FACTORS AFFECTING THE RESPONSE OF

DAIRY COWS TO ONCE DAILY MILKING

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of the requirements for the degree of

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ABSTRACT

The aims of this study were to evaluate the effect of once daily milking during mid-lactation and to identify factors which could be used to predict the response of dairy cows milked once daily. Factors studied included intramammary pressure (IMP), udder volume, residual milk and milk composition. A total of 32 Friesian cows, sixteen high breeding index (HBI) and sixteen low breeding index (LBI) (2-11 years old) were used in the study. Within each breeding index group, cows were further divided into 2 groups, cows were then allocated to either once daily milking (treatment group) or twice daily milking (control group) so the groups were balanced for breeding index and age.

The experimental period was divided into 3 main periods, a pre-treatment (3 weeks), a treatment (3 weeks) and a post-treatment period (7 weeks). During the pre-treatment period IMP (before morning and afternoon milking), empty udder volume after morning milking, residual milk and peak flow rate were measured. During the treatment period intramammary pressure was measured at 15 hr, 20 hr and 24 hr after milking. During the first three days of the post-treatment period IMP was measured before afternoon milking and udder volume after afternoon milking were measured. Milk yield and composition were measured during the whole period.

1) On average once daily milking reduced daily yields of milk, fat, and protein by 2.8 kg/cow (19%), 0.08 kg/cow (14%) and 0.08 kg/cow (16%) respectively in comparison with the control group.

2) Following cessation of the treatment, the average daily
production of cows previously milked once daily was 1.1 kg (7%) milk, 0.06 kg (9%) fat, 0.02 kg (4)
lactose less than that of the control group.

3) The decrease in milk yield for the individual cows over the
treatment period ranged from 12% to 46%.

4) The concentration of fat and protein increased while that of
lactose decreased during the treatment period in cows milked
once daily.

5) Measurement of the milk yield and milking times were not
sufficiently precise to allow the calculations of residual milk
by a novel method.

6) There was a significant relationship between IMP 15 hr
after previous milking (P < 0.05) and the decrease
in milk yield due to once daily milking.

7) The response to once daily milking was not related to other factors
such as IMP at 20 hr and 24 hr after milking, udder volume or
milk flow.

Possible reasons for the failure of these factors to predict
the response of cows to once daily milking are discussed.

It is concluded that further studies are needed to increase our
understanding of the contribution of residual milk to the physical and
chemical inhibition of milk secretion.
ACKNOWLEDGEMENT

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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCS</td>
<td>Body condition score</td>
</tr>
<tr>
<td>CO2</td>
<td>Carbon Dioxide</td>
</tr>
<tr>
<td>H2O</td>
<td>Water</td>
</tr>
<tr>
<td>HBI</td>
<td>High breeding index</td>
</tr>
<tr>
<td>Hg</td>
<td>Mercury</td>
</tr>
<tr>
<td>hr</td>
<td>Hour</td>
</tr>
<tr>
<td>IMP</td>
<td>Intramammary Pressure</td>
</tr>
<tr>
<td>kg</td>
<td>Kilogram</td>
</tr>
<tr>
<td>l</td>
<td>Litre</td>
</tr>
<tr>
<td>LBI</td>
<td>Low breeding index</td>
</tr>
<tr>
<td>mm</td>
<td>Millimeter</td>
</tr>
<tr>
<td>s.e</td>
<td>Standard error</td>
</tr>
<tr>
<td>U.S.A</td>
<td>United States of America</td>
</tr>
<tr>
<td>SCC</td>
<td>Somatic cell count</td>
</tr>
</tbody>
</table>
CHAPTER ONE

INTRODUCTION

It is well established in dairy cattle husbandry that, to achieve optimum milk production cows must be milked at least twice daily. During the last few decades, however, some dairy farmers in Northern Hemisphere have opted for thrice daily milking. In New Zealand and Australia, on the other hand, interest has been to reduce milking frequencies on a daily or weekly basis.

Reducing milking frequency inevitably results in production losses, however, the physiological mechanism regulating milk production under reduced milking regimes remain uncertain.

Previous studies suggested that the decrease in milk production when cows are milked less frequently is due to: 1) limited storage capacity of the udder (Tucker, 1961; Schmidt, 1971); 2) the presence of chemical inhibitors in the milk (Linzel and Peaker, 1971; Henderson and Peaker, 1984; 1987); 3) a reduced milking stimulus (Cowie et al., 1980); 4) increased residual milk yield due to inadequate duration of milk ejection relative to the amount of milk in the udder (Woolford et al., 1982). In none of these studies, however, have measurements of factors which may limit milk production been made in conjunction with response of the cows to once daily milking.

This thesis reviews the literature on factors which may influence the response of cows to once-a-day milking, and presents results of an
experiment to identify those factors which are considered most important in affecting milk production.
CHAPTER TWO

LITERATURE REVIEW

2:1 EFFECT OF MILKING FREQUENCY ON MILK YIELD AND COMPOSITION

2:1:1 Thrice Daily Milking

Although in principle it is possible to milk cows more frequently, milking three times a day is the greatest frequency found in practice. Thrice daily milking increased milk yield by 3% to 40% in comparison with twice daily milking (Ludwick et al., 1941; Morag, 1973; Pearson, 1979; Poole, 1982; De Peters, 1985). There was however, no significant change in milk composition (Henderson et al., 1983; Gisi et al., 1986). Amos et al. (1984) and Allen (1986) found that the increase in lactation yield was higher in heifers, in response to three times daily milking than in mature cows (25.2% vs 18.5%). Moreover the increases were higher in the late stage of lactation than in early lactation (Cash et al., 1950; Pellisier et al., 1978; Pearson et al., 1979). The increase in milk production when cows are milked three times daily arises from: 1) An increase in daily production which occurs immediately after the imposition of the treatment, 2) a higher peak production and 3) increased persistency resulting in longer lactations (Elliot, 1961; Poole, 1982).

The interest in milking three times daily in the Northern Hemisphere arises from the following needs: first, as a means of increasing output from fixed capital investments; secondly, to take advantage of
the increasing automation of the milking parlour and therefore reduced labour requirements; and thirdly, because modern cows are high yielding and milking three times reduces udder strain. A recent economic evaluation of three times daily milking in U.S.A showed that with current milk and feed prices, the regime would only be profitable if the increased cost of labour was very low compared to the increased milk yield (Culotta and Schmidt, 1988).

Consequently milking three times daily is unlikely to be economical in New Zealand where the cows are grazed on grass throughout the year and milk production per cow is relatively low. In addition automation of the milking parlour is minimal and high labour input is required in milking operations. Although it is clear that thrice daily milking increases milk production, its acceptance or rejection in practical dairy farming is, nonetheless, reliant upon economic rather than biological factors.

2:1:2 Twice Daily Milking

Even though more frequent milking results in greater production, twice daily milking has been practiced for centuries possibly since the beginning of the dairy industry. Until recently it was considered that optimum production from twice daily milked cows was achieved when intervals between milkings were 12 hr (Bryant, 1978). On the other hand, several studies indicated that milking intervals up to 16 hr did not significantly affect milk and fat yields (Table 2.1) (Adapted from Dodd, 1984).
There are however differences between animals in the response to these interval lengths. For example, heifers produced less when milked twice daily with intervals of 8 hr and 16 hr than when the intervals were equal (Schmidt, 1971, Dodd and Griffin, 1977).
Table 2.1 The yields of milk and fat produced by cows milked at equal and unequal intervals (Adapted from Dodd, 1984)

<table>
<thead>
<tr>
<th>Intervals(hr)</th>
<th>Cows(n)</th>
<th>Duration(days)</th>
<th>Milk(kg)</th>
<th>Fat(kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 : 12</td>
<td>7</td>
<td>232</td>
<td>2533</td>
<td>114</td>
</tr>
<tr>
<td>16 : 8</td>
<td>7</td>
<td>232</td>
<td>2477</td>
<td>112</td>
</tr>
<tr>
<td>12 : 12</td>
<td>11</td>
<td>264</td>
<td>2920</td>
<td>143</td>
</tr>
<tr>
<td>16 : 8</td>
<td>11</td>
<td>264</td>
<td>2973</td>
<td>146</td>
</tr>
<tr>
<td>12 : 12</td>
<td>7</td>
<td>complete</td>
<td>2541</td>
<td>96</td>
</tr>
<tr>
<td>14 : 10</td>
<td>7</td>
<td>lactation</td>
<td>2562</td>
<td>97</td>
</tr>
<tr>
<td>12 : 12</td>
<td>11</td>
<td>280</td>
<td>3203</td>
<td>129</td>
</tr>
<tr>
<td>15 : 9</td>
<td>11</td>
<td>280</td>
<td>3145</td>
<td>128</td>
</tr>
<tr>
<td>12 : 12</td>
<td>17</td>
<td>280</td>
<td>3593</td>
<td>143</td>
</tr>
<tr>
<td>16 : 8</td>
<td>17</td>
<td>280</td>
<td>3470</td>
<td>142</td>
</tr>
<tr>
<td>12 : 12</td>
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<td>305</td>
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<td>236</td>
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<td>14 : 10</td>
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<td>305</td>
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<td>243</td>
</tr>
<tr>
<td>16 : 8</td>
<td>35</td>
<td>305</td>
<td>6161</td>
<td>238</td>
</tr>
<tr>
<td>12.5 : 11.5</td>
<td>82</td>
<td>266</td>
<td>4910</td>
<td>186</td>
</tr>
<tr>
<td>14.5 : 9.5</td>
<td>82</td>
<td>266</td>
<td>4800</td>
<td>181</td>
</tr>
</tbody>
</table>
2:1:3 Milking Three Times in Two Days

Eldridge and Clark (1978) studied the effect of milking three times in two days on milk yield. Milk yield was reduced by 11% to 18% in comparison with milking twice daily. Copeman and Napper (1982) using identical twins observed that milking three times in two days at intervals of 11 hr and 18.5 hr resulted in 6.4% and 2.8% decrease in milk and milkfat yields respectively. Woolford et al. (1985) using similar intervals reported losses of 6.8% and 2.8% in milk and milkfat production respectively. Much larger losses were apparent for heifers in which production of milkfat was reduced by 12.9%.

2:1:4 Once Daily Milking

The effects of reducing milking frequency from twice to once a day have been examined in a limited number of experiments. Claesson et al. (1959) compared once daily milking with twice daily milking over complete lactations. They observed 50% and 40% losses in milk for the first and second lactation respectively. The percentage decrease was greater in early lactation and in younger cows. Furthermore the concentration of milkfat increased while that of lactose decreased for once daily milking compared to twice daily milking.

Short term experiments towards the end of lactation showed smaller reductions in milk and milkfat yields, ranging from 15% to 37% (Parker, 1965; Wilson, 1965; Bryant, 1978). The decrease in milk production when cows are milked once daily is due to a decrease in daily production as well as shorter lactations (Claesson et al., 1959;
There are variations between cows in their response to once daily milking which is not related to level of milk production (Parker, 1965). In Parker’s experiment a cow milked twice daily produced 29 kg of milkfat more than her twin mate milked once daily. In contrast another cow produced 1.3 kg less than her twin mate on once daily milking. Just what characteristics are involved in accounting for the variation in response to once daily milking are not known.

2:1:5 Missed Milkings

Milking regime has a great influence on the daily routine of the farmer. Occasionally the farmer might find that he has to miss a milking and it is therefore important for him to know the effect on production.

Omitting one milking per week showed no significant losses in production (Autrey et al., 1963; Wilson, 1965; Radcliffe et al., 1973; Maroske et al., 1984).

Missing two consecutive milkings however, caused a reasonable reduction in both milk yield and milk constituents. Radcliffe et al. (1973), observed a reduction of 14% for both milk and the main solids components when two consecutive milkings were missed. The effects were greatest early in lactation and in high producing cows. Moreover there were compositional changes resulting from missing two consecutive milkings which were similar to those occurring in cows with subclinical
mastitis. These include increases in pH, chloride concentration and somatic cell count (Bailey et al., 1973). These changes in milk composition were associated with poorer processing properties of the milk such as reduction in heat stability and curd firmness.
Several investigators have studied the effect of milking frequency on body weight (Parker, 1965; Pearson et al., 1979; Poole, 1982; De Peters et al., 1985). In short term studies, measurements of bodyweight showed that there was no difference between cows milked once or twice daily (Parker, 1965). Similar results were observed by Pearson et al. (1979) comparing twice and thrice daily milked cows.

