

## Effect of temporary alterations to milking frequency during the early post-partum period on milk production and body condition score in grazing dairy cows

C.V.C. PHYN<sup>1\*</sup>, J.K. KAY<sup>1</sup>, A.G. RIUS<sup>1</sup>, S.R. MORGAN<sup>1</sup>, C.S. ROACH<sup>1</sup>, T.M. GRALA<sup>2</sup>, and J.R. ROCHE<sup>1</sup>

<sup>1</sup>DairyNZ, Private Bag 3221, Hamilton 3240, New Zealand

<sup>2</sup>DairyNZ, C/- ViaLactia Biosciences (NZ) Ltd., P.O. Box 109-185, Newmarket, Auckland 1149, New Zealand

\*Corresponding author: claire.phyn@dairynz.co.nz

### ABSTRACT

This study investigated the immediate and long-term effects of temporary alterations to milking frequency post-partum in grazing cows. Multiparous Holstein-Friesian cows (n = 150) were assigned to one of five groups at calving: milked twice daily (2X, Control), or either once daily (1X) or thrice daily (3X) for three or six weeks, and 2X thereafter. During Weeks 1-6 post-calving, 1X for six weeks resulted in greater decreases in milk, fat, protein and lactose yields (P < 0.05) relative to 2X than 1X for three weeks. Furthermore, 1X for three or six weeks reduced fat, protein and lactose yields (P < 0.05), and tended to reduce milk yields (P = 0.08), for the remainder of lactation (Weeks 8-32). Body condition was greater for 1X cows at Weeks 6 and 8 (P < 0.05) and tended to be greater during Weeks 8-32 (P = 0.09). Milking 3X for three or six weeks increased milk yield (P < 0.05) relative to 2X during Weeks 1-6 and milk yields were numerically greater during Weeks 8-32 (P = 0.17); however, fat and protein yields were not affected. Once-daily milking for three or six weeks post-calving had lactation-long negative effects on milk production, whereas 3X increased milk yield but did not result in short- or long-term benefits in fat and protein yields.

**Keywords:** milking frequency; early lactation; dairy cow.

### INTRODUCTION

Temporary alterations to milking frequency (MF) can have long-term effects on milk production through changes in mammary function such as, secretory cell activity, proliferation, and/or death via apoptosis (Stelwagen, 2001; Hale *et al.*, 2003). These effects indicate that MF could be strategically manipulated to improve lactation persistency and increase the annual and lifetime productivity of dairy cows. Multiple studies (Hale *et al.*, 2003; Dahl *et al.*, 2004; Wall *et al.*, 2006) reported that short-term use of increased MF post-partum resulted in lactation-long increases in milk production; however, the majority of research has been conducted using North-American Holstein-Friesian cows managed in high-input, confinement systems. It is unclear whether positive long-term milk production effects can be obtained in pasture-based systems.

In comparison, decreasing MF to once daily (1X) during early lactation may provide labour, lifestyle and animal health benefits such as body condition score (BCS), energy status and reproduction (McNamara *et al.*, 2008), particularly in pasture-based systems, where seasonal calving results in a rapid influx of fresh cows, putting pressure on feed resources and staff and cow management processes. Limited data are available, however, on the long-term impacts of 1X milking as a short-term strategy during the post-partum period. Reports to date indicate that, in addition to an immediate 20-30% decrease in milk production, 1X milking post-

calving negatively effects subsequent production, especially when used for longer than three weeks (Rémond *et al.*, 1999; McNamara *et al.*, 2008).

Study objectives were to determine the immediate and long-term effects of temporary alterations to MF immediately post-partum on milk production and BCS in grazing cows.

### MATERIALS AND METHODS

This study was conducted at the DairyNZ Lye Farm, Hamilton, New Zealand from June 2009 to May 2010. All procedures had prior approval of the Ruakura Animal Ethics Committee, Hamilton, New Zealand.

