was 0.6 litres milk, 0.02 fat, 0.01 protein and 0.03 lactose. The total number of somatic cells secreted per day was not affected by milking frequency.

The interaction between the effects of infection and milking frequency are illustrated in Figures 4.1 (a, b, c and d). Once daily milking had a larger effect on the yield of milk and its constituents in IHSCC cows than ILSCC cows (interaction $P < 0.01$). The average decreases per cow in the ILSCC group were 14% for milk, 9% for fat, 10% for protein and 15% for lactose. On the other hand, the IHSCC group milked once daily had an average decrease of about 26%, 24%, 21% and 29% for milk, fat, protein and lactose respectively. Total number of somatic cells secreted per cow daily were not affected by milking frequency in either ILSCC or IHSCC cows (interaction $P < 0.5$).

Results of statistical analysis of data for milk composition shows that lactose concentration was lower in the IHSCC than the ILSCC group ($P < 0.01$; Table 4.2). Once daily milking decreased lactose concentration in both the IHSCC and ILSCC group but the effect was larger in the IHSCC group ($P < 0.001$). The protein concentration was significantly increased by once daily milking in both groups ($P < 0.001$), whereas that of fat was increased only in the ILSCC cows ($P < 0.01$).

4.3 POST-TREATMENT

The results for the post-treatment period are summarised in Table 4.3. IHSCC cows produced less protein ($P < 0.05$) during the post-treatment period. Figures 4.1 (a,b and d) show that once daily milked cows produced lower milk yield and its constituents compared to twice daily milked cows, one week after cessation of treatment, but these differences were not statistically significant. By the second week after cessation of treatment, the previously once daily milked ILSCC cows had slightly higher but non-significant yields of milk, fat, protein and lactose. Thus there was no significant carryover of the effect of once daily milking on yield both for the ILSCC and IHSCC cows.

Milk composition results (Table 4.3) show a carry over effect of once daily milking on protein ($P < 0.05$), but there was no significant interaction between the effects of once daily milking and initial SCC level. This carry over effect on protein concentration was observed only in the ILSCC cows. Lactose concentration was slightly lower for IHSCC once daily milked group ($P < 0.1$) giving a slightly significant interaction effect ($P < 0.1$). There was no carry over effect of once daily milking on the concentration of fat.

4.4 SOMATIC CELL COUNT

During 4 weeks of treatment, once daily milking significantly increased SCC ($P < 0.001$), the effect of which is illustrated in Figure 4.2. However, the interaction effect shows that once daily milking increased SCC of the IHSCC group but not the ILSCC group (interaction $P < 0.1$). Figure 4.2 shows a significantly higher SCC for the IHSCC compared to ILSCC once daily milked cows in the first and second weeks of treatment but no significant effect for the last two weeks of treatment.

Post-treatment period analysis showed no carry over effect of once daily milking on SCC (Table 4.3).

Table 4.3 Daily means for milk yield and composition (\pm SEM; covariance adjusted) and somatic cells in cows with high SCC or low SCC over a 2 week post-treatment period during which all cows were milked twice daily.

	ILSCC		IHSCC		Pooled ± SEM	Significance		
	Twice	Once	Twice	Once		Inf	Freq	InfxFreq
<u>Yield/cow</u>								
Milk (l)	12.6 ^a	12.6 ^a	12.2 ^a	12.1 ^a	0.35	NS	NS	NS
Fat (kg)	0.65 ^a	0.67 ^a	0.66 ^a	0.65 ^a	0.03	NS	NS	NS
Protein (kg)	0.50 ^a	0.50 ^a	0.47 ^b	0.48 ^b	0.01	*	NS	NS
Lactose (kg)	0.61 ^a	0.60 ^a	0.59 ^a	0.58 ^a	0.02	NS	NS	NS
Total somatic cells (x10 ⁹)	5.0 ^a	5.0 ^a	7.7 ^a	6.0 ^a	1.80	NS	NS	NS
<u>Composition</u>								
Fat (%)	5.21 ^a	5.34 ^a	5.38 ^a	5.53 ^a	0.13	NS	NS	NS
Protein (%)	3.91 ⁺	4.03 ⁺	3.92 ^a	4.00 ^a	0.04	NS	*	NS
Lactose (%)	4.79 ^a	4.81 ^a	4.82 ⁺	4.77 ⁺	0.02	NS	NS	+
SCC (Log ₁₀ /ml)	5.17 ^a	5.14 ^a	5.90 ^b	5.80 ^b	0.12	***	NS	NS