In contrast Poole (1982) and De Peters et al. (1985) in longer term studies observed that, the cows milked three times per day gained less weight than those milked twice daily. Similarly cows milked once daily over the whole lactation gained more weight compared to control cows (Claesson et al., 1959).

Data on feed intake showed that there was no immediate difference in feed intake between cows milked three or once compared to those milked twice daily (Parker, 1965; Pearson et al., 1979). However, in a long term study Poole (1982) observed an increase in food intake for the thrice daily milked group, arising in the second half of the treatment period. It has been suggested that the immediate increase in production in three times milked cows is due to either a preferential utilization of feed nutrients for milk production or the stimulation of a higher rate of tissue catabolism (Porter et al., 1966 and Amos et al., 1985).

No long term study has been done on the effect of once daily milking on feed intake. A study of this nature would be useful because if cows
milked once per day consume less feed then more cows could be carried to partially compensate for the loss in per cow production. Alternatively cows milked once daily and gaining weight at the end of the lactation may produce more in the next lactation because they may calve in better condition.
2.3 EFFECT OF MILKING FREQUENCY ON UDDER HEALTH

Udder health is of great economic importance to the farmers because every year they spend much time and money controlling and treating udder diseases. Therefore the effects of any new management technique on udder health need to be considered.

Jarret, (1977) suggested that thrice daily milking might result in reduction in severity of infection because of shorter incubation time for infectious organisms in the udder between milkings. Indeed Pearson et al. (1979) reported a reduction in Wisconsin Mastitis Test (WMT) scores when cows were milked thrice per day. Poole (1982) observed that incidence of mastitis was slightly less amongst the cows milked three times daily. In contrast Waterman et al. (1983) reported that the incidence of mastitis were independent of milking frequency. In this particular herd, the incidence of infection was always maintained at low levels, and on farms with a higher incidence of infection thrice daily milking may have a beneficial effect on mastitis.

Regarding the reduction of milking frequency, Natzke et al. (1965) noted an increase in mastitis in gland samples from cows which had missed an afternoon milking. Maroske et al. (1984) observed an increase in (WMT) scores but not clinical mastitis in cows which missed one milking. On the contrary (Autrey et al., 1963; Radcliffe et al., 1973; Woolford et al., 1985) found no mastitis resulting from omission of milkings.

Clearly the effect on udder health needs to be evaluated in any study
on the effect of milking frequency on milk production.
2.4 MILK DISTRIBUTION AND REMOVAL

Milk secretion is a continuous process, while milking occurs intermittently. Milk secreted between milkings is stored in the gland and must be available for rapid removal at milking.

2.4.1 Distribution of Milk in the Gland

There are four stages of milk removal with corresponding fractions referred to as foremilk, bulkmilk, stripplings and residual milk.

Foremilk is the milk present in the udder and teat cisterns and large ducts, and can be drawn off through the teat without milk ejection.

Bulk milk is the milk present within the alveoli and smaller ducts and is retained until it is forcibly ejected to the exterior through the neuroendocrine milk ejection reflex. Stripplings is the milk in the gland cistern which can be machine stripped by adding weight to the milking unit towards the end of milking. This action has the effect of reopening the passage between the teat and gland cisterns and thus allows milk flow to recommence (Williams and Mein, 1982; Woolford and Phillips, 1982). However bulk milk and stripplings may be one thing if a good, effective milking machine is used. Residual milk yield is the milk held in the alveoli and small ducts after milking and it can only be removed by injecting pharmacological doses of oxytocin and remilking the cows.
2.4.2 Milk Ejection

Milk-ejection is the mechanism by which preformed milk in the mammary glands is forcibly ejected from the alveoli towards the gland and teat cisterns under the influence of milk ejection reflex during suckling or milking.

Tactile stimulation of the udder during suckling or by the action of the teat cup during machine milking activates pressure sensitive receptors in the dermis of the teat and the stimuli are transformed into nerve impulses. The nerve impulses travel via the spinothalamic tract and dorsal longitudinal fasciculus to the paraventricular and supraoptic nuclei in the hypothalamus (Grosvenor and Mena, 1974). The neurons within these two nuclei synthesise and package oxytocin in vesicles which are transported from the cell body down the axons and stored within the posterior pituitary (neurohypophysis) (Goodman and Grosvenor, 1983). Upon stimulation the axon terminals discharge oxytocin into the circulation where it travels through the jugular vein to the heart and lungs then back to the heart and finally to all remaining parts of the body including the mammary gland where it binds to specific receptors located on the myoepithelial cells and causes them to contract (Cowie et al., 1980)

2.4.3 Factors Affecting Milk Flow Rates

The rate of milk flow of dairy cows is determined by the physiological
factors of the cow and the physical properties of the milking machine. Only physiological factors will be dealt with here.

2.4.3.1 Milk yield and intramammary pressure

Within cows the rate of milk flow is correlated with the volume of milk in the udder at the beginning of milking. For example larger volumes of milk, resulting from extended milking intervals, will increase milk flow rate (Dodd and Foot; 1947; Sandvick, 1957; Johansson and Malven, 1960). Similarly milk flow rate is higher in early than in late lactation (Johansson and Malven, 1960). Milk flow rate also increases with age mainly due to increasing milk production (Johansson and Malven, 1960). Cows selected for higher milk production have higher peak flow rates than lower yielding cows (Davey et al., 1983; Peterson, et al., 1986). This is at least part because of a high genetic correlation between milk yield and peak flow rate (Sandvick, 1957; Touchberry and Markos, 1970; Miller, 1976).

The accumulation of milk in the udder increases intramammary pressure (Schmidt, 1971). When the udder is filled with milk, a positive pressure exists within the mammary gland. During milking the pressure below the teat is reduced to the level of vacuum in the milking machine. This creates a pressure difference across the teat orifice and causes it to open and milk to flow. The greater the pressure difference the faster the flow.
2.4.3 Teat diameter

For cows which do not differ in either milk volume or in intramammary pressure, the most important anatomical factor determining the rate of milk flow is the nature of the teat opening (Schmidt, 1971). This includes the diameter and length of the teat canal and the tonus of the circular muscle and connective tissue surrounding the meatus.

The rate of milk flow through the canal is proportional to the diameter of the orifice which in turn is controlled by the tonus of the surrounding muscle. Correlations of 0.3-0.5 between teat canal diameter and peak flow rate were obtained in cows of different ages (Johansson, 1959). Baxter et al. (1950) compared the rate of flow during ordinary machine milking of eight quarters of cows. The peak flow rate ranged from 0.5 to 1.3 kg/minute. When a teat cannula was inserted to standardise the teat canal diameter the variation in peak flow rate was greatly reduced and ranged from 1.1 to 1.3 kg/minute. Johansson and Malven (1960) observed that for each increase in the diameter of the orifice of 1 mm the peak flow increased by 0.6 kg/minute.

In an attempt to determine the relative importance of the tonus of sphincter muscles Buhr (1958) observed that anesthesia of the teat increased the rate of milk flow. Anesthesia supposedly incapacitated the sympathetic nerves that control the tonus of circular muscle around the streak canal (Schmidt, 1971).
2.5 FACTORS AFFECTING THE RESPONSE TO VARIATIONS IN MILKING FREQUENCY

2.5.1 Intramammary Pressure and Udder Volume

Intramammary pressure (IMP) within the alveoli is the factor which is most likely to affect milk secretion in extended milking intervals. However, IMP has been measured through cannulae placed in the teat or gland cistern of dairy animals (Schmidt, 1971). This method probably does not measure exactly the pressure developed in the alveoli and the small collecting ducts of the udder. Furthermore IMP recorded through cannulae may be unreliable because udder manipulations connected with teat cannulation may result in some milk ejection and elevation of the IMP (Witzel and McDonald, 1965).

2.5.1.1 Changes of IMP between milkings

Measurement of IMP between milking intervals showed an initial rapid increase over the first 1 to 2 hr after milking (Korkman, 1953; Schmidt, 1960; Tucker, et al., 1961). This is hydrostatic pressure which is caused by the residual milk moving from the alveoli and smaller ducts into the cisterns (Korkman, 1953). This phase is then followed by a slow increase in pressure, as milk secreted drains into the teat and gland cisterns. Finally, development of pressure in the gland probably occurs as the storage capacity of the gland is approached.

The rate of increase in pressure per unit of milk secreted is lower in high yielding cows, in rear glands and in early lactation (Dodd and
Griffin, 1977). Heifers have low IMP compared to older cows when the accumulated milk is small, but higher when the milk volume is larger (Korkman, 1953). This is probably because in heifers the collecting ducts, gland and teat cisterns are smaller relative to the amount of milk produced than in the older cows in which storage tissues are possibly stretched with age. Moreover the storage tissues of the rear glands and the high producing cows may also be more developed than that of the fore glands and low producing cows for the same reasons.

2:5:1:2 Effect of IMP on milk secretion

It is well established that IMP does not influence milk secretion rate when milking intervals are less than 12 hr (Turner, 1953; Turner, 1955; Elliot, 1960; Porter et al., 1966; Henderson, 1984). Therefore it is probable that relieving IMP is not responsible for the increase in milk yield on three times milking regime. Schmidt (1960), Tucker et al. (1961) observed that IMP started to inhibit milk production 16 hr after milking (Fig. 2.1). However, the time needed for the intramammary pressure to inhibit milk secretion varies between cows depending on age, udder volume, level of milk yield and the amount of residual milk (Turner, 1955; Johanson and Malven, 1960; Tucker et al., 1961).
Figure 2.1 Relationship of udder pressure and secretion rate
(Adapted from Schmidt, 1971)
2.5.2 Udder Volume

Differences in udder volume have been observed at different ages and stage of lactation (Davis et al., 1983; Davey et al., 1983). Although the relationship between udder volume and the susceptibility to production loss on once daily milking has not been studied, it might be presumed that cows with large udder volumes might be able to tolerate extended milking intervals better than those with smaller udder volumes. There is no doubt that the greater losses observed in heifers when milked three times in two days, relative to mature cows (Woolford et al., 1985) were related to their smaller udder volumes. Davis and Hughson, (1988) showed that among New Zealand Jersey cows, many animals posseses the udder capacity to contain 24 hr worth of secretion. Because functional udder capacity is correlated with empty udder volume (Peaker, 1980; Davis and Hughson, 1988) the later might also be related to the ability of cows to tolerate extended milking intervals.

2.5.3 Udder Productivity

Davis et al. (1983) found that the differences in production between HBI and LBI Friesian cows were due to higher udder productivity in HBI cows. Udder productivity (per ml of tissue) seems to be under the influence of galactopoietic hormones or key enzyme activities in the udder. High udder productivity is likely to increase the rate of udder filling. This may mean that HBI Friesians will be more sensitive to extended milking intervals than LBI Friesians.
2.5.4 Residual Milk Yield

Residual milk is the milk left in the gland after the teat cups have been removed. It is expressed as a percentage of the total amount of milk removed from the gland at the milking. Values ranging from 5% to 50% and 25% to 50% of the total milk and milkfat respectively have been observed (Johansson, 1952; Koshi and Peterson, 1955; Davis and Hughson, 1988). Reasons for the variation are unknown, but probably involve differences in the rate and pattern of oxytocin secretion and in the characteristics of milk flow in the gland. In addition percentage residual milk is lower in high producing than low producing cows (Anderson et al. 1968; Ludri, 1984).

At milking, the release of oxytocin causes the contraction of the myoepithelial cells around the alveoli and thus increasing the pressure within the lumen forcing milk towards the gland and teat cisterns. The volume of the milk in the alveoli is reduced further as milk flows under pressure towards the teat to replace milk removed at milking. Apparently the contraction of the myoepithelium is insufficient or not sustained for a sufficient period to cause complete collapse of the alveoli and expulsion of all the milk from the lumen. Thus, some milk always remains in the gland. Injection of a pharmacological dose of oxytocin at the end of milking does, however, force this residual milk into the gland and teat cisterns from whence it can be removed. The amount of milk or milkfat left behind in the secreting region of the gland after milking is completed, may be responsible for inhibiting secretion rate when milking intervals are extended (Elliot, 1961; Brandsma, 1978; Woolford et al., 1985; Peaker and Blatchford, 1988).
The action may be directly on the rate of milkfat secretion since the concentration of fat is extremely high. It also reduces the storage capacity of the udder (Davis and Hughson, 1988).