Multiparous Holstein-Friesian and Holstein-Friesian x Jersey cows (n = 150) were randomly assigned to one of five treatments at calving (mean  $\pm$  standard deviation; 15 July 2009  $\pm$  10.6 days). Treatments were: Milked twice daily (2X) for the entire lactation (Control), Milked 1X for either three or six weeks, and 2X thereafter, or Milked thrice daily (3X) for either three or six weeks, and 2X thereafter. Daily milking times were 07:00 h for 1X (24 hour interval), 07:00 h and 15:00 h for 2X (16/8 hour interval), and 07:00 h, 15:00 h and 22:00 h for 3X (9/8/7 hour interval).

All cows were offered a generous pasture allowance (30 to 40 kg dry matter (DM)/cow/day to target post-grazing residuals of 1,800 kg DM/ha), and grazed in 1X, 2X and 3X milking mobs in the same paddock separated by a wire. Cows on different MF were moved separately to the dairy for

**TABLE 1:** Mean milk production of cows milked either twice daily (2X; Control), Once daily (1X) for three or six weeks post-calving and 2X thereafter (1X\_3wks and 1X\_6wks, respectively), or Thrice daily (3X) for three or six weeks post-calving and 2X thereafter (3X\_3wks and 3X\_6wks, respectively). SED = Standard error of difference. Use  $SED \times \sqrt{0.75}$  to compare the mean of 1X or 3X treatments with the 2X Control. Bolding of P value indicates significance ( $P \leq 0.05$ ).

Milk production	Week post-calving	Treatment					SED	P value		Milking frequency x Duration
		1X_3wks	1X_6wks	2X	3X_3wks	3X_6wks		1X versus 2X	3X versus 2X	
Milk (kg/d)	1 to 3	19.2	18.3	22.2	24.1	23.5	0.86	<b>&lt;0.01</b>	<b>0.04</b>	0.78
	4 to 6	24.1	20.2	25.3	26.1	27.6	1.01	<b>&lt;0.01</b>	0.06	<b>&lt;0.01</b>
	1 to 6	21.7	19.7	23.7	25.1	25.6	0.89	<b>&lt;0.01</b>	<b>0.04</b>	<b>0.03</b>
	8 to 32	20.3	20.0	21.3	22.6	21.9	0.77	0.08	0.17	0.81
Fat (%)	1 to 3	4.85	4.80	5.02	4.67	4.78	0.14	0.11	<b>0.02</b>	0.43
	4 to 6	4.48	4.45	4.66	4.32	4.19	0.14	0.10	<b>&lt;0.01</b>	0.63
	1 to 6	4.66	4.61	4.83	4.48	4.47	0.12	<b>0.05</b>	<b>&lt;0.01</b>	0.81
	8 to 32	4.63	4.55	4.77	4.42	4.35	0.13	0.12	<b>&lt;0.01</b>	0.98
Protein (%)	1 to 3	4.04	4.02	3.95	3.85	3.90	0.06	0.14	0.16	0.42
	4 to 6	3.64	3.60	3.56	3.53	3.38	0.05	0.15	<b>0.02</b>	0.13
	1 to 6	3.84	3.81	3.75	3.68	3.63	0.05	0.11	<b>0.03</b>	0.86
	8 to 32	3.69	3.64	3.72	3.63	3.57	0.06	0.31	<b>0.02</b>	0.96
Lactose (%)	1 to 3	4.68	4.67	4.80	4.66	4.66	0.03	<b>&lt;0.01</b>	<b>&lt;0.01</b>	0.94
	4 to 6	4.86	4.78	4.88	4.83	4.76	0.02	<b>&lt;0.01</b>	<b>&lt;0.01</b>	0.91
	1 to 6	4.77	4.73	4.84	4.75	4.71	0.02	<b>&lt;0.01</b>	<b>&lt;0.01</b>	0.91
	8 to 32	4.84	4.86	4.87	4.83	4.85	0.03	0.29	0.20	0.99
Fat (kg/d)	1 to 3	0.92	0.87	1.10	1.10	1.11	0.05	<b>&lt;0.01</b>	0.94	0.42
	4 to 6	1.07	0.88	1.17	1.12	1.15	0.05	<b>&lt;0.01</b>	0.40	<b>&lt;0.01</b>
	1 to 6	1.00	0.87	1.14	1.11	1.13	0.05	<b>&lt;0.01</b>	0.66	<b>0.03</b>
	8 to 32	0.92	0.89	1.00	0.97	0.94	0.03	<b>&lt;0.01</b>	0.12	0.93
Protein (kg/d)	1 to 3	0.76	0.72	0.86	0.90	0.89	0.04	<b>&lt;0.01</b>	0.21	0.56
	4 to 6	0.88	0.73	0.90	0.92	0.93	0.03	<b>&lt;0.01</b>	0.30	<b>&lt;0.01</b>
	1 to 6	0.82	0.72	0.88	0.91	0.91	0.03	<b>&lt;0.01</b>	0.22	<b>0.03</b>
	8 to 32	0.75	0.72	0.79	0.82	0.82	0.02	<b>&lt;0.01</b>	0.80	0.79
Lactose (kg/d)	1 to 3	0.90	0.86	1.07	1.13	1.10	0.04	<b>&lt;0.01</b>	0.21	0.82
	4 to 6	1.17	0.97	1.23	1.26	1.31	0.05	<b>&lt;0.01</b>	0.17	<b>&lt;0.01</b>
	1 to 6	1.04	0.92	1.15	1.20	1.21	0.04	<b>&lt;0.01</b>	0.17	<b>0.03</b>
	8 to 32	0.98	0.97	1.04	1.09	1.07	0.04	<b>0.05</b>	0.22	0.89

