

## **Effect of Breed, Season of the Year and Stage of Lactation on the Response of Dairy Cows to Increased Milking Frequency**

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**Abstract.** The effect of breed, season of the year and stage of lactation on the response (milk secretion rate) of exotic dairy cows to thrice-daily milking was studied in 20 Friesian and 10 Jersey cows using the technique of half-udder study. The results indicated that there was a positive response to increased milking frequency in both breeds. The response by the Friesian during the winter and early summer seasons was similar, regardless of stage of lactation. During summer, the response of the Jersey cows at the post-peak stage of lactation was greater ( $P < 0.05$ ) than that of the Friesians, but it was lower ( $P < 0.01$ ) at the early stage. The response during the post-peak stage was always higher than during the early stage. These increases in rate of milk secretion were due to the increase in secretion rate of the morning milking followed the extra mid-night milking of only the half udder milked thrice daily, suggesting a local regulatory mechanism for controlling milk secretion, that respond rapidly to milk removal.

### **Introduction**

The time to be allowed between milkings is a decisive element of dairy management, manifesting itself in the choice both of number of milkings completed in a 24-hour day and of milking intervals. In the cows, studies of milk secretion over different milking intervals have indicated that the rate of milk secretion is constant for at least 12 h [1,2] or 16 h [3]. However, this view is difficult to reconcile with the fact that thrice milking instead of twice daily increases milk yield by up to 40% with average increase of 20% [4]. There is, therefore, a stimulatory effect of increasing the frequency of milking [5,6,7]. Two times daily milking is the common practice in most dairy farms. Three times daily milking is used in some farms to increase milk yield, especially when cows are high producers, and to reduce the udder strain.

Factors such as breed, season of the year, age of the animal, stage of lactation [8] and level of nutrition [9] that affect milk yield may, hence, affect the animal's response to any practical manipulation including increasing milking frequency. It is very important under certain conditions to reinvestigate the response of the dairy animals to more frequent milking, especially if the climates are different. This study was thus conducted to investigate the effect of increased milking frequency to thrice daily instead of twice on milk secretion rate in Friesian and Jersey cows raised under the semi-arid climate of Saudi Arabia, taking into account the effect of both seasons of the year and stage of lactation.

### Materials and Methods

Four groups of 5 Friesian cows each at their third lactation were used to study the effect of season of the year and stage of lactation on the response of dairy cows to thrice-daily milking. Each group compromised one stage of lactation, early (week 2) or post-peak (weeks 14-16), within one season of the year 1992 (winter or early summer). Another two groups of Jersey cows (only during the summer season) were used to study the effect of breed on the above response. The animals were housed in a semi-closed concrete barn, with a good ventilation and constant lighting. Each cow was held in a separate stall by a chain halter. The main experiment began at the same time for all cows within the same season and lasted for a period of six weeks, preceded by a 4-day adaptation period in which each half of the cow's udder (the left hind with the right front glands 'control glands', and the right hind with the left front glands 'test glands') was milked separately by a mobile milking machine, twice a day at 8.00 h and 16.00 h. Due to some difficulty in milking both udder's halves separately at the same time, test glands were chosen randomly to be milked before the control ones throughout the experimental period.

This effect of milking at different times was taken into account when comparing both udder's halves within a specific period. Then the experiment was divided into three experimental periods. The milking routine during the first (pre-treatment, 12 days) and third (post-treatment, 13 days) periods were as in the adaptation period. During the second (treatment, 13 days) period, only the test glands were milked an extra milking at 00.00 h, while the control glands were milked twice daily as in the other two groups. After the last morning milking of each experimental period, cows were joined the rest of the milking herd in an open playground where no milking was

carried out until the afternoon milking of the next day, when they were brought back to the barn to be milked. Milk yield accumulated during this 36-h rest period will be used in another article as an index of the functional capacity of the udder. Milk yields of the day following the rest periods and of the morning milking of the next day in both treatment and post-treatment periods were subsequently disregarded in a later statistical analysis to eliminate the effect of the previous day.