2:5.4:1 Milking frequency and amount of residual milk

Very few experiments have examined the relationship between residual milk yield and milking frequency. Deshmuck and Ludri (1979) observed that the quantity of residual milk was greater with twice daily milking than at three and four times milking.

In a study where milk ejection was enhanced by administering oxytocin immediately before teatcup attachment, and therefore lowered residual milk levels, cows milked once daily produced similarly to those milked twice daily (Woolford et al., 1982). This study suggest that, the residual milk left in the secreting region after milking plays some role in inhibiting milk secretion when cows were milked once daily.

2.5.4.2 Milk Flow Rates and Residual Milk

The initial premise that there is an inverse relationship between milking speed and milk yield or persistency has not been established unequivocally. It has been suggested that slow milking cows have lower persistency and produce less milk because they have high residual milk yield. Dodd and Foot, (1953) and Johansson and Malven (1960) found that lactation length was increased by 8 days and persistency index by 10 days for every 0.4 kg per minute increase in the maximum flow rate. Moreover in an experiment whereby the direct relationship between
residual milk and milk flow rate was determined, Rajamman (1966) observed that faster milking cows had lower residual milk.

2.5.4.3 The Intensity and duration of milk ejection

The amount of residual milk yield and milk fat in the udder is highly influenced by the intensity and duration of milk ejection (Woolford et al., 1985). Milk ejection is evoked by the reflex release of oxytocin (Folley and Knaggs, 1966; Cleverly and Folley, 1970; Momongan and Schmidt, 1970). Measurement of oxytocin concentration in the blood showed that, physiological releases of oxytocin up to 1-3 pmol/l were sufficient to evoke maximum ejection (Momongan and Schmidt, 1970; Landgraf et al., 1982; Schams et al., 1984). The amount of oxytocin and the pattern of release vary between cows (Schams et al., 1984). Furthermore Schams et al. (1984) and Phillips (1984) reported that correct timing of oxytocin release is more important for milk ejection and milk removal than the absolute value. These characteristics may determine the amount of residual milk remaining after milking.
2.6 THE MECHANISM BY WHICH MILKING FREQUENCY INFLUENCES MILK PRODUCTION

Several studies have been carried out in an effort to elucidate the physiological mechanisms responsible for the changes which occur in milk yield when cows are subjected to different milking regimes. There appears to be a number of factors involved and the contribution made by each probably varies depending on interval length and duration of the treatment.

2.6.1 Intramammary Pressure

The hydrostatic pressure, needed to oppose the osmotic movement of water into milk (Peaker, 1978), is much greater than the intramammary pressure involved in inhibiting milk secretion (Peaker, 1980). For many years intramammary pressure has been recognised to be a major factor in arresting milk secretion in long milking intervals. However, the mechanism of action was not established. Recent studies by Peaker, (1978; 1980), suggested that it is mammary distension rather than increased intramammary pressure *per se* which reduces milk secretion when milking intervals are extended.

The udder, because its tissues have a degree of elasticity, expands as milk accumulates between milkings, but because the tissues are not infinitely elastic the pressure also rises. Further accumulation of milk builds up pressure in the lumen of the alveoli and further mammary distension. Peaker (1980) suggested that the stretching of the alveoli cell interferes with their metabolism and as a result milk secretion
falls. If this is correct then the relationship between the intramammary pressure and the rate of milk secretion will not be simple. Nevertheless without a simple method of measuring secretory cell distension, intramammary pressure may still provide an indirect indication of the effect of milk accumulation on secretion. It seems reasonable to expect that the increase in pressure will be transmitted throughout the secretory system and that the pressure in the alveoli and collecting ducts will be similar.

2.6.2 Chemical Inhibitors

Linzell and Peaker (1971) proposed that a chemical inhibitor in milk modulates the rate of milk secretion. This hypothesis explains the increase in the rate of milk secretion that occurs when the frequency of milk removal is increased (Henderson et al., 1983; Henderson and Peaker, 1984). These studies also indicated that the site of action of the inhibitor is in the secretory alveoli.

The presence of milk constituents capable of inhibiting milk synthesis is supported by recent studies on the effect of milk fractions on lactose and casein synthesis by rabbit mammary explants in organ culture and in lactating goats (Wilde and Gamble, 1985; Wilde et al., 1987; 1988).
2.6.3 Hormonal Effects

2.6.3.1 Mammary Gland Growth

Milk production is a function of the number of secretory cells in the udder and their productivity. It was originally believed that the development of mammary gland is essentially complete at parturition (Cowie et al., 1980). However in goats, when extra milking stimulus was given, mammary growth took place in early lactation (Davis et al., 1980; Anderson et al. 1981).

Henderson et al. (1987), after several months of milking one half of a goat’s udder thrice daily and the other half twice daily, observed that the more frequently milked gland was larger than that milked twice daily. This suggests that either growth or reduced regression of the gland had taken place. Similarly there is also a possibility that in cows milked once daily the rate of secretory cell regression, which normally takes place as lactation advances, is increased and hence the progressive depression in milk yield (Claesson et al., 1959).

2.6.3.2 Galactopoiesis

In many species withdrawal of milking stimulus results in reduced milk secretion (Cowie et al., 1980). Thus, the continued secretion of prolactin is responsible for maintainance of lactation in many non-ruminant species while growth hormone may play the same role in ruminants (Cowie et al., 1980). It is tempting to suggest that the change in milking stimulus and hence the release of galactopoietic
hormones in cows milked once daily and three times daily may partly contribute to the change in milk production. However, applying an extra milking to one udder half had no effect on milk yield of the other udder half (Elliot, 1961; Morag, 1973; Henderson et al., 1983). Since both sides received blood supply of similar hormone content, the increase in production was not mediated by the release of a systemic hormone. Moreover, measurements of concentration of growth hormone and prolactin showed that there was no difference between cows milked three times and two times daily (Kazmer et al., 1986).

2.6.4 Mammary Blood Flow

Blood flow to the mammary gland determines the amount of nutrients available to the mammary gland for milk synthesis (Davis and Collier, 1985). It was originally believed that mammary distension following accumulation of milk caused a reduction in blood flow to the udder (Tucker et al., 1961; Pearly, et al., 1973; Fleet and Peaker, 1978). However, more recently Peaker, (1980) suggested that stretching of the secretory cells reduces their metabolic activity so they produce less CO₂ and other vasodilatory metabolites. As a result the diameters of the blood vessels constrict and blood flow falls. In whatever the mechanism, a fall in blood flow would mean a decrease in the delivery of precursors to the secretory cells and therefore reduced milk synthesis.

Wilde et al. (1987) observed an increase in secretory cell productivity as a result of milking thrice daily. Increase in cell productivity may necessitate an increase supply of nutrients per unit
of tissue, probably mediated by a relative increase in udder blood flow (Davis and Collier, 1985).

2.5.5 Reabsorption of secretion

Bailey et al. (1955), Claesson et al. (1959), Wheelock et al. (1965) observed that, with extended milking intervals of up to 24 hr lactose concentration in milk decreases. Lactose is believed to escape through gaps opened up between the secretory cells as the epithelium is stretched. It would be accompanied by reabsorption of water because of the need to maintain milk in osmotic equilibrium with blood (Wheelock, et al., 1965) and therefore contribute to the reduction in milk yield in once daily milked cows.

The possibility of reabsorption of milk from the gland between milkings was also suggested by Tucker (1961) and Porter et al. (1966) as an explanation for the decrease in yield when cows were milked twice per day as compared to thrice per day. It was suggested that reabsorption may take place in high yielding cows (above 31 kg) while secretion continues to occur at a reduced rate.

2.7.6 Milk Composition

There exist differences between breeds in the composition of their milk and in general the higher the lactation milk yield, the lower the fat and protein concentration of milk (Crabtree, 1984). High yielding cows may suffer more when milked less frequently because of udder distension. It is likely that Jerseys and other breeds with relatively
concentrated milks are less susceptible to production loss with extended milking intervals. Indeed, those species which have naturally long intervals between suckling such as rabbits (24 hr), tree shrew (48 hr) and sea lions (8 days) all produce milk with relatively high nutrient concentration (Oftedal, 1984).

2.8 Objectives of the Present Study

The review of literature indicates that the response of cows to milking frequencies is determined by several interacting factors. The most important factors influencing milk secretion in reduced milking frequency seem to be those which interfere with the stretching of the udder as milk accumulates.

The role of IMP in regulating milk secretion in long milking intervals is well established (Section 2.5.1.2). Korkman (1953) observed that the increase in IMP per unit of secreted milk was an individually inherited characteristic. Therefore it appeared worthwhile to investigate if a relationship existed between the IMP during the milking interval of once daily milking and the response of cows to once daily milking. Furthermore the effect of IMP is mediated through mammary distension (Tucker et al. 1961; Peaker, 1980). It is speculated that cows with smaller udder volumes, for example heifers, will be more susceptible to production loss on once daily milking. For this reason, the influence of udder volume and age on the response of cows to once daily milking was studied.

Because lactation milk yield is negatively correlated to the
concentration of solids in the milk (Crabtree, 1984) it is expected that cows with more concentrated milk will be more tolerant to once daily milking. Therefore this study looked at milk composition in relation to the susceptibility of cows to production loss when milked once daily.

The level of residual milk directly influences the rate of milkfat secretion since the concentration of fat is extremely high. It also reduces the storage capacity of the udder which is important to the relationship between milk yield and interval. Furthermore the difference in the amount of residual milk between cows may result from differences in milking characteristics. Therefore peak flow rate, being the best measure of milk flow rate (Griffin and Dodd, 1962), was also studied. The assumption was that cows with fast milking cows will have low residual milk and thus less affected when milked once daily.

The difference in production between HBI and LBI Friesian cows is due to higher udder productivity in HBI cows (Davis et al., 1983). High udder productivity is likely to increase the rate of udder filling. This may mean that HBI Friesian cows will be more sensitive to extended milking intervals. Thus, it seemed appropriate to determine if breeding index influenced response of cows to once daily milking.
3.1 ANIMALS

A total of 32 Friesian cows on the Dairy Cattle Research Unit at Massey University were used. They included 16 high breeding index (HBI) and 16 low breeding index (LBI) cows. Half of the cows from each breeding index group were in their first lactation, having calved at about 2 years of age while the remaining half were mature cows with ages between 3 and 11 years. Cows within BI, and age were allocated to either once daily or twice daily milking regimes. Details of the average age, days of lactation, milk yield and breeding index of the cows in the two milking regimes are presented in Table 3.1.
Table 3.1 Mean (± s.e) age, day of lactation at start of experiment, and milk yield of cows assigned to once daily or twice daily milking regimes.

<table>
<thead>
<tr>
<th>Milking Frequency</th>
<th>Once daily</th>
<th>Twice daily</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>4.3 (±0.42)</td>
<td>4.4 (±0.45)</td>
</tr>
<tr>
<td>Lactation (days)</td>
<td>89.0 (±2.68)</td>
<td>91.0 (±1.86)</td>
</tr>
<tr>
<td>Milk Yield (kg)</td>
<td>15.6 (±0.57)</td>
<td>16.1 (±0.36)</td>
</tr>
<tr>
<td>HBI</td>
<td>132 (±1.84)</td>
<td>134 (±1.05)</td>
</tr>
<tr>
<td>LBI</td>
<td>106 (±1.45)</td>
<td>108 (±0.90)</td>
</tr>
</tbody>
</table>

3.2 EXPERIMENTAL DESIGN

The experiment was a 2x2 factorial design. The treatments imposed were milking frequency (twice and once a day milking) and breeding index (HBI and LBI), to give four treatments combinations, HBIx1, HBIx2, LBIx1 and LBIx2. The experiment was divided into three periods, a preliminary period, a treatment period and a post-treatment period.
The experiment was carried out over 14 weeks during November, 1987 to January, 1988. The length of time of the three periods and the various measurements made on the cows are indicated in Table 3.2

Table 3.2 Summary of the measurements made during the three periods of the experiment.