milking. Cows in 1X or 3X treatments were moved into the 2X mob after completing their three or six weeks of decreased or increased MF, respectively, and grazed together for the remainder of the experiment. Pasture silage was offered to maintain pasture residuals when pasture availability was limited: 3.6 kg DM/cow/day for 40 days during July and August 2009, and 5.0 kg DM/cow/day for 21 days in April 2010. In addition, from two weeks prior to predicted calving date, cows were offered 2 kg DM/cow/day of a pelleted maize-barley concentrate in the paddock. Following calving, cows were individually offered concentrate during the morning milking of 4 kg DM concentrate/cow/day until 1 November 2009 then 2 kg DM/cow/day until 24 November 2009.

Individual milk yield and composition (fat, protein and lactose) were determined daily and

weekly, respectively. Body condition score (1-10 scale; Roche *et al.*, 2004) was recorded weekly until September and fortnightly thereafter.

Data were analysed using mixed models fitted with REML in GenStat (version 13.2; VSN International, 2010) including treatment and contrasts to test MF (1X, 2X or 3X), duration (three or six weeks) and their interaction as fixed effects with cow as a random effect. Mean body condition score at calving of  $5.1 \pm 0.5$  BCS units was used as a covariate for analysis of BCS data. T-tests were used for individual treatment comparisons if significant MF x Duration interactions were detected. Least squared means and the standard error of the difference between means (SED) are presented. Data are considered significant when  $P \leq 0.05$  and declared a trend when  $P < 0.15$ .