Data of milk yield were used to calculate hourly secretion rate which was analyzed statistically by GLM, fixed-model procedures [10], including breed of the cow, season of the year (when experiment was made), udder's half, stage of lactation, treatment (twice vs. thrice daily milking), experimental period (pre-treatment vs. treatment vs. post-treatment), time of milking and all possible existed interactions with the treatment effect. The effect of the cow was absorbed [10] before the model. Multiple comparisons were made using the Least-squares means (LS-MEANS) procedure of the same computerized program of SAS. Due to non-significant differences among milk yields between pre- and post-treatment periods (twice-daily milking) within each half of the udder, and also, due to the presence of the frequent milking (treatment) period between these two periods, data were pooled for the latter two periods into one period, namely control period, and were used in the subsequent analysis.

### Results and Discussion

This study was conducted first during January and February of winter 1992, and again during the early summer months of May and June. Average minimum and maximum ambient temperatures were respectively 8.5 and 22.4°C during the winter months and 18.6 and 38.4°C during the summer months. Average morning and afternoon relative humidities were 76.9 and 58.7% during the winter and 48.6 and 18.6 during the summer period.

Table 1 shows the daily average secretion rate of milk obtained from both halves of the udder during the twice and thrice daily milking periods at early and post-peak stages of lactation in winter (Friesian cows) and early summer (Friesian and Jersey cows) seasons. There was always an increase in milk secretion from the test glands above the control glands throughout the twice- as well as the thrice-daily milking periods. This indicated that each quarter of the udder has its own individual reaction in the overall process of milk letdown [11, p.28]. Consequently, after milking the secretion, storage and next milking letdown may take place at different conditions for each quarter of the udder. This detail may be used to explain the well-known observation that mastitis of the cow generally appears in one quarter of the udder.

**Table 1. Milk secretion rates (mean  $\pm$  s.e.) during twice- and thrice- daily milking at two stages of lactation during two seasons of the year in Friesian and Jersey cows**

	Early	Post-Peak	All stages
<b>Friesian cows (winter)</b>			
<b>Secretion rate (l/h)</b>			
Twice daily period:			
Control glands	0.263 $\pm$ 0.005 <sup>Aa</sup>	0.248 $\pm$ 0.006 <sup>Aa</sup>	0.255 $\pm$ 0.006 <sup>A</sup>
Test glands	0.293 $\pm$ 0.008 <sup>Ba</sup>	0.291 $\pm$ 0.008 <sup>Ba</sup>	0.292 $\pm$ 0.006 <sup>B</sup>
Thrice daily period:			
Control glands	0.256 $\pm$ 0.010 <sup>Aa</sup>	0.244 $\pm$ 0.007 <sup>Aa</sup>	0.250 $\pm$ 0.008 <sup>A</sup>
Test glands	0.358 $\pm$ 0.009 <sup>Ca</sup>	0.370 $\pm$ 0.009 <sup>Ca</sup>	0.364 $\pm$ 0.006 <sup>C</sup>
<b>Response (%)</b>			
Milking time	11.41 $\pm$ 1.252 <sup>Aa</sup>	18.15 $\pm$ 1.264 <sup>Ab</sup>	14.78 $\pm$ 1.012 <sup>A</sup>
Milking thrice	22.18 $\pm$ 1.802 <sup>Ba</sup>	27.15 $\pm$ 1.964 <sup>BEb</sup>	24.66 $\pm$ 1.012 <sup>B</sup>
Both	39.84 $\pm$ 2.278 <sup>Ca</sup>	51.64 $\pm$ 2.902 <sup>Cb</sup>	45.74 $\pm$ 2.313 <sup>C</sup>
<b>Friesian cows (summer)</b>			
<b>Secretion rate (l/h)</b>			
Twice daily period:			
Control glands	0.238 $\pm$ 0.004 <sup>Aa</sup>	0.179 $\pm$ 0.004 <sup>Ab</sup>	0.208 $\pm$ 1.006 <sup>A</sup>
Test glands	0.266 $\pm$ 0.009 <sup>Ba</sup>	0.206 $\pm$ 0.009 <sup>Bb</sup>	0.236 $\pm$ 0.007 <sup>B</sup>
Thrice daily period:			
Control glands	0.237 $\pm$ 0.009 <sup>Aa</sup>	0.179 $\pm$ 0.009 <sup>Ab</sup>	0.208 $\pm$ 0.008 <sup>A</sup>
Test glands	0.315 $\pm$ 0.008 <sup>Cab</sup>	0.260 $\pm$ 0.008 <sup>Cb</sup>	0.287 $\pm$ 0.007 <sup>C</sup>
<b>Response (%)</b>			
Milking time	11.76 $\pm$ 1.109 <sup>Aa</sup>	18.44 $\pm$ 1.164 <sup>Ab</sup>	15.10 $\pm$ 1.272 <sup>A</sup>
Milking thrice	19.42 $\pm$ 1.652 <sup>Ba</sup>	25.60 $\pm$ 2.204 <sup>Bb</sup>	22.51 $\pm$ 1.612 <sup>BE</sup>
Both	32.91 $\pm$ 2.002 <sup>Da</sup>	45.25 $\pm$ 2.274 <sup>Dh</sup>	39.08 $\pm$ 1.802 <sup>D</sup>
<b>Jersey cows (summer)</b>			
<b>Secretion rate (l/h)</b>			
Twice daily period:			
Control glands	0.195 $\pm$ 0.004 <sup>Aa</sup>	0.136 $\pm$ 0.005 <sup>Ab</sup>	0.165 $\pm$ 0.004 <sup>A</sup>
Test glands	0.214 $\pm$ 0.009 <sup>Aac</sup>	0.162 $\pm$ 0.009 <sup>Bb</sup>	0.188 $\pm$ 0.008 <sup>A</sup>
Thrice daily period:			
Control glands	0.194 $\pm$ 0.009 <sup>Aa</sup>	0.139 $\pm$ 0.011 <sup>Ab</sup>	0.167 $\pm$ 0.008 <sup>A</sup>
Test glands	0.237 $\pm$ 0.010 <sup>Ba</sup>	0.210 $\pm$ 0.010 <sup>Cb</sup>	0.224 $\pm$ 0.009 <sup>B</sup>
<b>Response (%)</b>			
Milking time	9.74 $\pm$ 1.052 <sup>Aa</sup>	19.12 $\pm$ 1.351 <sup>Ab</sup>	14.43 $\pm$ 1.002 <sup>A</sup>
Milking thrice	10.75 $\pm$ 1.259 <sup>Aa</sup>	29.63 $\pm$ 2.214 <sup>Eb</sup>	20.19 $\pm$ 1.432 <sup>E</sup>
Both	22.16 $\pm$ 1.734 <sup>Ba</sup>	50.00 $\pm$ 2.929 <sup>Cb</sup>	36.08 $\pm$ 2.024 <sup>D</sup>