<table>
<thead>
<tr>
<th>Period</th>
<th>Duration</th>
<th>Measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(weeks)</td>
<td></td>
</tr>
<tr>
<td>Pre-treatment</td>
<td>3</td>
<td>Udder volume</td>
</tr>
<tr>
<td>(Milked twice</td>
<td></td>
<td>Milk yield and composition</td>
</tr>
<tr>
<td>daily)</td>
<td></td>
<td>Intramammary pressure</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Residual milk yield</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Milking characteristics</td>
</tr>
<tr>
<td>Treatment</td>
<td>3</td>
<td>Milk yield and composition</td>
</tr>
<tr>
<td>(Milked twice/once</td>
<td></td>
<td>Intramammary Pressure</td>
</tr>
<tr>
<td>daily)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-treatment</td>
<td>7</td>
<td>Milk yield and composition</td>
</tr>
<tr>
<td>(Milked twice</td>
<td></td>
<td>Intramammary pressure</td>
</tr>
<tr>
<td>daily)</td>
<td></td>
<td>Udder Volume</td>
</tr>
</tbody>
</table>
3.3 MANAGEMENT OF THE EXPERIMENTAL ANIMALS

The experimental cows were grazed together with the main herd on ryegrass/clover pastures throughout the experiment. The experimental cows were also milked with the main herd at 0600 hr and 1500 hr, i.e. the night interval was approximately 15 hr and the day interval was approximately 9 hr. Cows milked once a day were milked at 1500 hr.

3.4 MEASUREMENTS

3.4.1 Milking characteristics

All milking characteristics were measured at two consecutive morning milkings and the mean results were used in statistical analyses.

Milk flow rates were measured by recording cumulative milk volumes collected in milk sampling meters (True-test Distributors Ltd). Readings were taken every half-minute, from the time the last teat cup was put on until the teat cups were removed by automatic cup remover. Time was determined with a stop-watch. A graph of cumulative milk yield against time was plotted for each cow. Maximum flow rate (L/minute) for each cow was calculated from the slope of the linear portion of the graph for each day and calculating the mean value for the two days. Total cups on time was taken as the interval from the time the milking unit was attached to the udder until it was removed.

Average milk flow rate was calculated by dividing the total milk yield by the total cups on time.
3.4.2 Intramammary Pressure

Intramammary pressure was measured using an instrument designed and constructed by Dr. R.C. O’Driscoll (1987), Department of Physics, Massey University. The design of the equipment was based on a Tympanometer principle described by Phillips, (1955). Briefly, this instrument measured the pressure inside the udder by applying a measured force to a small portion of the distended surface of the udder, sufficient to flatten the area of contact without causing an indentation. The external force applied to the pressure plate was then equal and opposite to the intramammary pressure acting on the inside of the udder. It is assumed that the pressure measured by this means will be proportional to intraalveolar pressure but the extent relationship has not been established.

The instrument was sensitive to temperature and therefore had to be adjusted to zero before taking every measurement. Separate readings were taken for rear and fore gland and analysed separately. The frequency of IMP measurement is indicated on Table 3.3.
Table 3.3 Summary showing the frequency of IMP measurement during the three periods of the experiment

<table>
<thead>
<tr>
<th>Period</th>
<th>Week (number)</th>
<th>Frequency (test days/week)</th>
<th>Morning</th>
<th>Noon</th>
<th>Afternoon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-treatment</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3</td>
<td>-</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>4-6</td>
<td>5</td>
<td>2</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Post-treatment</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

3.4.3 Residual Milk Yield

Residual milk yield was measured directly, and also estimated by calculations.

Direct measurement

Residual milk left in the udder after a morning milking was measured by the oxytocin method on 16 cows, eight HBI and eight LBI which were randomly selected from the 32 cows of the experiment. The selected cows were grazed separately from the herd on the evening before residual milk yields were measured. They were brought in for milking
after all other cows had been milked. After the routine machine
milking each of the 16 cows were injected subcutaneously with 10 i.u
oxytocin and remilked immediately. The residual milk was weighed and
sampled. Percentage residual milk yield was calculated as fraction of
the total yield times 100.

**Calculated residual milk yield**

Residual milk yield was calculated from milk yield data using a formula
shown below as developed by Dr D.D.S. Mackenzie, (personal
communication) (see appendix 2)

\[
R = \frac{(M_1 - S_1)}{(M_2 - S_1)} \quad \text{OR} \quad R = \frac{(M_2 - S_1)}{(M_1 - S_2)}
\]

where

- \( R \) = residual milk as a fraction of total in
  the gland in litres
- \( M_1 \) = Milk recovered at a.m milking in litres
- \( M_2 \) = Milk recovered at p.m milking in litres
- \( S \) = Rate of milk secretion in litres/hr
  (assumed to be constant over 24 hr).
- \( I_1 \) = Time interval from p.m to a.m milking in hours
- \( I_2 \) = Time interval from a.m to p.m milking in hours

3.4.4 Udder Volume

Udder volumes were measured by the method described by Davis et al.
(1983). Briefly, post-milking udder height (rear gland to base of rear
teat), width (average width measured approximately 8 cm above front and
rear teats), and length (from base of rear teat to anterior junction of
udder with abdomen) were measured. Half of the product of these measurements were taken as an estimate of udder volume. Udder volume measurements were made on two consecutive days after morning milking during the first week of the preliminary period. The mean for each cow for these two days was treated as one value in the analysis of variance. After the experimental period measurements were made on two consecutive days after afternoon milking and the mean calculated for each cow. Measurements were made by two operators on each cow before the experiment and by one operator after the experiment.

3.4.5 Milk Yield and Composition

During the whole period of 14 weeks measurements of milk yield and composition were carried out at the frequencies shown in Table 3.3. Milk samples collected at consecutive afternoon and morning milking were analysed separately for milk composition using a Milkoscan 140 A/B (A/S N Foss electric Denmark).

Daily yields of milk, fat, protein and lactose were then calculated by adding together the yields obtained at consecutive evening and morning milkings. The mean yields of milk were calculated for a) the one week of the pre-treatment period period b) the first 3 days of the treatment period c) the remaining 12 days of the treatment period d) seven weeks of post-treatment period.

These mean values were used in subsequent statistical analysis. Milk composition was treated in a similar manner.
Table 3.4 Summary showing the frequency of milk yield and composition measurements during the three periods of the experiment

<table>
<thead>
<tr>
<th>Period</th>
<th>Duration (weeks)</th>
<th>Week (number)</th>
<th>Frequency (test days/week)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-treatment Period</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Treatment Period</td>
<td>3</td>
<td>4-6</td>
<td>5</td>
</tr>
<tr>
<td>Post-treatment Period</td>
<td>7</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9-14</td>
<td>1</td>
</tr>
</tbody>
</table>

3.4.6 Body Weight and Condition Score

Cows were weighed and condition scored on the first day of the experimental period and two days after the end of the experiment. The condition score system used was that described by Scott and Smeaton, (1980).
3.4.7 Somatic Cell Count

Somatic cell count (SCC) was measured to determine the effect of milking once daily on udder health. Samples of milk were collected separately for each of the 32 cows on the first day of milking once daily and on the last day of milking once daily and analysed for SCC.

3.5 STATISTICAL ANALYSIS

All data were analysed using the Statistical Analysis System (SAS) computing package (SAS Institute, 1985).

The error structure of the means for IMP and milk yield and composition may differ due to differences in the number of observations contributing to each mean.

Yields of milk, fat, protein, and lactose and IMP, udder volume, milking characteristics and concentration of fat, protein and lactose were analysed by analysis of variance. The model used to define the data was:

\[ Y_{ijkl} = U + a_i + b_j + c_k + a.b + a.c + b.c + X_{ijk} + e_{ijkm}. \]

where

\[
Y_{ijk} = \text{an observation of milk yield, fat, protein, lactose}
\]

IMP, udder volume or milking characteristics for
the lth individual in the ith age class the jth BI class
and the kth milking frequency,

\[ U = \text{the unknown population mean of milk yield, fat yield,} \]
\[ \text{protein yield, lactose yield, IMP, udder volume and} \]
\[ \text{peak flow rate, average flowrate, machine time and} \]
\[ \text{concentration of fat, protein and lactose,} \]

\[ a_i = \text{the effect of the ith age (i =1 (2 years), 2 (3+ years)}. \]

\[ b_j = \text{effect of the jth BI (j = 1 (HBI) or 2 (LBI),} \]

\[ c_k = \text{effect of the kth milking frequency} \]
\[ (k = 1 \text{ (once daily milking) 2 (twice daily milking).} \]

\[ a.b = \text{the interactive effect of the ith age with the jth} \]
\[ \text{BI group} \]

\[ a.c = \text{the interactive effect of the jth BI group with kth} \]
\[ \text{milking frequency group} \]

\[ b.c = \text{the interactive effect of the ith age group with the kth} \]
\[ \text{milking frequency group} \]

\[ e_{ijkl} = \text{the random error associated with Yijkl observation} \]
\[ \text{which is assumed to be normally distributed with mean 0} \]
\[ \text{and variance } q^2 \]
LW and BCS were analysed by analysis of variance. The model used to define the data was:

\[ Y_{ij} = U + a_i + e_{ij}, \]

where

- \( Y_{ij} = \) the observation on the \( j \)th individual exposed to the \( i \)th treatment,
- \( U = \) the unknown population mean,
- \( a_i = \) the effect of the \( i \)th treatment, and
- \( e_{ij} = \) the random error associated with the \( j \)th individual exposed to the \( i \)th treatment assuming that \( e_{ij} \) is normally distributed with mean 0 and variance \( q^2 \).

The decrease in milk yield

The percentage decrease in milk yield due to once daily milking was determined as explained in appendix 1. A model was then developed to predict the decrease in milk yield due to once daily milking.

\[ Y_i = b_0 + b_{1Pi} + b_{2Vi} + b_{3Mpi} + b_{4MYi} + e_i, \]

where

- \( Y_i = \) observation on individuals for fall in milk yield (\( Y_i \)),
bo = average fall in milk yield when peak flow rate, udder volume, IMP are not considered,

\[ b_1 (b_2, b_3, b_4) = \text{regression of PF(UV, IMP, MY)} \]
\[ \text{on drop in milk yield,} \]

\[ e_i = \text{the random error associated with the } i\text{th individual and is assumed that } e_i \text{ is normally distributed with mean 0 and variance } q_2. \]
CHAPTER 4

RESULTS

4.1 Pre-treatment Period

The results for the pretreatment period are summarised in Table 4.1.

Cows assigned to twice daily milking produced a little more milk, fat, protein and lactose than those assigned to once daily milking although the differences were not significant.

High breeding index (HBI) cows produced significantly greater yields of milk ($P < 0.05$), fat ($P < 0.001$), protein ($P < 0.01$), and lactose ($P < 0.01$) than low breeding index (LBI) cows. Milk from HBI cows also had significantly higher fat ($P < 0.01$) and lactose ($P < 0.05$) concentration than milk from LBI cows. Milking characteristics i.e peak flow rate, machine time, and average flow rate did not differ significantly between breeding index group ($P < 0.05$).

Mature cows produced significantly greater yields of milk ($P < 0.01$), fat (0.001), protein (0.01) and lactose ($P < 0.05$) than heifers. Heifers gave milk with significantly higher lactose concentration ($P < 0.05$) than milk from mature cows. Udder volumes of mature cows were significantly larger than that of heifers ($P < 0.01$). Mature cows took significantly ($P < 0.05$) longer time to milk (8.44 min) than younger cows (6.32 min). Other milking characteristics i.e the average machine time and peak flow rate did not differ significantly between age groups. Just before morning milking intramammary pressure (IMP) in the
rear gland was significantly ($P < 0.05$) higher in heifers than for mature cows.

During this pre-treatment period the yields of milk, fat, protein and lactose were decreasing significantly at a rate of $0.08 \, \text{kg/day}$, $0.004 \, \text{kg/day}$, $0.0007 \, \text{kg/day}$, $0.0009 \, \text{kg/day}$ respectively. The contribution of the decrease to total coefficient of variation was $10\%$, $5\%$, $6\%$, and $9\%$ for yields of milk, fat, protein and lactose respectively.

There was a significant interaction between breeding index groups and milking frequency groups for protein yield ($P < 0.05$) which meant that HBI cows assigned to once daily milking regime produced significantly ($P < 0.05$) more protein than LBI cows assigned to once daily milking.