*** Significant difference at probability < 0.001

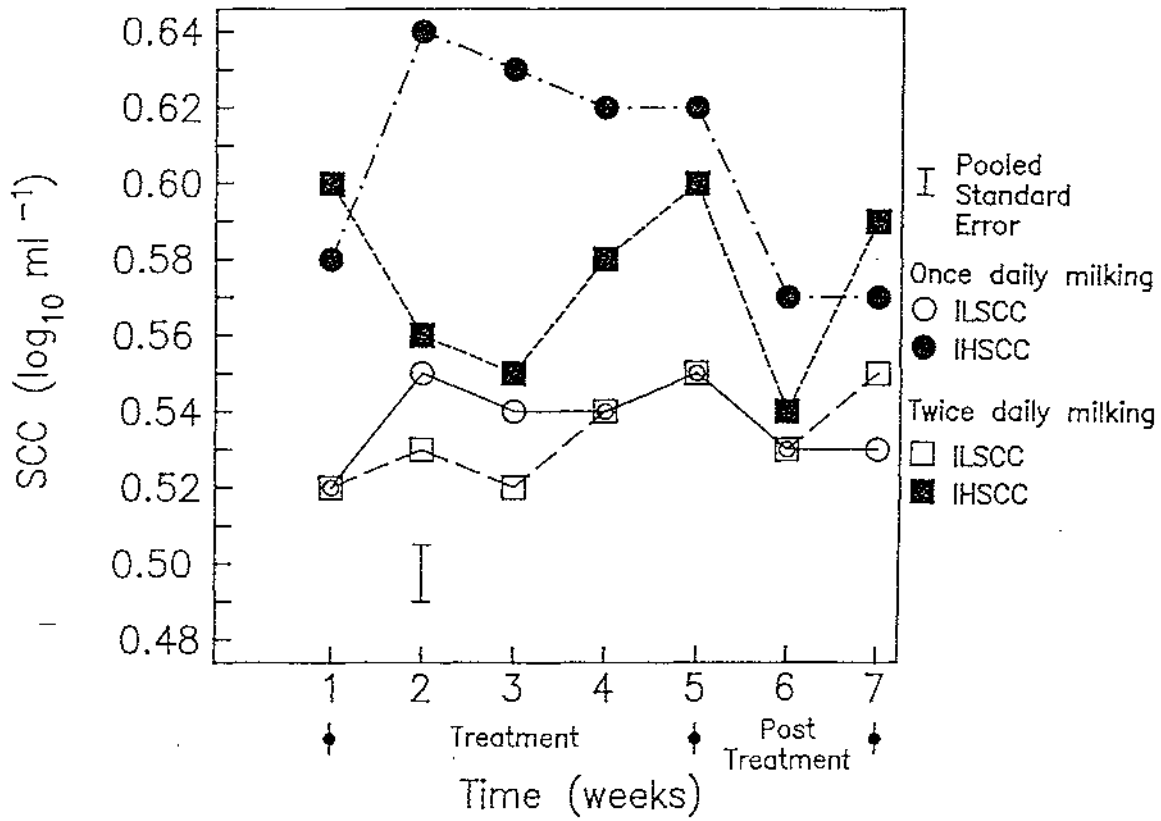
* Significant difference at probability < 0.05

+ Significant difference at probability < 0.1

NS No significance

Note: Means within the same line but with different superscripts are significantly different at probability < 0.05

Figure 4.2 Somatic cell count of cows with high or low initial somatic cell count during a four week period during which half the cows from each group were milked twice daily and the other half once daily, and during a 2 week post-treatment period when all cows were milked twice daily.



CHAPTER 5

GENERAL DISCUSSION AND CONCLUSION

CHAPTER FIVE

5.1 SCC - PREDICTIVE ABILITY FOR INFECTED AND UNINFECTED QUARTERS

The current opinion for a positive diagnosis of mastitis is to fulfil two criteria, a positive bacteriological test and the presence of inflammatory change as demonstrated by an indirect test (Tolle, 1971). Thus, provided the samples are not contaminated, isolation of bacteria from milk samples indicates mastitis infection.