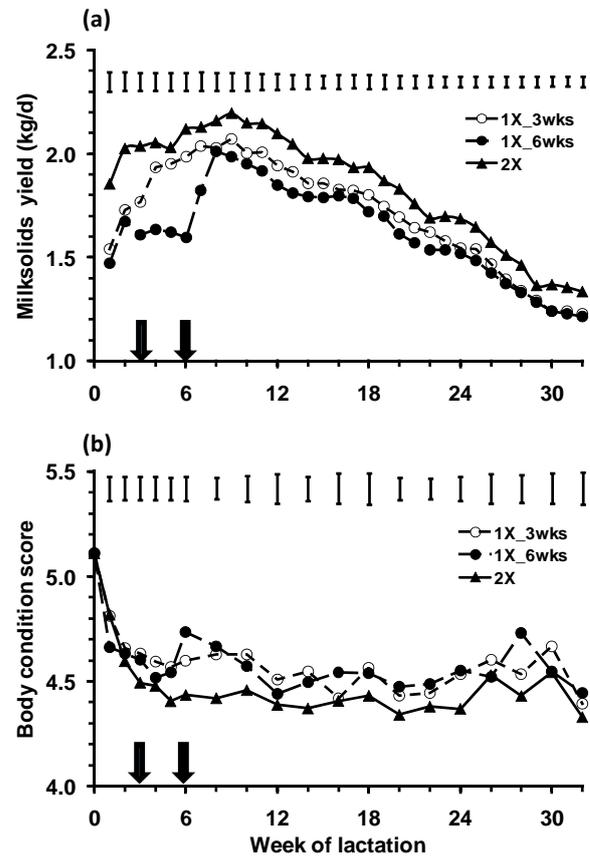
## RESULTS AND DISCUSSION

### Effects of once-daily milking on milk production

Milk production data during and after the three- or six-week periods of altered MF are presented in Table 1. During the first three weeks post-calving, cows milked 1X had lower yields of milk (-15.4%), fat (-19.2%), protein (-14.0%), and lactose (-17.5%) compared with cows milked 2X. There were MF x Duration interactions during Weeks 4-6 post-calving. When cows milked 1X for three weeks were changed to 2X milking, milk and milk component yields during Weeks 4-6 increased ( $P < 0.05$ ) relative to cows remaining on 1X milking for six weeks (Table 1; Figure 1a). During Weeks 1-6 post-calving, cows milked 1X for six weeks produced less ( $P < 0.05$ ) milk, fat, protein, and lactose compared with cows milked 1X for three weeks. Relative to 2X, 1X milking for six weeks decreased ( $P \leq 0.05$ ) milk (-18.8%), fat (-23.2%), protein (-17.7%), and lactose (-20.4%) yields. The size of these immediate milk production losses are comparable to previous studies, where cows were milked 1X for three, four or six weeks following calving (Rémond *et al.*, 1999; McNamara *et al.*, 2008; Schlamberger *et al.*, 2010). Furthermore, losses in milk production increased with duration of 1X milking post-calving, as reported by Rémond *et al.* (1999). However, Rémond *et al.* (1999) and Schlamberger *et al.* (2010) reported increases in milk fat and protein concentrations that partially offset losses in milk yield during 1X milking. In the present study, protein concentration tended to increase and milk fat concentration decreased during Weeks 1-6 post-calving. Therefore, the greatest immediate losses from 1X milking were for milk fat yield rather than milk volume.

Milking cows 1X for a short duration post-calving also resulted in long-term negative effects on milk production. During Weeks 8-32, when all cows were milked 2X, cows milked 1X post-calving had lower fat (-9.3%), protein (-7.0%), and lactose (-6.0%) yields, and tended to have lower milk yields (-5.5%), relative to those continuously milked 2X (Table 1). The temporal pattern of milksolids (MS; fat + protein) yields in Figure 1a demonstrates that the negative carry-over effect was still present at Week 32 of lactation (-1.22 kg/d: -8.7%). Furthermore, there were no MF x Duration interactions during Weeks 8-32, indicating that milking 1X for three or six weeks resulted in similar long-term decreases in milk and milk component yields. This result differs from Rémond *et al.* (1999), who reported that cows milked 1X for six weeks had a greater and longer residual milk yield loss than cows milked 1X for three weeks. Rémond *et al.* (1999) also observed significant negative carry-over losses in fat and protein yields when

**FIGURE 1:** (a) Mean milksolids (fat + protein) yield (kg/cow/day) and (b) mean body condition score of cows milked either twice daily (2X Control), or Once daily (1X) for three or six weeks post-calving and 2X thereafter (1X\_3wks and 1X\_6wks, respectively). Arrows indicate the end of the three-week and six-week treatment periods. Error bars indicate the standard error of the difference between means.



cows were milked 1X for six weeks but not when they were milked 1X for three weeks. However, more recent studies demonstrated that 1X milking for four weeks post-calving (NcNamara *et al.*, 2008; Schlamberger *et al.*, 2010) caused long-term reductions in milk, fat and protein yields. Combined with the results of the present study, these findings indicate that 1X milking for three weeks or longer during the early post-partum period will lead to sustained losses in milk and milk component yields.