4.1.2 Treatment Period One

The results for the treatment period one are summarised in Table 4.2.

Figures 4.1-4.4 show that it took about three days for the effect of milking once per day to be fully realized. The treatment period was thus divided into two periods, the first consisting of days 1-3 and the second days 4-21.

Milking once daily reduced significantly yields of milk ($P < 0.001$), protein ($P < 0.001$) and lactose ($P < 0.001$). The yield of fat was also reduced though not significantly ($P > 0.05$). The average decrease per cow were $2.2 \, \text{kg (15 \%)}$, $9 \%$, $0.07 \, \text{kg (14 \%)}$ and $0.13 \, \text{kg (18 \%)}$ for milk, fat, protein and lactose respectively. The concentration of
lactose also decreased significantly ($P < 0.05$) in once daily milked cows. Just before morning milking IMP of cows milked twice per day was higher, though not significantly, than that of cows milked once daily. The trend was reversed however at noon and before the afternoon milking when cows milked once daily had significantly higher IMP than cows milked twice daily.

Breeding index group i.e 16 HBI and 16 LBI and age groups i.e heifers and mature cows were analysed together regardless of the milking frequency imposed. During the first three days of the treatment period HBI cows gave significantly higher yields of milk ($P < 0.05$), fat ($P < 0.01$), protein ($P < 0.05$) and lactose ($P < 0.05$) than LBI cows. HBI cows produced milk with significantly higher contents of lactose ($P < 0.01$) than milk from LBI cows. The intramammary pressure before afternoon milking was significantly higher in both rear ($P < 0.05$) and fore glands ($P < 0.001$) of LBI than HBI cows.

Mature cows produced significantly higher yields of milk ($P < 0.01$), and protein ($P < 0.05$) than heifers. On the other hand heifers produced milk with significantly higher concentration of lactose ($P < 0.01$) than milk from mature cows. The IMP before afternoon milking was significantly higher in both rear ($P < 0.01$) and fore glands ($P < 0.001$) of heifers than mature cows.

Yields of milk, protein and lactose showed a significant daily decrease over the first three days of the treatment period and the rate of decrease was $0.08$ kg/day, $0.002$ kg/day, $0.003$ kg/day for milk, protein and lactose respectively. The contribution of the decrease to
coefficient variation were 13%, 7%, and 6% for milk, protein, and lactose respectively. Similarly IMP of both rear and fore glands at afternoon milking showed a daily significant decrease over this period at a rate of 0.08 mm H2O/day and 0.09 mm H2O/day for rear and fore gland respectively. The contribution of this decrease to coefficient of variation was 2% and 4% for rear and fore glands respectively.

There was a significant interaction between age and milking frequency for milk (P <0.05) and lactose (P <0.05) and in this case heifers milked once daily produced significantly less milk and lactose than mature cows milked once daily. There was a significant age and treatment interaction for IMP of both rear (P <0.001) and fore (P <0.01) glands at noon and before afternoon milking in that heifers milked once daily had significantly higher IMP than mature cows milked once daily. There was a significant interaction between BI and age for IMP of the fore gland (P <0.05) in the morning and afternoon and in both cases HBI heifers had significantly lower IMP than LBI heifers whereas there was no corresponding difference for mature cows.

4.1.3 Treatment Period Two

The results for the treatment period two are summarized in Table 4.3.

Cows milked once daily produced less milk (P <0.001), fat (P <0.001), protein (P <0.001) and lactose (P <0.001) than cows milked twice daily. The decrease was 3.4 kg (23%), 0.1 kg (17%), 0.09 kg (18%) and 0.23 kg (25%) for yields of milk, fat, protein and lactose respectively.
Milk from cows milked once daily had significantly higher fat (P < 0.001) and lower lactose (P < 0.001) concentration than those milked twice daily. Before morning milking cows milked twice daily had significantly higher IMP at rear gland (P < 0.001) than cows milked once daily, while cows milked once daily had significantly higher IMP at both rear and fore glands (P < 0.001) both at noon and before the afternoon milking. During this treatment period HBI cows produced significantly more milk (P < 0.001), fat (P < 0.001), protein (P < 0.001) and lactose (P < 0.001) than LBI cows. Fat and protein and lactose concentrations were significantly higher in HBI than LBI cows.

Mature cows gave significantly more milk (P < 0.001), fat (P < 0.001), protein (P < 0.001) and lactose (P < 0.001) than heifers. Before the afternoon milking IMP was significantly higher in the fore glands of heifers than mature cows (P < 0.001).

In both groups of cows the yields of milk (P < 0.001), fat (P < 0.05), protein (P < 0.001) and lactose (P < 0.001) decreased with time. The rate of decrease during the 12 days was 0.07 kg/day, 0.002 kg/day, 0.002 kg/day, 0.003 kg/day for yields of milk, fat, protein and lactose respectively. The contribution of the decrease to total coefficient of variation was 9%, 1%, 4%, 7% for yields of milk, fat, protein and lactose respectively. Similarly IMP for both glands showed a daily decrease in the morning and at noon but only in the fore gland (P < 0.05) in the afternoon. The contribution of the decrease to total coefficient variation was 3% and 3% before morning milking for rear and fore gland respectively, 4% and 3% at noon for rear and fore gland respectively and 2% for the fore gland before the afternoon.
There was a significant interaction between age and milking frequency for yields of milk ($P < 0.001$), fat ($P < 0.01$), protein ($P < 0.001$) and lactose ($P < 0.001$) meaning that production by mature cows milked once daily fell significantly more than that of heifers milked once per day. Similarly there was an interaction between age and BI for yields of milk ($P < 0.01$), fat ($P < 0.01$) and lactose ($P < 0.001$). In this case HBI mature cows produced significantly higher yields of milk, fat, and lactose than LBI mature cows.

There was significant interaction between age and milking frequency for IMP of the rear gland at noon ($P < 0.01$) and both glands in the afternoon ($P < 0.001$) in that heifers milked once daily had higher IMP than mature cows milked once daily but this difference did not occur on twice daily milking. There was also an interaction between age and BI for IMP of the fore glands in the morning ($P < 0.05$) and both glands in the afternoon ($P < 0.01$) in that HBI heifers had significantly lower IMP than LBI heifers but this difference did not occur for mature cows.
4.1.3 Post-treatment Period

The results for post-treatment period are summarized on Table 4.4.

Figures 4.1-4.4 show that there was a carry over of the effect of once daily milking on the yield of milk and its components after the cessation of once daily milking. It took about seven weeks until cows milked once a day fully recovered to produce similarly to the control group.

During the seven weeks after cessation of the treatment cows previously milked once daily milking cows still produced about 1.1 kg (7%), 0.06 kg (9%), 0.02 kg (4%) and 0.05 (7%) less yields of milk, fat, protein and lactose respectively than the control group. The concentration of protein in milk from cows previously milked twice daily was significantly ($P < 0.01$) higher than that from cows previously milked once daily, while lactose concentration continued to be significantly lower ($P < 0.001$) in cows previously milked once daily compared to that of cows milked twice daily.

HBI cows continued to produce significantly more fat ($P <0.001$), protein ($P <0.01$) and lactose ($P <0.05$) than LBI cows. HBI cows gave milk with significantly higher concentration of fat ($P < 0.05$), protein ($P < 0.01$) and lactose ($P < 0.01$) than milk from LBI.

Mature cows produced significantly higher yields than heifers over the post-treatment period. Similarly heifers produced milk with significantly higher concentration of lactose ($P <0.001$) than mature
cows. Udder volumes were significantly larger (P < 0.01) in mature cows than heifers.

The yields of milk and lactose continued to decrease as lactation proceeded and this contributed to the total coefficient of variation of 9% and 8% for milk and lactose respectively.

There was a significant interaction between BI and milking frequency for IMP for rear glands in the afternoon, the HBI cows previously milked once daily had significantly higher (P < 0.05) IMP than LBI cows previously milked once daily, a difference which was not seen in cows milked twice daily.

4.1.4 Body Weight and Body Condition Score

The mean bodyweights and body condition score for both groups of cows before and after treatment are shown in Table 4.5. There was no significant difference in body weight and condition score between cows milked twice and once per day both before and after treatment.
4.1.5 Somatic Cell Count

At the end of the treatment period, the SCC of eight cows milked daily and nine milked twice daily were greater than 300,000 cells/ml. A Chi squared statistic was used to test the null hypothesis and it was found that there was no significant difference between groups on the proportion of cows with high somatic cell count.

4.2 Prediction of the decrease in Yields of Milk

The decrease in milk yield due to milking once daily varied between cows and ranged from 1.5 kg to 6.6 kg (calculated as in appendix 1). Analysis of variance (Table 5.2) showed that the IMP of the rear gland measured 15 hr after last milking (before morning milking) was the only significant (P < 0.05) factor in predicting the response of dairy cows to once daily milking. Other factors such as IMP of the fore gland, udder volume, peak flow rate, breeding index, age, milk yield (pre-treatment period) and milk solids were not significant factors in predicting the response of cows to once daily milking.
Table 4.1  Least square means (± standard errors) for various milking traits for 32 cows according to BI group, age, treatment and stage of lactation during the pre-treatment period.

<table>
<thead>
<tr>
<th>Breeding Index</th>
<th>Age</th>
<th>Milking Frequency</th>
<th>Stage Lactation</th>
<th>Interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HBI</td>
<td>LBI</td>
<td>Sig</td>
<td>Heifers</td>
</tr>
<tr>
<td>Milk Yield (kg)</td>
<td>16.0 ± 0.41</td>
<td>15.2 ± 0.45</td>
<td>*</td>
<td>14.8 ± 0.46</td>
</tr>
<tr>
<td>Fat Yield (kg)</td>
<td>0.71 ± 0.02</td>
<td>0.59 ± 0.02</td>
<td>***</td>
<td>0.60 ± 0.02</td>
</tr>
<tr>
<td>Protein Yield (kg)</td>
<td>0.54 ± 0.01</td>
<td>0.48 ± 0.01</td>
<td>**</td>
<td>0.48 ± 0.01</td>
</tr>
<tr>
<td>Lactose Yield (kg)</td>
<td>0.79 ± 0.02</td>
<td>0.74 ± 0.02</td>
<td>**</td>
<td>0.74 ± 0.02</td>
</tr>
</tbody>
</table>

| Milk Composition (g/100 ml) | Fat | 4.4 ± 0.11 | 3.9 ± 0.11 | ** | 4.1 ± 0.12 | 4.2 ± 0.09 | NS | 4.1 ± 0.11 | 4.2 ± 0.11 | NS | NS | NS |
|                            | Protein | 3.3 ± 0.06 | 3.1 ± 0.06 | NS | 3.2 ± 0.06 | 3.3 ± 0.05 | NS | 3.2 ± 0.06 | 3.2 ± 0.06 | NS | NS | NS |
|                            | Lactose | 4.9 ± 0.02 | 4.8 ± 0.02 | * | 4.9 ± 0.03 | 4.8 ± 0.02 | * | 4.9 ± 0.02 | 4.9 ± 0.02 | NS | NS | NS |

| Udder Volume (L) | 9.0 ± 1.11 | 10.7 ± 1.13 | NS | 7.3 ± 1.25 | 12.4 ± 0.99 | ** | 9.6 ± 1.11 | 10.1 ± 1.13 | NS | NS | NS |

| Peak Flow Rate (L/min) | 2.06 ± 0.17 | 1.83 ± 0.17 | NS | 2.06 ± 0.19 | 1.83 ± 0.15 | NS | 1.98 ± 0.17 | 1.91 ± 0.17 | NS | NS | NS |
| Machine Time (min)     | 7.35 ± 0.57 | 7.41 ± 0.57 | NS | 6.32 ± 0.64 | 8.44 ± 0.49 | * | 7.56 ± 0.57 | 7.21 ± 0.57 | NS | NS | NS |
| Average Flow (L/min)   | 1.64 ± 0.13 | 1.48 ± 0.13 | NS | 1.70 ± 0.14 | 1.42 ± 0.11 | NS | 1.65 ± 0.13 | 1.47 ± 0.13 | NS | NS | NS |
| fMP (mm H2O)           | Rear gland | 10.7 ± 0.88 | 12.7 ± 0.87 | NS | 13.0 ± 0.98 | 10.3 ± 0.78 | * | 11.7 ± 0.88 | 11.6 ± 0.87 | NS | NS | NS |
|                         | Morning | 10.8 ± 0.95 | 12.9 ± 0.94 | NS | 13.1 ± 1.05 | 10.6 ± 0.84 | NS | 11.5 ± 0.95 | 12.2 ± 0.94 | NS | NS | NS |
|                         | Afternoon | 9.3 ± 0.58 | 8.9 ± 0.58 | NS | 8.6 ± 0.65 | 9.5 ± 0.51 | NS | 8.9 ± 0.58 | 9.2 ± 0.58 | NS | NS | NS |
|                         | Fore gland | 9.4 ± 0.64 | 9.1 ± 0.64 | NS | 9.3 ± 0.72 | 9.2 ± 0.56 | NS | 8.8 ± 0.64 | 9.6 ± 0.64 | NS | NS | NS |

The following symbols are used throughout the results to indicate the level of significance of difference between means:

***  significant difference at the probability < 0.001
**  significant difference at the probability < 0.01
*   significant difference at the probability < 0.05
NS  no significant difference
Table 4.2: Least square means (± standard errors) for various milking traits for 32 cows according to BI groups, age, milking frequency and stage of lactation during treatment period one.