In the present study SCC has been used as an inflammatory detection parameter for the identification of cows which were most likely to be infected. Bacteriological assays conducted at the start of the experiment showed that cows which had SCC below the threshold value exhibited bacterial infection in some of their quarters (Table 4.1). In fact, about 52% of all the quarters in the initially low SCC group (ILSCC group) (the average for the once and twice daily milked cows), were infected with minor pathogens, but no major pathogen was isolated. Similarly, not all quarters in cows which had initial high SCC values were in fact infected. Only 83% of quarters showed bacterial growth, leaving 17% with no infection. These results are not surprising since bacteriological tests were done using quarter milk samples whilst composite milk samples were used for determination of somatic cells. Clearly, the effect of bacterial infection of the udder on SCC will depend, among other factors, on the number of udder quarters which are infected at the time. While the relationship between bacteriological status of a quarter and SCC of milk from the same quarter is relatively straightforward (Holdaway, 1990), the relationship between infection of udder quarters and somatic cell count of composite samples is complicated by the fact that the cow may be infected in one, two, three or four quarters (Natzke *et al.*, 1972). Therefore the high SCC milk from infected quarters was likely to be diluted by lower SCC from uninfected quarters within the infected udder and this may partly explain the isolation of bacteria from some cows which had low SCC. Likewise, the opposite would be true for a healthy quarter in a severely

infected udder. There are also variations in cellular response elicited by udder pathogens, especially those between minor and major pathogens (Tolle, 1975; Schultz, 1977). Major pathogens cause greater cellular response compared to minor pathogens and this may partly account for the isolation of minor pathogens in 52% of quarters of cows which had low SCC.

A number of researchers have studied the association between bacterial infection of the udder and SCC of composite milk samples. The mean SCC for milk from the cows which were uninfected, infected with minor pathogens, or infected with major pathogens were 170,000, 227,000 and 998,000 cells/ml respectively (Schultz, 1977). A recent study by Holdaway (1990) showed highly significant associations between SCC of composite milk samples and bacteriological status, even when infection was limited to a single quarter within the udder. However, the ability of SCC of composite milk samples to identify infected and uninfected cows at a chosen threshold value depends on the infection criterion that is used. Two bacterial classifications may be used (Holdaway, 1990):

1. Quarters infected with major pathogens are deemed to be infected, whereas bacteriological negative quarters and quarters harbouring minor pathogens are regarded as uninfected.
2. Quarters infected with major or minor pathogens are deemed to be infected and bacteriologically negative quarters regarded as uninfected.

The results obtained from this study shows that under bacteriological classification 1, all cows in the ILSCC cows would be correctly classified as uninfected, whereas only 33% uninfected cows in the IHSCC would be misclassified as being infected (Table 4.1). Using a similar bacteriological classification for composite milk samples and a critical threshold value of 185,000 cells/ml, Holdaway (1990) found that SCC misclassified about 25% of the samples as false positive or false negative infection. In the same study, when quarter milk samples and a critical threshold value of 245,000 cells per ml were used, about 20% of quarters were misclassified. Thus,

even where quarter milk samples are used, an agreement of 100% between SCC and bacteriology cannot be expected since inflammation, as indicated by an increase in SCC, can be caused by mechanisms other than an infection and presence of infection does not necessarily mean a significant increase in SCC. In another study only 6% of composite milk samples from cows which were harbouring major pathogens fell into the somatic cell count range of 0 to 200,000 cells per ml, while 60% of milk samples from uninfected cows fell within this range (Duirs and McMillan, 1979). In the present trial, no major pathogen was isolated from composite milk samples with SCC below 250,000 cells/ml. The results reported in this study therefore agree well with the cited data, showing a high degree of accuracy in predicting bacterial infection under classification 1.

Bacteriological classification 2, however, would result in 73% (average for once and twice daily milked cows) of bacteriologically positive samples from cows which had SCC below the threshold to be misclassified as false negative infection, and only 6% of bacteriologically negative cows in the high somatic cell count group misclassified as false positive infection (Table 4.1). If this type of classification is to be used, a much lower threshold value would have been required in order to improve the discrimination power of SCC.

The results from this trial confirms the finding of the cited studies that the use of SCC of composite milk samples provides a reasonably accurate prediction of the infection status of the cow, although differences between trials may arise with respect to definition of infection and the choice of threshold values.