The immediate and carry-over effects of 1X milking on milk production are controlled by local, intra-mammary signals (Stelwagen, 2001). These mechanisms are yet to be fully characterised, but sustained milk production losses may be due to decreased mammary secretory cell activity or number. Once-daily milking reduces the activity of key mammary enzymes involved in fat and lactose synthesis (Farr *et al.*, 1995), and it is possible that decreased mammary secretory cell activity is maintained even when cows are switched to 2X

milking. In support of this premise, Grala *et al.* (2011) reported that transcription of milk synthesis genes was down-regulated in cows milked 1X post-calving relative to those milked 2X, and that expression remained lower after the 1X cows were switched to 2X. Furthermore, expression of mammary genes involved in cell death via apoptosis was greater in cows milked 1X relative to 2X, both during and after the reduced MF (Grala *et al.*, 2011). These results indicate that both reduced mammary secretory cell number and activity contribute to the long-term negative production effects following a short duration of 1X milking in early lactation.

In the present study, the immediate and residual effects of short-term 1X milking led to decreased ( $P < 0.001$ ) cumulative milk and milk component yields between Weeks 1-32, (224 days in milk; C.V.C. Phyn, Unpublished data). Cows milked 1X for three weeks produced 7% less total MS between Weeks 1-32, while cows milked 1X for six weeks produced 12% less total MS than cows milked 2X (382.5, 363.40, and 413.2 kg MS/cow, respectively; SED of 12.52 kg MS/cow). Use of 1X milking during the early post-calving period will, therefore, lead to losses in total milk revenue per cow.

Losses in milk income will be particularly evident at higher MS payouts; however, in a commercial farm these negative effects may be outweighed by benefits to labour requirements, farmer lifestyle and animal health. Milking 1X during the busy seasonal calving period allows farmers more time to concentrate on other aspects of the farm system, such as grazing management and cow health. Improvements in these areas may partially compensate for the negative effects of decreased MF on milk production; however, any positive effects of improved feeding are separate from those of MF (Kay *et al.*, 2011).

In the present study, cows were well-fed on pasture supplemented with concentrate. Comparable immediate and carry-over production losses from milking 1X for three weeks during early lactation were also reported (Kay *et al.*, 2011) under best practise grazing management, but immediate losses were reduced if cows were severely restricted. These results indicate that, while relative production losses from milking 1X post-calving may be smaller in lower-producing herds, 1X causes further reductions in the ability of cows to produce milk. Nevertheless, other strategies may be employed to minimise the total milk revenue loss on-farm, such as applying 1X milking on a herd-basis for the first three to six weeks after the start of calving, using a shorter duration of less than three weeks, or only during the colostrum period.

### Effects of thrice-daily milking on milk production

During the first three weeks post-calving, cows milked 3X had increased milk yields (+7.2%) relative to 2X (Table 1). However, fat and protein concentrations were decreased, resulting in no differences in milk component yields. During Weeks 1-6 post-calving, milking cows 3X for six weeks did not increase milk yields relative to 3X for three weeks ( $P > 0.05$ ) but increased milk yields (+7.7%,  $P \leq 0.05$ ) relative to 2X. These results indicate that increasing the duration of 3X milking beyond three weeks post-calving did not lead to further improvements in milk production.