<table>
<thead>
<tr>
<th>Breeding Index</th>
<th>Age</th>
<th>Milking Frequency</th>
<th>Stage Lactation</th>
<th>Interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBI LBI Sig</td>
<td>Heifers Mature Sig</td>
<td>Twice Once Sig</td>
<td>Sig</td>
<td>Age x TRT</td>
</tr>
<tr>
<td>Milk Yield (kg)</td>
<td>14.0 ± 0.39 13.4 ± 0.39 *</td>
<td>13.2 ± 0.44 14.2 ± 0.34 **</td>
<td>14.8 ± 0.39 12.5 ± 0.39 ***</td>
<td>**</td>
</tr>
<tr>
<td>Fat Yield (kg)</td>
<td>0.67 ± 0.03 0.54 ± 0.03 **</td>
<td>0.57 ± 0.03 0.65 ± 0.03 NS</td>
<td>0.64 ± 0.03 0.58 ± 0.03 NS</td>
<td>NS</td>
</tr>
<tr>
<td>Protein Yield (kg)</td>
<td>0.49 ± 0.01 0.44 ± 0.01 *</td>
<td>0.45 ± 0.02 0.49 ± 0.01 *</td>
<td>0.50 ± 0.02 0.43 ± 0.02 ***</td>
<td>*</td>
</tr>
<tr>
<td>Lactose Yield (kg)</td>
<td>0.68 ± 0.02 0.64 ± 0.02 *</td>
<td>0.64 ± 0.02 0.67 ± 0.01 NS</td>
<td>0.72 ± 0.02 0.59 ± 0.02 ***</td>
<td>NS</td>
</tr>
<tr>
<td>Milk Composition (g/100 ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>4.7 ± 0.23 4.2 ± 0.23 NS</td>
<td>4.3 ± 0.26 4.6 ± 0.20 NS</td>
<td>4.3 ± 0.23 4.6 ± 0.23 NS</td>
<td>NS</td>
</tr>
<tr>
<td>Protein</td>
<td>3.6 ± 0.06 3.3 ± 0.06 NS</td>
<td>3.4 ± 0.07 3.5 ± 0.06 NS</td>
<td>3.4 ± 0.07 3.5 ± 0.07 NS</td>
<td>NS</td>
</tr>
<tr>
<td>Lactose</td>
<td>4.9 ± 0.03 4.8 ± 0.03 *</td>
<td>4.9 ± 0.03 4.7 ± 0.02 *</td>
<td>4.9 ± 0.03 4.8 ± 0.03 *</td>
<td>NS</td>
</tr>
<tr>
<td>UDIM (mm H2O)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rear gland</td>
<td>15.0 ± 0.80 15.6 ± 0.80 NS</td>
<td>15.4 ± 0.89 15.2 ± 0.70 NS</td>
<td>16.0 ± 0.80 14.6 ± 0.80 NS</td>
<td>NS</td>
</tr>
<tr>
<td>Morning</td>
<td>12.5 ± 0.72 14.6 ± 0.72 NS</td>
<td>14.2 ± 0.80 12.9 ± 0.62 NS</td>
<td>13.3 ± 0.71 13.8 ± 0.71 NS</td>
<td>NS</td>
</tr>
<tr>
<td>Fore gland</td>
<td>17.0 ± 0.93 17.0 ± 0.90 NS</td>
<td>16.9 ± 1.01 17.0 ± 0.82 NS</td>
<td>11.4 ± 0.92 22.6 ± 0.90 ***</td>
<td>NS</td>
</tr>
<tr>
<td>Noon</td>
<td>14.7 ± 0.96 15.5 ± 0.93 NS</td>
<td>16.6 ± 1.04 13.6 ± 0.84 NS</td>
<td>10.2 ± 0.95 20.0 ± 0.95 ***</td>
<td>NS</td>
</tr>
<tr>
<td>Rear gland</td>
<td>19.8 ± 0.95 23.3 ± 0.95 *</td>
<td>23.6 ± 1.06 19.5 ± 0.83 **</td>
<td>14.7 ± 0.95 28.4 ± 0.95 ***</td>
<td>NS</td>
</tr>
<tr>
<td>Afternoon</td>
<td>16.7 ± 0.87 22.0 ± 0.87 ***</td>
<td>22.1 ± 0.98 16.6 ± 0.76 ***</td>
<td>12.8 ± 0.87 25.9 ± 0.87 ***</td>
<td>NS</td>
</tr>
<tr>
<td>Fore gland</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4.3  Least square means (± standard errors) for various milking traits for 32 cows daily according to BI groups, age, milking frequency and stage of lactation during the treatment period 2.

<table>
<thead>
<tr>
<th>Breeding Index</th>
<th>Age</th>
<th>Milking Frequency</th>
<th>Stage Lactation</th>
<th>Interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HBI</td>
<td>LBI</td>
<td>Sig</td>
<td>TWICE</td>
</tr>
<tr>
<td>Milk Yield (kg)</td>
<td>13.2 ± 0.18</td>
<td>12.7 ± 0.18</td>
<td>***</td>
<td>14.6 ± 0.18</td>
</tr>
<tr>
<td>Fat Yield (kg)</td>
<td>0.64 ± 0.01</td>
<td>0.53 ± 0.01</td>
<td>***</td>
<td>0.64 ± 0.01</td>
</tr>
<tr>
<td>Protein Yield (kg)</td>
<td>0.47 ± 0.01</td>
<td>0.42 ± 0.01</td>
<td>***</td>
<td>0.49 ± 0.01</td>
</tr>
<tr>
<td>Lactose Yield (kg)</td>
<td>0.64 ± 0.01</td>
<td>0.61 ± 0.01</td>
<td>***</td>
<td>0.71 ± 0.01</td>
</tr>
<tr>
<td>rMP (mm H2O)</td>
<td>12.2 ± 0.52</td>
<td>12.9 ± 0.53</td>
<td>NS</td>
<td>14.3 ± 0.52</td>
</tr>
<tr>
<td>Rear</td>
<td>12.1 ± 0.59</td>
<td>13.0 ± 0.46</td>
<td>NS</td>
<td>12.2 ± 0.45</td>
</tr>
<tr>
<td>Morning</td>
<td>12.5 ± 0.51</td>
<td>11.0 ± 0.40</td>
<td>NS</td>
<td>10.7 ± 0.85</td>
</tr>
<tr>
<td>Noon</td>
<td>12.5 ± 0.80</td>
<td>13.0 ± 0.65</td>
<td>NS</td>
<td>9.1 ± 0.73</td>
</tr>
<tr>
<td>Afternoon</td>
<td>13.6 ± 0.51</td>
<td>16.5 ± 0.72</td>
<td>NS</td>
<td>12.8 ± 0.51</td>
</tr>
<tr>
<td>Milk Composition (g/100 ml)</td>
<td>4.8 ± 0.09</td>
<td>4.2 ± 0.09</td>
<td>***</td>
<td>4.4 ± 0.09</td>
</tr>
<tr>
<td>Fat</td>
<td>4.3 ± 0.10</td>
<td>4.8 ± 0.07</td>
<td>**</td>
<td>4.4 ± 0.09</td>
</tr>
<tr>
<td>Protein</td>
<td>3.5 ± 0.03</td>
<td>3.5 ± 0.03</td>
<td>NS</td>
<td>3.4 ± 0.03</td>
</tr>
<tr>
<td>Lactose</td>
<td>4.9 ± 0.03</td>
<td>4.7 ± 0.02</td>
<td>***</td>
<td>4.9 ± 0.03</td>
</tr>
</tbody>
</table>
Table 4.4  Least square means (± standard error) for various milking trait according to BI, age, milking frequency and stage of lactation during the post-treatment period.

<table>
<thead>
<tr>
<th>Breeding Index</th>
<th>Age</th>
<th>Milking Frequency</th>
<th>Stage</th>
<th>Interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HBI</td>
<td>LBI</td>
<td>Sig</td>
<td>Heifers</td>
</tr>
<tr>
<td>Milk Yield (kg)</td>
<td>14.3 ± 0.44</td>
<td>13.6 ± 0.50</td>
<td>NS</td>
<td>13.1 ± 0.53</td>
</tr>
<tr>
<td>Fat Yield (kg)</td>
<td>0.64 ± 0.02</td>
<td>0.54 ± 0.02</td>
<td>***</td>
<td>0.55 ± 0.02</td>
</tr>
<tr>
<td>Protein Yield (kg)</td>
<td>0.54 ± 0.01</td>
<td>0.48 ± 0.02</td>
<td>**</td>
<td>0.48 ± 0.02</td>
</tr>
<tr>
<td>Lactose Yield (kg)</td>
<td>0.72 ± 0.02</td>
<td>0.66 ± 0.03</td>
<td>*</td>
<td>0.66 ± 0.03</td>
</tr>
<tr>
<td>Milk Composition</td>
<td>(g/100 ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>4.7 ± 0.13</td>
<td>4.3 ± 0.14</td>
<td>*</td>
<td>4.5 ± 0.15</td>
</tr>
<tr>
<td>Protein</td>
<td>3.6 ± 0.05</td>
<td>3.4 ± 0.05</td>
<td>**</td>
<td>3.5 ± 0.05</td>
</tr>
<tr>
<td>Lactose</td>
<td>4.8 ± 0.04</td>
<td>4.6 ± 0.04</td>
<td>**</td>
<td>4.8 ± 0.04</td>
</tr>
<tr>
<td>IMP (mm H₂O)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rear gland</td>
<td>10.5 ± 0.72</td>
<td>10.6 ± 0.74</td>
<td>NS</td>
<td>9.9 ± 0.82</td>
</tr>
<tr>
<td>Afternoon</td>
<td>9.4 ± 0.65</td>
<td>10.2 ± 0.67</td>
<td>NS</td>
<td>9.4 ± 0.74</td>
</tr>
<tr>
<td>Front gland</td>
<td>6.8 ± 0.68</td>
<td>8.0 ± 0.70</td>
<td>NS</td>
<td>5.4 ± 0.77</td>
</tr>
</tbody>
</table>

V1 - 4
Table 4.5  Means (± standard errors) for body weight and body condition score (BCS) of the 32 cows before and after treatment.