Different superscripts (small among the same row; capital within the same column) indicate significant difference ( $P < 0.05$ ) between means; otherwise indicate similarity.

Differences among the glands in their function and/or structure lead to different milk letdown [12]. In this present study, the difference in the gland function or response could be due to that the test glands were consistently milked [13,14] before the control glands, and, in the case of thrice-daily milking, due to the effect of increased milking frequency [15]. Morage [16] reported a 20% reduction in yield in ewes when the half udders were milked at different times as compared with coincidental milking. This effect was abolished when oxytocin was administered before milking, suggesting that the decline in yield was due to an inhibition of milk ejection in the control glands.

Furthermore, the test glands in both breeds showed significant increase ( $P < 0.01$ ) in secretion rate of milk during the treatment period over the control period, solely to the effect of increased milking frequency, since no difference were observed in the control glands due to the period of experiment (Table 1). This response to thrice daily milking was much more higher than that due to milking at different times. However, the response to both effects together (different udder's halves) was higher than the sum of their individual effects, with a marked difference during the winter season. This synergetic effect could be attributed to increased mammary hormonal receptors induced mainly by thrice milking [17] with more removal of milk from the test glands than the control (residual milk). The more reliable comparison for the response to increased milking frequency in the present work is to compare the same udder half (test glands) between the twice and thrice daily milking periods to eliminate any variations other than those due to thrice-daily milking.