A numerical milk yield increase (+4.4%,  $P = 0.17$ ) was evident during Weeks 8-32 when cows milked 3X post-calving were switched to 2X for the rest of lactation. This finding is consistent with earlier results (Phillips *et al.*, 1980) that indicated milking 3X post-calving may benefit subsequent 2X production provided nutrition was not limiting; however, there has been limited research conducted on the use of increased MF in grazing systems. In the present study, cows were offered a generous pasture allowance supplemented with a moderate rate of concentrate (4 kg DM/cow/day). The relatively small increase in immediate and then subsequent milk yields, and the decrease in milk fat and protein contents, may reflect an inability of cows to consume sufficient pasture during early lactation. These effects may be magnified during 3X milking, due to reduced grazing time because cows are removed for a third milking. Similarly, McNamara *et al.* (2008) reported that 3X milking during the first four weeks of lactation did not affect immediate or long-term milk production, possibly due to reduced access to total mixed ration and, consequently, dry matter intake. Higher energy density diets may be required during increased MF post-calving because the milk yield rise is not necessarily accompanied by an increase in feed intake and consequently, cows mobilise more body reserves (Andersen *et al.*, 2003).

The immediate and carry-over responses to increased MF may be greater if cows are milked four times daily (4X) post-calving. Several studies (Hale *et al.*, 2003; Dahl *et al.*, 2004; Wall *et al.*, 2006) have confirmed immediate and lactation-long increases in milk yield from milking high-producing cows 4X for three weeks post-calving in confinement systems. The positive effects of increased MF on milk production may reflect greater proliferation or differentiation of mammary cells, extracellular remodelling or creation of new blood vessels (Hale *et al.*, 2003; Connor *et al.*, 2008). Grala *et al.* (2011) reported that increases in milk yield from 3X milking post-calving (Table 1) were not associated with changes in milk synthesis gene expression, indicating that mammary cell

activity was not increased. Thus, the mechanisms regulating milk production responses to increased MF are still to be fully elucidated.

### Effects of altered milking frequency on body condition score

The BCS profiles of cows milked 1X or 2X post-calving are presented in Figure 1b. Milking cows 1X for three or six weeks did not affect BCS loss during the first four weeks post-calving. Body condition score tended to be greater ( $P = 0.10$ ) in cows milked 1X post-calving at Week 5 (+0.15 units), and was greater at Weeks 6 and 8 (+0.23 units) relative to cows milked 2X. During the first five weeks post-calving, cows milked 1X lost 0.55 BCS units, whereas cows milked 2X lost 0.7 BCS units ( $P = 0.10$ ). The trend ( $P = 0.07$ ) for a MF x Duration interaction at Week 6 post-calving indicated that the increase in BCS tended to be greater for cows milked 1X for six weeks than for cows milked 1X for three weeks (+0.30 versus +0.16 units, respectively); however, there were no interactions at Week 8. Mean BCS during Weeks 8-32 of lactation also tended to be greater (+0.14 units;  $P = 0.09$ ) in cows milked 1X post-calving, but by late lactation (Week 32) BCS was not significantly different relative to cows milked 2X post-calving (4.4 versus 4.3 units, respectively; Figure 1b). These results support previous reports that short-term 1X milking following calving improved BCS and cow energy status (Rémond *et al.*, 1999; McNamara *et al.*, 2008; Schlamberger *et al.*, 2010). In comparison, cows milked 3X had a greater ( $P < 0.05$ ) BCS loss than cows milked 2X during the first three weeks post-calving (0.8 versus 0.6 units, respectively), but not thereafter. Increased BCS loss immediately post-calving implies that cows milked 3X mobilised more body reserves to support their greater milk production, as reported previously (Andersen *et al.*, 2003).

Milking 1X for three or six weeks post-calving had lactation-long negative effects on milk production, but improved BCS profiles from Weeks 5-6 of lactation onwards. Potential losses in milk production should be considered relative to the possible benefits of 1X on farm and labour management, farmer lifestyle and animal health. In comparison, 3X milking increased milk yield, but did not result in short- or long-term benefits in fat and protein yields.

### ACKNOWLEDGEMENTS

The authors gratefully acknowledge the contribution and dedication of the DairyNZ Lye Farm technical and farm teams, especially A. Napper and B. Sugar, for implementation of the experiment, and B. Dow for statistical analysis. This study was funded by New Zealand dairy farmers

through DairyNZ Inc. and by the Foundation for Research, Science and Technology (contract DRCX0801).