<table>
<thead>
<tr>
<th>Milking frequency</th>
<th>Twice daily</th>
<th>Once daily</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (kg)</td>
<td>444 ± 16.4</td>
<td>471 ± 16.4</td>
<td>NS</td>
</tr>
<tr>
<td>Final body weight (kg)</td>
<td>428 ± 15.4</td>
<td>458 ± 15.4</td>
<td>NS</td>
</tr>
<tr>
<td>Final BCS</td>
<td>4.8 ± 1.4</td>
<td>5.2 ± 1.4</td>
<td>NS</td>
</tr>
<tr>
<td>Initial BCS</td>
<td>4.8 ± 1.9</td>
<td>5.3 ± 1.9</td>
<td>NS</td>
</tr>
</tbody>
</table>
Figure 4.1 The mean weekly milk yield for cows milked twice daily during the pre-treatment and post-treatment period and once (•) or twice (+) daily during the treatment period.
Figure 4.2 The mean weekly fat yield for cows milked twice daily during the pre-treatment and post-treatment period and once (\*) or twice (+) daily during the treatment period.
Figure 4.3 The mean weekly protein yield for cows milked twice daily during the pre treatment and post treatment period and once (★) or twice (+) daily during the treatment period.
Figure 4.4 The mean weekly lactose yield for cows milked twice daily during the pre treatment and post treatment period and once (*) or twice (+) daily during the treatment period.
CHAPTER FIVE

DISCUSSION

5.1 EFFECTS OF ONCE DAILY MILKING

5.1.1 Effects on Milk Production

Over the 3 weeks of once daily milking, daily milk yield decreased by 2.8 kg/cow (19%) relative to the control group. Yields of milkfat, protein and lactose were also reduced by 0.08 kg/cow (14%), 0.08 kg/cow (16%), and 0.16 kg/cow (21%) respectively. The decrease in milk yield is similar to that observed by Bryant (1978) of 18% but is less than those observed by others (Claesson et al., 1959; Parker, 1965; Wilson, 1965) of 31-50%.

Following cessation of the treatment cows previously milked once daily continued to produce less than the control group and it took about 7 weeks to fully recover. During this period daily yields of milk, fat, protein and lactose for treatment group were 1.1 kg/cow (7%), 0.06 kg/cow (9%), 0.02 kg/cow (4%) and 0.05 kg/cow (7%) respectively less than the control group. The total decrease in production over both the 3 weeks (treatment period) and 7 weeks (post-treatment period) is shown on Table 5.1. The residual effect of once daily milking has also been reported in single long milking intervals and missed milkings (Wheelock et al., 1965; 1966; Wilson, 1965). The apparent failure of secretory activity to recover after cessation of once daily milking implies that any damage to the secretory cells from udder distension or chemical
inhibitors is not easily reversed (Peaker, 1980). Furthermore a recent study by Wilde et al. (1989) indicated that incomplete milking over a long period caused a partial secretory cellular involution. The partially involuted secretory cells may need time to adjust to increased milking stimulus.

Differences between experiments in the extent of decrease in production may arise from variation in factors such as duration of treatment (short term or full lactation), stage of lactation (when treatment was imposed) and level of feeding. These factors are explained below.

Duration of the treatment

The reduction in milk yield increased with time on treatment (Figure 4.1). Thus the decrease in milk production due to once daily milking, in the first three days of the treatment was smaller than that over the remaining 18 days of the treatment period (15% vs 23%). Similarly Wilson (1965) observed that the full effect of the change to once daily milking was not realized for several milkings. Claesson et al. (1959) over the whole lactation observed that milk yield was reduced by an average of 50%. However during the first two weeks of experiment, the decrease was only 20% but increased to 54% over the last two weeks. The progressive decline in milk yield with time shows that reduced milking frequency may not only limit secretion in the terminal phases of each milking interval but also depress secretory potential. The immediate drop in production in the first few days of once daily milking, is probably the result of both udder distension and incomplete milking. The longer interval between milkings is inevitably associated
with greater udder distension. Changing from twice daily milking to once daily milking also means additional milk to harvest within the effective milk ejection time. Consequently the amount of residual milk and milk fat retained in the alveoli will increase significantly, partly probably because the period of ejection is too short to allow complete removal of all the milk. Residual milk has an inhibitory effect on subsequent milk secretion (Elliot, 1961, Wilde et al., 1989). Recently this inhibitory effect has been attributed to a protein which accumulates in milk after secretion (Wilde et al., 1987).

**Level of Feeding**

Although feed intake was not measured in this study, the dry conditions which prevailed during the treatment period probably restricted feed intake by reducing pasture quality and quantity. Thus the yield of the control group fell by 11% during the treatment period in comparison with their yield during the pretreatment period. Under restricted levels of feeding Bryant, (1978) observed a depression of 18% in milk production by cows milked once daily compared to a 31-37% fall when cows were fully fed (Parker, 1965; Wilson, 1965). It is probable that when feed intake is limited the control cows were not able to produce to their maximum ability and therefore the difference between the two groups becomes smaller.

**Stage of Lactation**

Stage of lactation possibly affects the extent to which milk yield is depressed by once daily milking. When once daily milking was carried
out during later part of lactation milk yield decreased by 31% to 37% (Parker, 1965; Wilson, 1965). The present experiment was carried out after peak lactation. Turner (1955) observed that the depression in milk secretion rate during a 14 hr as compared to a 10 hr interval was 0.6% in early lactation and 6.3% in late lactation. Henderson and Peaker (1983) observed that with goats the inhibitory effect due to milk accumulation was higher over the later stages of lactation. Possible reasons include greater mammary distension as a result of a loss of internal functional capacity with advancing lactation (Peaker, 1980), increasing sensitivity to distension, greater production of or sensitivity to local chemical factors (Linzel and Parker, 1971) or loss of the sensitivity of the neuroendocrine reflex for oxytocin (Wachs et al. 1984). Further research is needed to distinguish between these possibilities.
Table 5.1 Average decrease in daily production traits due to once daily milking over 3 weeks (treatment period) and 7 weeks (post-treatment period)

<table>
<thead>
<tr>
<th>Milking Frequency</th>
<th>Decrease Amount kg/cow</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Twice daily</td>
<td>Once daily</td>
<td></td>
</tr>
<tr>
<td>Milk (kg)</td>
<td>14.6</td>
<td>12.7</td>
</tr>
<tr>
<td>Fat (kg)</td>
<td>0.63</td>
<td>0.55</td>
</tr>
<tr>
<td>Protein (kg)</td>
<td>0.51</td>
<td>0.46</td>
</tr>
<tr>
<td>Lactose (kg)</td>
<td>0.71</td>
<td>0.62</td>
</tr>
</tbody>
</table>

5.1.2 Effect on Milk Composition

Milking once daily increased milkfat and protein concentration while the concentration of lactose fell (Table 4.2 and 4.3). These changes in milk composition are in agreement with previous studies (Claesson et al., 1959; Wilson 1965). The different trends in the concentration of individual milk constituents, following milking intervals of 18 hr or longer, can be explained by changes that occur to the milk during
storage in the udder. The permeability of the glandular epithelium is increased either by the pressure forcing the epithelial cells apart or by a chemical factor causing the tight junctions to loosen. Lactose and potassium are then reabsorbed and sodium and chloride diffuse into the alveoli down their concentration gradients while still maintaining osmotic equilibrium (Dodd, 1984). It is probable that the ability of the secretory cells to exchange sodium ions for potassium ions at the apical membrane is also impaired which will also lead to a fall in potassium and increase in sodium concentration in the milk. The reabsorption of lactose will be accompanied by the reabsorption of water (Wheelock et al., 1965) which will contribute to the decrease in milk yield. The reabsorption of water reduces the volume of milk within the gland so the concentration of large molecules such as the proteins, and fat globules which cannot pass between the epithelial cells is increased.

5.1.3 Effect on Body Weight and Condition Score

Considering that milk production decreased when cows were milked once daily, it was expected that the treatment group would gain weight or body condition score over the treatment period. However as shown in Table 4.5 this was not the case. A comparable finding was made by Parker (1965). In contrast Claesson et al. (1959) over the whole lactation, observed that cows milked once daily gained more weight than the control cows. The absence of a significant effect of milking once daily on body weight and condition score in the present study was probably due to the short duration of the treatment period. Indeed in
the experiment of Claesson et al. (1959) the increase weight gain of
the group milked once daily was more marked when the treatment was
throughout the whole lactation than when it was stopped after 140 days
of lactation.

5.1.4 Effects on Udder Health

It has been argued that milking cows more frequently helps to flush out
the pathogens before an infection is established (Neave, 1971). Pathogens placed in the teat multiply and establish infection within
12-18 hr of entering the teat sinus (Dodd, 1971). Therefore
establishment of infection is more likely in cows milked once daily if
pathogens happen to enter the teat during milking. Natzke et al.
(1965) reported an increase in mastitis in cows which had missed an
afternoon milking. In the present study there was no difference in
udder health of the two groups as indicated by SCC. This could be
attributed to the efficient mastitis control system on this farm, for
example cows identified as having mastitis were the last to be milked
in order to avoid transfer of pathogens from one cow to another and
teats were sprayed with bacteriocidal solution after each milking.

5.1.5 Effect on Intramammary Pressure

Intramammary pressure was higher in cows milked once daily than the
control group at noon and before afternoon milking but not at the
morning. IMP in the rear gland was always higher than that in the fore
gland in both groups of cows (Appendix 5) possibly reflecting the
higher proportion of milk in the rear than fore gland. The trend in
the IMP is in agreement with earlier studies (Schmidt, 1960; Tucker, 1961). Direct comparison of the values between these experiments however, is not possible because of different methods of measurement used. In the earlier experiments IMP was measured in the cannulated teat. The pressure recorded using this technique comprises, when the gland contains sufficient milk, the hydrostatic pressure of a column of milk in the gland plus the pressure exerted by the walls of the gland (Korkman, 1953) and is therefore, likely to be higher than that recorded in the present study. The relationship between the values obtained by the methods is of considerable interest but outside the scope of the present study.

5.1.6 Effect on Udder Volume

In the present study the average decline in the udder volumes of once and twice daily milked groups were not significantly different i.e. 24 and 26% respectively. This percentage decrease is large for three weeks and probably includes errors involved in the measurement of udder volume. In addition the decrease in milk yield (11%) in both groups over the period of the experiment would have led to a fall in residual milk in the gland.

The pattern of udder volume change over lactation is well defined. Commonly udder volume declines during lactation, the decline being relatively rapid for the first 18-20 weeks of lactation (Davis et al., 1985). Reasons for the decline in udder volume are not known but inadequate milk removal (Davis et al. (1985) and reduced milking frequency (Davis and Hughson, 1988) are likely to accelerate the rate
of udder regression. Conversely 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 cells (Wilde et al. (1987). Therefore it appears that a longer treatment period, than that applied in this experiment, is needed before a noticeable change in the size of the udder due to once daily milking could take place.
5.2 Predicting the Fall in Milk Production in Response to Once Daily Milking

This study was undertaken to determine if factors believed to be responsible for the drop in production such as IMP, post-milking udder volume before treatment, residual milk, and milk composition could be used to predict the response of cows to once daily milking.

Earlier studies reported variations among cows in their response to once daily milking (Claesson et al., 1959; Parker, 1965; Bryant, 1978) and in the present study percentage drop in milk production varied from 12-46% between individual cows. This variation was found to be significantly (P < 0.05) related to IMP measured 15 hr after milking (Table 5.2). No other factor, however, was useful, either individually or in combination with other variables, in predicting the response to once daily milking. Possible reasons for the failure to detect significant relationship are discussed below.
Table 5.2 Analysis of variance of factors affecting the response of cows to once daily milking

<table>
<thead>
<tr>
<th>Factor</th>
<th>Probability</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.86</td>
<td>NS</td>
</tr>
<tr>
<td>BI</td>
<td>0.11</td>
<td>NS</td>
</tr>
<tr>
<td>Peak flow rate</td>
<td>0.53</td>
<td>NS</td>
</tr>
<tr>
<td>Udder volume</td>
<td>0.49</td>
<td>NS</td>
</tr>
<tr>
<td>Milk yield (before treatment)</td>
<td>0.08</td>
<td>NS</td>
</tr>
<tr>
<td>IMP (morning)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rear</td>
<td>0.01</td>
<td>*</td>
</tr>
<tr>
<td>Fore</td>
<td>0.53</td>
<td>NS</td>
</tr>
<tr>
<td>Milk solids (gm/100ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(fat, protein lactose)</td>
<td>0.09</td>
<td>NS</td>
</tr>
</tbody>
</table>
5.2.1 Reliability of the Factors measured to Predict Production Loss arising from once daily milking