The positive effect of thrice daily milking which appeared only in the test glands could be through removal or deactivation of a putative local inhibitor of milk secretion [7,18] which affects directly the mammary secretory cells and reduces their efficiency for milk production. Therefore, milking should reduce inhibitor levels so as to reestablish an optimal rate of secretion. Frequent milking might influence the action of a hormone or a growth factor through alteration in its mammary receptors, with higher response with complete milk ejection reflex as with the test glands (milked first). In goats, there was a significant increase in the number of prolactin receptor per each mammary cell only in the glands milked three times daily for 4 weeks compared to those milked twice [17]. This prolactin receptor modulation by milking frequency was found to be related directly to an autocrine milk constituent [19], and any variations in response to milking frequency could be ascribed to variations in induction of these receptors. Prolactin maintains milk synthesis at least in part by inhibiting epithelial cell loss and maintaining cellular differentiation [20]. Decreased intramammary pressure by milking frequency could play a small part [4,11, p.41] in the above response. The increase in milk secretion of the test glands milked thrice

instead of twice is not dependent on the presence of residual milk, since the intervals among the three daily milkings were equal [21].

Half udder studies showed that thrice daily milking produced 8-52% more milk than twice daily milking [22,23,24,25] depending upon the breed, stage of lactation, length of the experimental period and the technique used. The response found in the present study was within 10-30% (Table 1). Kulsreshtha and Razdan [26] found breed differences in response to either 2,3 or 4x daily milking for monthly total milk yield. Whole udder study [27] indicated advantages of 17.3 and 6.3% in 305 d-first lactation yield of Holstein and Jersey cows, respectively, milked three times daily compared with milking twice daily, and this positive response was affected significantly ( $p < 0.01$ ) by season of calving in both breeds.

In cows, the effect of stage of lactation was significant [25] with inconsistent results. Increased response to thrice-daily milking of one half of the udder [25] or the whole udder [28-32] with advancing lactation was reported, but it was higher during early stage than during latter stages as reported by Shinde [25] in half-udder study and by Kopecky and Grolig [33] and Polovtsev [34] in a whole udder study. The present study showed the former trend of stage of lactation. Shinde [35] found that increasing milking frequency appeared to increase milk yield by promoting proliferation and activity of secretory cell during early lactation and reducing the rate of involution after peak. Residual milk found to be decreased by both thrice-daily milking and stage of lactation [36]. Body-weight changes in early lactation could be implicated in production response [37].

In the present work, the response during summer due to thrice daily milking was higher ( $P < 0.01$ ) for the Friesian cows than that for the Jerseys during the early stage of lactation, while it was less ( $P < .05$ ) during the post-peak stage. Therefore, the variation among the two breeds in response to thrice daily milking is only during a certain stage of lactation. Thrice milked glands of the Jerseys may have become more persistent during the post-peak stage of lactation than those of the Friesians, resulting in higher response at this later stage to increased milking frequency. The late stages of lactation could have greater concentration of the milk secretion inhibitor, and frequent milking may reduce this effective concentration to levels that are less effective compared with the early stage of lactation, hence the higher rate of secretion in the former. Speight and Fairlie [38] found that the increase in milk yield with 3x daily milking compared with twice daily milking in the previous year varied with month of the year.

During thrice-daily milking there were considerable variations in the rate of

milk secretion throughout the day (Table 2). These variations cannot be related to differences in residual milk volume with equal milking intervals [24] and must be diurnal. The increase in secretion rate, and hence in total daily milk yield, was accounted for entirely by the increased secretion rate during the 16 h period (i.e. the sum of the two 8 h periods interposed by the extra milking). The higher secretion rate of the morning milking was the result of the third milking at mid-night. This rapid increase in milk yield has also been reported by Linnerud *et al.* [39], Elliot [23], Morag [24] and Hillerton *et al.* [7]. The stimulation of yield was rapidly reversed when twice-daily milking was restored. These rapid changes suggest that the increased output is achieved from existing secretory cell rather than by cell division [40].

**Table 2.** Diurnal variation in milk secretion rate (mean  $\pm$  s.e., l/h) in Friesian and Jersey cows milked thrice daily (test glands only) at two stages of lactation and seasons of the year