### REFERENCES

- Andersen, J.B.; Friggens, N.C.; Sejrsen, K.; Sorensen, M.T.; Munksgaard, L.; Ingvarsen, K.L. 2003: The effects of low vs. high concentrate level in the diet on performance in cows milked two or three times daily in early lactation. *Livestock Science* **81**: 119-128.
- Connor, E.E.; Siferd, S.; Elsasser, T.H.; Clover, C.; Van Tassell, C.P.; Sonstegard, T.S.; Fernandes, V.; Capuco, A.V. 2008: Effects of increased milking frequency on gene expression in the bovine mammary gland. *BMC Genomics* doi:10.1186/1471-2164-9-362.
- Dahl, G.E.; Wallace, R.L.; Shanks, R.D.; Lueking, D. 2004: Hot topic: Effects of frequent milking in early lactation on milk yield and udder health. *Journal of Dairy Science* **87**: 882-885.
- Farr, V.C.; Stelwagen, K.; Kerr, M.A.; Davis, S.R.; Eichler, S.J. 1995: Effect of once daily milking (ODM) on enzyme activities in the bovine mammary gland. *Proceedings of the New Zealand Society of Animal Production* **55**: 12-13.
- Grala, T.M.; Phyn, C.V.C.; Kay, J.K.; Rius, A.G.; Littlejohn, M.D.; Snell, R.G.; Roche, J.R. 2011: Temporary alterations to milking frequency, immediately post-calving, modifies expression of milk synthesis and apoptosis genes in the mammary gland. *Proceedings of the New Zealand Society of Animal Production* **71**: 3-8.
- Hale, S.A.; Capuco, A.V.; Erdman, R.A. 2003: Milk yield and mammary growth effects due to increased milking frequency during early lactation. *Journal of Dairy Science* **86**: 2061-2071.
- Kay, J.K.; Phyn, C.V.C.; Rius, A.G.; Morgan, S.R.; Grala, T.M.; Roche, J.R. 2011: Effect of milking frequency and nutrition in early lactation on milk production and body condition score. *Proceedings of the New Zealand Society of Animal Production* **71**: 37-41.
- McNamara, S.; Murphy, J.J.; O'Mara, F.P.; Rath, M.; Mee, J.F. 2008: Effect of milking frequency on energy metabolism, milk production and reproductive performance of dairy cows. *Livestock Science* **117**: 70-78.
- Phillips, D.S.M.; Woolford, M.W.; Copeman, P.J.A. 1980: The implications of milking management strategies involving variations of milking frequency in the immediate post-partum period. *Proceedings of the New Zealand Society of Animal Production* **40**: 166-174.
- Rémond, B.; Coulon, J.B.; Nicloux, M.; Levieux, D. 1999: Effect of once-a-day milking in early lactation on milk production and nutritional status of dairy cows. *Annales de Zootechnie* **48**: 341-352.
- Roche, J.R.; Dillon, P.G.; Stockdale, C.R.; Baumgard, L.H.; VanBaale, M.J. 2004: Relationships among international body condition scoring systems. *Journal of Dairy Science* **87**: 3076-3079.
- Schlamberger, G.; Wiedemann, S.; Viturro, E.; Meyer, H.H.D.; Kaske, M. 2010: Effects of continuous milking during the dry period or once daily milking in the first 4 weeks of lactation on metabolism and productivity of dairy cows. *Journal of Dairy Science* **93**(6): 2471-2485.
- Stelwagen, K. 2001: Effect of milking frequency on mammary functioning and shape of the lactation curve. *Journal of Dairy Science* **84** (Electronic supplement):E204-E211.
- VSN International 2010: GenStat for Windows 13th Edition. VSN International, Hemel Hempstead, UK. www.vsn.co.uk
- Wall, E.H.; Crawford, H.M.; Ellis, S.E.; Dahl, G.E.; McFadden, T.B. 2006: Mammary response to exogenous prolactin or frequency milking during early lactation in dairy cows. *Journal of Dairy Science* **89**: 4640-4648.