5.2.1.1 Intramammary pressure

According to a scheme proposed by Peaker (1980), accumulation of milk in the udder causes mammary distension and an increase in IMP. Further mammary distension leads to a loss of secretory and metabolic activities and therefore reduced milk yield in extended milking intervals. The time given for the pressure to be reached would be expected to vary with breed, udder volume, and udder productivity and residual milk yield. In cows it has been shown that secretion rate starts falling 16-18 hr after last milking (Wheelock et al., 1966; Wheelock, 1980). Therefore intramammary pressure between 12 and 24 hr after milking would be expected to be related to the response to once daily milking. In the present study IMP were taken at 15 hr, 20 hr, and 24 hr after last milking. Only IMP of the rear gland in the morning (15 hr since the previous milking) was significantly related to the response to once daily milking. Thus cows in which the pressure rose most rapidly were more likely to suffer a reduction in milk yield. The failure to detect a similar relationship for the fore gland may be due to the occurrence of milk ejection following manipulation of the rear quarter (Korkman, 1953). The lack of a relationship between milk yield response and the IMP recorded at noon and in the afternoon, 20 hr and 24 hr after last milkings may be due to reabsorption of lactose and water tending to reduce IMP (Fleet and Peaker, 1978). Since the speed of reabsorption may vary between cows, the IMP recorded 20 hr to 24 hr after milking may not reflect the damage caused by udder distension.
5.2.1.2 Udder Volume

Functional udder capacity was observed to be variable among cows (22.6-56.5 hr) (Davis and Hughson, 1988). Similarly in the present study, udder volume of the 16 cows milked once daily varied from 6-23 litres. The increase in pressure per unit of secreted milk obviously depends mainly on udder volume and elasticity. The present study shows that udder volume could not be used to predict the loss in production due to once daily milking. This may be attributed to the following reasons. There is lack of knowledge about the distribution of the storage space in the udder or the extent to which milk production varies across different regions of the udder. It has been suggested that some areas may be grossly under utilized in terms of storage (Woolford., et al (1985). If this is true then udder volume might not be a reliable factor for prediction. Furthermore in Friesian cows milk yield is related to udder productivity rather than udder volume (Davis et al., 1985). Therefore although a recent study by Davis and Hughson (1988) has shown promising results in the ability of Jersey cows to tolerate extended milking intervals this might not be the same in Friesian cows.

Finally udder volume was measured by a technique described by (Davis et al., 1983). This technique is valid when the shape of the udder approximates a wedge shape. The validity of this assumption was not verified in this study and also, because of the shortage of animals, no effort was made to select cows with udders approximating a wedge shape. Therefore it is possible that errors in the estimation of udder volumes may have obscured any relationship with response to once daily milking.
5.2.1.3 Peak Flow Rate

Peak flow rate was included in this study because of its possible relationship with residual milk yield. However, there was no significant relationship between peak flow rate and the response of cows to once daily milking (Table 5.2). The relationship between residual milk and milk flow rate is still a controversial subject (Sandvick, 1957; Rajamman et al., 1966). The quantity of residual milk partly depends on the effectiveness of milk ejection (Elliot, 1961). Although high peak flow rate is important in efficient milk removal, recent studies by Sagi et al. (1980) and Schams et al. (1984) show that the amount and pattern of oxytocin release may play a major role in the amount of residual milk at the end of milking. These studies showed that there was no relationship between patterns or absolute concentrations of oxytocin and milk flow characteristics. An investigation exploring the relationship between the pattern of oxytocin release and volume of residual milk would be useful.

5.2.1.4 Milk Composition

The variation in milk solid concentration in milk within a breed is large. About 50% of the variation between individuals within a breed is genetic (Rook and Thomas, 1980). A range of milk fat percentage within a breed could be over 20g/kg milk while variation in protein concentration is less but may be over 10g/kg of milk. Lactose values vary less than those for fat and protein (Rook and Thomas, 1980). It was suggested that cows producing higher concentrated milk might suffer less when milked once daily than those with less concentrated milk.
(Davis and Hughson, 1988). The present study did not show significant relationship between the concentration of milk solids and the response to once daily milking (Table 5.2). Furthermore since a chemical inhibitor has been identified as a constituent of the whey proteins (Wilde et al., 1987; Wilde et al., 1988) it may be speculated that cows producing milk with high concentration of the inhibitor in their milk would suffer more under a once daily milking regime.

5.2.1.5 Residual milk yield

Residual milk yield could not be included in the model due to the failure to obtain reasonable values from the calculations (Appendix 2). This is a reflection of the sensitivity of the calculated values to small variations in the volume of milk harvested and the length of the milking intervals. Thus to obtain repeatable estimates of residual milk it may be necessary to maintain much more constant levels of feeding and perhaps make the intervals between milking more uneven.

The inhibitory effect of residual milk secretion in cows and goats is well established (Elliot, 1961; Henderson and Peaker, 1983; 1984) and seems to play an important role in controlling the rate of milk secretion. Woolford et al. (1982) concluded that the reduction in milk yield of cows milked once daily was almost entirely due to the increase in residual milk. Residual milk volume for most cows amounted to the equivalent of 1 to 3 hr worth of secretion (Davis and Hughson, 1988). A consistent variation in the proportion of residual milk in individual glands was found to be inversely related to their rate of
milk secretion (Peaker and Blatchford, 1988). The presence of residual milk yield may inhibit secretion rate in two ways. First by increasing the physical distension as milk accumulates, and secondly, by increasing chemical feed-back to the secretory cell which would affect milk secretion through reduced milk secretion (Henderson et al., 1983) or increased permeability of mammary cell epithelium (Peaker, 1980). This description indicates that, in order to predict the susceptibility of cows to production loss on once daily milking, some account needs to be taken of both residual milk and the feed back inhibitor. Because of the sensitivity of the formula developed to calculate residual milk, it would be better to determine residual milk volume by the direct method using exogenous oxytocin injected intravenously. It is important that residual milk yield after once daily milking is measured to test the hypothesis that the decrease in production is partly because milk ejection is inadequate and thus milking is incomplete. Another important area of study is the relationship between residual milk and the concentration of the chemical inhibitor in the milk.
CHAPTER SIX
CONCLUSION

Over the three weeks of once daily milking and the seven weeks post-treatment period daily yields of milk, fat and protein and lactose decreased by an average of 2 kg/cow (13%), 0.08 kg/cow (12.7%) and 0.05 kg/cow (10%), 0.09 (12.6%) respectively compared to twice daily milking. As is in other studies, the response to once daily milking varied between cows and ranged from 12% to 46%. The decrease in milk production when the cows were milked once daily was probably the result of both udder distension and incomplete milking. Incomplete milking probably resulted from a shorter duration of milk ejection relative to the amount of milk present in the udder. As a result the secretory potential was depressed and this explains the progressive decline in milk yield with time when cows are milked once daily. Decreased lactose concentration in milk from the cows milked once daily further supports the suggestion that the structure of secretory epithelium was altered when milking intervals were extended beyond 18 hr.

Cows in which the pressure rose most rapidly were likely to suffer a greater reduction in milk yield. Although udder volume and milk composition did not show a significant relationship with the response of cows to once daily milking in the present study this does not rule out their involvement in inhibiting milk secretion over extended milking intervals as already discussed. Another study involving larger number of animals, and more accurate measurement of udder volume would increase the likelihood of finding a significant relationship between milk composition and udder volume with the response of cows to once
daily milking. A study is also required to increase our understanding of the contribution of residual milk, for example do animals with high amount of residual milk reach maximum IMP earlier than those with low residual milk or does this depend on the udder volume and productivity. Similarly how much does residual milk contribute to the building up of the chemical feed back inhibitor in milk.
REFERENCES


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Appendix 1

Regression coefficients of yields of milk and days for individual cows milked twice daily over the treatment period showed that there was no significant change in milk production during this period. Therefore the decrease in yields of milk, fat, protein and lactose for each of the 16 cows milked once daily was determined as the difference between mean yield during the treatment period and mean yield for the one week before once daily milking began.

Appendix 2

Formula for calculating Residual Milk Residual milk was calculated from measures of milk yield and interval length over a 4 successive milkings where M1 to M4 were the yields of milk collected at times T1 to T4 and the milking intervals were

\[ I_1 \text{ the time from } T_1 \text{ to } T_2 \]
\[ I_2 \text{ the time from } T_2 \text{ to } T_3 \]
\[ I_3 \text{ the time from } T_3 \text{ to } T_4 \]
\[ I_4 \text{ the time from } T_4 \text{ to } T_5 \]

Assume

\[ C_1 \text{ to } C_4 \text{ were the volume of residual milk left in the gland at } T_1 \text{ to } T_4 \]

and that

\[ S \text{ is the rate of milk secretion (ml/min) which is constant from } T_1 \text{ to } T_4 \]

Then

\[ R = \frac{C_1}{M_1 + C_1} = \frac{C_2}{M_2 + C_2} = \frac{C_3}{M_3 + C_3} = \frac{C_4}{M_4 + C_4} \]
or rearranging,

\[
\begin{align*}
\text{RM}_1 &= \text{C}_1; \quad \text{RM}_2 = \text{C}_2; \quad \text{RM}_3 = \text{C}_3; \quad \text{RM}_4 = \text{C}_4 \\
1 - R &= 1 - R \\
1 - R &= 1 - R \\
1 - R &= 1 - R \\
1 - R &= 1 - R
\end{align*}
\]

substituting for \( C_1 \) and \( C_2 \) then

\[
\begin{align*}
\text{M}_2 &= \text{S}_{I1} + \frac{\text{RM}_1 - \text{RM}_2}{1 - R} \\
\text{M}_2 &= \text{S}_{I1} + \frac{\text{R}(\text{M}_1 - \text{M}_2)}{1 - R} \\
\text{M}_2 - \text{SI}_{I1} &= \frac{\text{R}(\text{M}_1 - \text{M}_2)}{1 - R} \\
(1 - R)(\text{M}_2 - \text{SI}_{I1}) &= \text{R}(\text{M}_1 - \text{M}_2) \\
\text{M}_2 - \text{SI}_{I1} - \text{RM}_2 + \text{RS}_I &= \frac{\text{RM}_1 - \text{M}_2}{\text{M}_1 - \text{SI}_{I1}} \\
\text{M}_2 - \text{SI}_{I1} &= \frac{\text{RM}_1 - \text{RM}_2 + \text{RM}_2 - \text{RS}_I}{\text{M}_1 - \text{SI}_{I1}} \\
\text{M}_2 - \text{SI}_{I1} &= \frac{\text{RM}_1 - \text{RS}_I}{\text{M}_1 - \text{SI}_{I1}}
\end{align*}
\]

\[
\begin{align*}
\text{R} &= \frac{(\text{M}_2 - \text{SI}_{I1})}{(\text{M}_1 - \text{SI}_{I1})} \\
\text{similarly}
\end{align*}
\]

\[
\begin{align*}
= \frac{(\text{M}_3 - \text{SI}_2)}{(\text{M}_2 - \text{SI}_{I1})} \quad \text{(Estimate A)} \\
= \frac{(\text{M}_4 - \text{SI}_3)}{(\text{M}_3 - \text{SI}_3)} \quad \text{(Estimate B)}
\end{align*}
\]
Appendix 3

Calculated Percentage Residual Milk

<table>
<thead>
<tr>
<th>Cow No</th>
<th>Estimate A</th>
<th>Estimate B</th>
</tr>
</thead>
<tbody>
<tr>
<td>151</td>
<td>19.00</td>
<td>20.00</td>
</tr>
<tr>
<td>152</td>
<td>39.09</td>
<td>41.91</td>
</tr>
<tr>
<td>153</td>
<td>-6.51</td>
<td>-10.06</td>
</tr>
<tr>
<td>159</td>
<td>36.29</td>
<td>36.17</td>
</tr>
<tr>
<td>160</td>
<td>12.54</td>
<td>13.11</td>
</tr>
<tr>
<td>163</td>
<td>-11.64</td>
<td>-14.10</td>
</tr>
<tr>
<td>165</td>
<td>264.18</td>
<td>686.24</td>
</tr>
<tr>
<td>167</td>
<td>9.55</td>
<td>8.17</td>
</tr>
<tr>
<td>169</td>
<td>1.18</td>
<td>0.23</td>
</tr>
<tr>
<td>170</td>
<td>25.11</td>
<td>23.28</td>
</tr>
<tr>
<td>171</td>
<td>48.45</td>
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<tr>
<td>175</td>
<td>5.09</td>
<td>5.96</td>
</tr>
<tr>
<td>179</td>
<td>10.22</td>
<td>7.92</td>
</tr>
<tr>
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Appendix 4  
The mean daily IMP at morning, noon and afternoon for cows milked once or twice daily.