5.2 INCIDENCE OF INFECTION

At the end of treatment, in the IHSCC group, once daily milked cows showed a slight increase in the incidence of infection ($P < 0.1$), whereas the twice daily milked cows showed a significant decrease in the incidence of infection ($P < 0.05$). Mastitis develops once bacteria pass through the teat canal and invade the secretory tissue of

the mammary gland (Mattila, 1985). Frequent removal of milk is believed to suppress the number of bacteria and their toxins through the wash out effects of milking (Mackenzie *et al.*, 1990). Infection developed in 8% of quarter inoculated with pathogen when animals were milked twice daily and 23% of quarters when the first post-inoculation milking was omitted (Newbould and Neave, 1965). It is therefore likely that once daily milking increased the chances for any bacteria present in the teat to multiply, invade the secretory tissue of the udder, causing new infection. Udder distention due to the accumulation of milk during the period of once daily milk could also have increased the risks of teat contamination and hence new infection.

An interesting observation from these results is that the cows which had low SCC showed no significant change in the incidence of infection over the treatment period. Bakken (1981) indicated that a healthy quarter on an already infected udder is at higher risk of becoming infected than another healthy quarter in an udder with no infection. Results from this trial supports this observation. Although the percentage of infected quarters increased in the IHSCC once daily milked cows, the percentage of cows infected at the start and end of the experiment remains the same (Table 4.1). It would appear from this account that the risks of infection due to extended milking intervals and cross infection from infected quarters was higher in the IHSCC once daily milked cows, and results in a significantly higher infection rate in this group than in the ILSCC once daily milked group. The most important conclusion which can be drawn from these results is that cows with low incidence of mastitis are less susceptible to new infection than those with higher incidence of mastitis, even when milked once daily. The occurrence of two cases of clinical mastitis in quarters that were already infected in IHSCC once daily milked cows shows an increase in severity of infection possibly due to increase in the concentration of bacteria and their toxins with longer milking intervals. On the other hand, a significant decrease in the incidence of infection in the twice daily IHSCC cows can be explained by the occurrence of spontaneous recovery which can take place normally in subclinically infected quarters as well as the washing out effect of more frequent milking (Kingwill *et al.*, 1977).

5.3 MILK YIELD AND COMPOSITION

Over the 4 weeks of once daily milking, the daily milk, fat, protein and lactose yields, as expected, decreased significantly relative to the twice daily milked group. The decrease in milk and milk components when cows are milked once a day are similar to those observed in the previous studies (Bryant, 1980; Njaritta, 1989; Carruthers and Copeman, 1990; Morris *et al.*, 1991), but there are variations in the extent of yield depressions between studies. These variations are presumably brought about by differences in the duration of treatment, stage of lactation, age of the cows, level of feeding, genetic factors and prevalence of infection within the cows used in different studies. The immediate decrease in production following once daily milking shows that the effect of reduced milking frequency on production is rapid and may be fully realised within one week of treatment. However, continued rate of decline in milk yield with time was similar in both the once and twice daily milked cows and was probably due to the "normal" involuntary changes in some lobules during advancing lactation (Lascelles and Lee, 1978). Although feed intake was not measured in this study, the dry weather conditions which prevailed during the treatment period probably restricted feed intake by reducing pasture quality and quantity and possibly affected the daily milk yields.

Several mechanisms involved in the inhibition of milk secretion following longer milking intervals have been proposed. The control of milk secretion by milking frequency may be a result of changes in the degree of concentration-dependent feedback inhibition exerted by a constituent of the whey proteins (Wilde *et al.*, 1988). This suggests that the concentration of the milk must increase as milk accumulates in order to reduce the rate of secretion as the gland fills. Conversely, milking should reduce the concentration of the inhibitor so as to restore a faster rate of secretion. Therefore, once daily milking, by eliminating the removal of the inhibitor at the afternoon milking probably reduced milk yield by inducing a high degree of autocrine feedback for much of the day. Experimental studies in which additional residual milk has been left in the gland after each milking (Elliott, 1961) have shown the inhibitory effect on milk secretion. On the other hand, Linzell (1955) in his paper on

contraction of the mammary myoepithelium in the mouse noted that, in all but empty glands, milk flowed back into the alveoli from the ducts when contraction had ended. Changing from twice to once daily milking means additional milk to harvest within the effective milk ejection time. As a result the amount of residual milk retained in the alveoli might have been increased significantly because the period of ejection was too short to allow complete removal of all the milk secreted for 24 hours. Consequently, this may have increased the inhibitory effect on milk secretion.

A recent study by Wilde *et al.* (1989) indicated that incomplete milking may cause a partial secretory cellular involution. Since milk yield is a function of the number of secretory cells and their activity, there may be no apparent relation between total milk yield secreted and the proportion of residual milk. The differences in yield that were observed between once and twice daily milked cows might have been masked by the differences in cell numbers.