Stage of lactation	Milk secretion rates between these times			
	08.00 - 16.00 h	16.00 - 08.00 h	16.00 - 00.00 h	00.00 - 08.00 h
<b>Friesian cows (winter)</b>				
Early	0.320 $\pm$ 0.015 <sup>Aa</sup>	0.378 $\pm$ 0.015 <sup>Ab</sup>	0.349 $\pm$ 0.015 <sup>Aa</sup>	0.406 $\pm$ 0.017 <sup>Ac</sup>
Late	0.312 $\pm$ 0.011 <sup>Aa</sup>	0.400 $\pm$ 0.011 <sup>Ab</sup>	0.334 $\pm$ 0.010 <sup>Aa</sup>	0.465 $\pm$ 0.012 <sup>Bc</sup>
Overall	0.316 $\pm$ 0.012 <sup>a</sup>	0.389 $\pm$ 0.011 <sup>b</sup>	0.342 $\pm$ 0.009 <sup>a</sup>	0.436 $\pm$ 0.011 <sup>c</sup>
<b>Friesian cows (summer)</b>				
Early	0.293 $\pm$ 0.010 <sup>Aa</sup>	0.331 $\pm$ 0.010 <sup>Bb</sup>	0.287 $\pm$ 0.012 <sup>Ba</sup>	0.374 $\pm$ 0.014 <sup>Cc</sup>
Late	0.232 $\pm$ 0.007 <sup>Ba</sup>	0.274 $\pm$ 0.007 <sup>Cb</sup>	0.226 $\pm$ 0.009 <sup>Ca</sup>	0.322 $\pm$ 0.011 <sup>Dc</sup>
Overall	0.263 $\pm$ 0.008 <sup>a</sup>	0.303 $\pm$ 0.008 <sup>b</sup>	0.257 $\pm$ 0.008 <sup>a</sup>	0.348 $\pm$ 0.009 <sup>c</sup>
<b>Jersey cows (summer)</b>				
Early	0.208 $\pm$ 0.008 <sup>BCa</sup>	0.252 $\pm$ 0.008 <sup>Cb</sup>	0.210 $\pm$ 0.012 <sup>Ca</sup>	0.293 $\pm$ 0.010 <sup>Ec</sup>
Late	0.183 $\pm$ 0.011 <sup>Ca</sup>	0.223 $\pm$ 0.011 <sup>Db</sup>	0.198 $\pm$ 0.015 <sup>Ca</sup>	0.248 $\pm$ 0.013 <sup>Fc</sup>
Overall	0.196 $\pm$ 0.008 <sup>a</sup>	0.238 $\pm$ 0.007 <sup>b</sup>	0.204 $\pm$ 0.008 <sup>a</sup>	0.271 $\pm$ 0.009 <sup>c</sup>

Different superscripts (small among the same row; capital within the same column) indicate significant difference ( $P < 0.01$ ) between means; otherwise indicate similarity.

This study confirms that the mechanism by which the rate of milk secretion is increased by thrice milking instead of twice daily is entirely local to the gland receiving the extra milking, acting rapidly within hours. Therefore, milk secretion is not determined by systemic factors such as hormonal or substrate supply but is determined at the level of the mammary gland [41]. However, successful milk ejection reflex could somehow affect such a mechanism.

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## تأثير السلالة وفصل السنة ومرحلة الحليب على استجابة أبقار الحليب لزيادة عدد مرات الحلابة

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ملخص البحث. درس تأثير السلالة وفصل السنة ومرحلة الحليب على استجابة (معدل إفراز الحليب) أبقار الحليب الأصلية للحلابة ثلاث مرات بدلاً من مرتين يوميًا، وذلك باستخدام أسلوب نصف الضرع في ٢٠ بقرة فريزيان و١٠ أبقار جيرسي. أشارت النتائج بأن هناك استجابة موجبة لزيادة عدد مرات الحلابة في كلتا السلالتين، وأنها متساوية بين فصلي الشتاء والصيف بصرف النظر عن مرحلة الحليب. إلا أن استجابة أبقار الجيرسي خلال فصل الصيف في مرحلة ما بعد ذروة الإنتاج كانت أعلى مما لأبقار الفريزيان، بينما العكس كان في مرحلة الإنتاج المبكرة. وعمومًا كانت استجابة الأبقار خلال مرحلة ما بعد ذروة الإنتاج أعلى دائمًا من تلك خلال المرحلة المبكرة. وتعزى الزيادة في معدل إفراز الحليب نتيجة زيادة عدد مرات الحلابة إلى الزيادة المعنوية في إنتاج حلبة الصباح التي تلت حلبة منتصف الليل الإضافية، وذلك فقط في نصف الضرع الذي حلب ثلاث مرات، مما يستدل منه على وجود نظام داخلي مستقل وخاص بكل غدة يتحكم في معدل إفرازها من الحليب ويستجيب سريعًا لعملية تفرغ الحليب.