Njaritta (1989) reported higher intramammary pressure in cows milked once daily. Although intramammary pressure was not recorded in the present study, it is likely that once daily milking caused an increase in average pressure, which in turn depressed the rate of secretion. Udder volume which determine the milk storage capacity of the udder could also have been important in explaining the present results.

The effect of once daily milking on yields was larger for IHSCC than ILSCC (interaction $P < 0.01$). The decrease in milk yield caused by mastitis is well established, and reviews covering the effect of mastitis on milk yield have been conducted by Janzen (1970), Hoare (1982) and Munro *et al.* (1984). When the mammary gland becomes colonised by pathogenic bacteria, the udder tissue undergoes pathological changes and synthesis of milk is reduced. The highly significant interaction (SCC x milking frequency) observed in this study suggests that the functional impairment to the secretory cells caused by once daily milking acted multiplicatively with the impairment caused by infection, not simply in additive fashion. Changes in the concentrations of fat, protein and lactose follow the trends observed in other studies (Claesson *et al.*, 1959; Wilson, 1965; Bryant, 1980; Holmes

et al., 1992). They reflect changes in both the synthetic activity of the secretory epithelial cells and the activity and integrity of the epithelium. Thus once daily milking and/or infection increased the permeability of the glandular epithelium either by higher intramammary pressure forcing the epithelial cells apart, or by chemical factors causing the tight junctions to loosen.

The cause of the observed changes in milk yield and concentrations of milk components are complex, but can be explained in terms of the model for milk secretion proposed by Linzell and Peaker (Linzell and Peaker, 1971; Peaker, 1978). A decrease in lactose synthesis, which is the major osmoregulatory component of milk leads directly to a decrease in milk yield, since the decrease in lactose synthesis is inevitably accompanied by decrease in water secreted (Wheelock *et al.*, 1965). Further reductions in milk yield in cows with higher SCC occurred due to greater losses of lactose and water into the interstitium through leaks in the epithelium caused not only by once daily milking, but also bacterial infection. The decreases in lactose concentration, however, are probably due, in part, to an inability of the epithelium to maintain normal sodium and potassium concentrations in milk. Thus as sodium ions increased in the milk, the lactose concentration decreased because the milk and surrounding fluids remain isotonic. The larger decrease in lactose concentration in the IHSCC group is due to exacerbation of effects by the combination of infection plus once daily milking, either because the activity of epithelial cells is impaired or the movement of sodium into the milk via leaks is in excess of the capacity of the cells to remove it. The slight rise in fat concentration on once daily milking suggests that fat synthesis is less affected than lactose synthesis. In contrast, the interpretation of the changes in protein concentration is complicated by the potential for variation in both the rate of synthesis of those proteins synthesised in the gland and the movement of serum proteins into the milk (Hurley and Rejmain, 1986; Welty *et al.*, 1976). It is also possible that the reduction of milk volume following losses of lactose and water through leaks increased the concentration of large molecules such as those of protein and fat globules which cannot pass easily between the epithelial cells.

Milk yield recovered rapidly and completely when twice daily milking resumed. In fact, ILSCC cows previously milked once daily had a slightly higher yield of milk, fat, protein and lactose by the second week after cessation of treatment (difference not significant). These results agree with previous studies, in which about 2 weeks of once daily milking had no effect on subsequent yield (Carruthers *et al.*, 1989; Wilde and Knight, 1990). Other reports show a carry over effect of once daily milking on yield for at least up to seven weeks (eg Njaritta, 1989). The complete recovery of milk yield after the resumption of twice daily milking implies that a short period of once daily milking had no long lasting detrimental effect on the secretory ability of the mammary epithelial cells of these cows. The carry-over effect of once daily milking on protein and lactose concentration for ILSCC and IHSCC cows respectively, shows that the mechanisms regulating the concentration of these components take time to recover.

5.4 SOMATIC CELLS

Once daily milking significantly increased SCC of IHSCC ($P < 0.001$) but not that of ILSCC group (interaction $P < 0.1$). Previous studies considered the effects of milking frequency or infection on SCC but not the interaction between the two. The increase in SCC with bacterial infection is well established (Giesecke and Van den Heever, 1974; Holdaway, 1990). Bovine mastitis is a result of colonisation of the mammary gland by pathogenic bacteria, causing inflammation. One of the manifestations of inflammation of the bovine mammary gland is the migration of PMN to the site of injury (Paape *et al.*, 1978). PMN (the dominant cell population in mastitic milk) which migrate from the blood and enter the milk, are important in defending the udder against invading bacteria. Once daily milking has also been shown to increase the SCC of milk in a short term trial and a full lactation study (Mackenzie *et al.*, 1990; Holmes *et al.*, 1992). In both studies, clinical mastitis was low and incidence of infection measured bacteriologically in the later study showed no difference between once and twice daily milked groups. It was concluded in those

studies that the observed SCC increases were due to physical and/or physiological changes in secretory tissue brought about by milking once a day.

A simple explanation of the observed increase in SCC in the IHSCC group milked once daily would, therefore be, due to a slight increase in the number of infected quarters during treatment period and also physiological changes brought about by once daily milking. However, this explanation cannot fully explain the results since the total somatic cells secreted per day measured during the same period was not affected by once daily milking in both treatment groups, despite the recorded increase in infection for the IHSCC group milked once daily. While infection caused an increase in SCC, cellular response elicited by different pathogens also tend to vary. In at least one experiment, once daily milking caused no significant change in SCC (Njaritta, 1989). It is evident from the present results that the slight increase in the incidence of infection in the IHSCC once daily milked cows and/or once daily milking did not cause a significant change in total number of somatic cells infiltrating the mammary gland. However, higher reduction in milk yields were recorded for IHSCC (26%) compared to ILSCC (14%) once daily milked cows. It is therefore reasonable to associate an observed increase in SCC in IHSCC with the 'concentration effect' due to reduced milk volume. Further research is needed to confirm these results.

Whatever the mechanism involved, the most important observation is that once daily milking increased the SCC of cows which had higher SCC but not those with low SCC. These results have implications for mastitis detection and once daily milking in late lactation as a normal practice in New Zealand dairy farming. They do emphasise that SCC can be used with a relatively high degree of accuracy for detection of mastitis cows whether milked once or twice daily. The fact that once daily milking increased further the SCC of cows with higher incidence of infection may even increase the accuracy with which subclinically infected cows can be distinguished from those which are not infected.

In conclusion, mastitis, mainly caused by udder infection, continues to be a disease of economic importance to the dairy industry. The primary objective of any indirect method intended for the diagnosis of mastitis must rely on an accurate determination of inflammation. While SCC of milk samples has been widely adopted as a convenient measurement for monitoring the progress of mastitis in a herd and also embodied in some milk payment schemes, its interpretation depends on the consideration of many other factors which may also influence the level of somatic cells in milk.

During the present trial, the effects of once daily milking on milk yield, composition and SCC for IHSCC and ILSCC cows in late lactation was investigated. Incidence of infection was also measured at the beginning and the end of the experiment. The results show that, at the end of the 5 weeks treatment period, the incidence of infection was slightly increased for IHSCC cows milked once daily. Two clinical mastitis quarters were recorded for IHSCC once daily milked cows, indicating an increase in severity of infection. These results suggest the existence of an association between once daily milking and the new infection rate, at least for cows with higher incidence of mastitis. This association and the mechanisms involved in causing new infection in quarters milked once daily needs further investigation. For a significant change in SCC to occur in composite milk samples, the change of SCC in the individual quarter foremilk samples due to infection must be large enough to overcome the dilution effect of the milk from the uninfected quarter and vice versa. The results of the present trial confirms earlier findings that SCC is able to distinguish infected from uninfected cows depending on the definition of infection and the threshold value for SCC used.

Once daily milking decreased the yields of milk, fat, protein and lactose in both ILSCC and IHSCC cows, but the extent of decrease was higher for the IHSCC group on once daily milking. The loss of milk production attributed to once daily milking or mastitis is quite considerable by any account, but this particular set of data has shown a significant interaction between the effects of mastitis and once daily milking on milk yield. Thus the presence of subclinical infection combined with once daily

milking has a larger effect on milk production than would be expected in healthy cows milked once daily. The results also showed a rapid yield recovery when the once daily milked cows were brought back to twice a day milking. Apart from decreasing milk production, once daily milking caused some changes in milk composition. The combination of lower milk solids and compositional changes which are associated with an increase in SCC due to either infection and/or once daily milking can affect the quantity and quality of dairy products. While these changes have been demonstrated experimentally, their importance with respect to commercial production is yet to be explored.

The Livestock Improvement Corporation currently offers a somatic cell counting service for individual cows. The results obtained in this trial suggest that SCC in late lactation would provide the dairy farmer with accurate and reliable information regarding the extent of subclinical udder infection regardless of milking frequency.

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