

Once-daily milking during a feed deficit improves energy status in early lactating dairy cows

JK Kay^{a*}, CVC Phyn^a, AG Rius^a, SR Morgan^a, TM Grala^b and JR Roche^a

^aDairyNZ, Private Bag 3211, Hamilton 3240, New Zealand; ^bDairyNZ, % University of Auckland, 3A Symonds Street, Auckland 1010, New Zealand

*Corresponding author. Email: jane.kay@dairyNZ.co.nz

Abstract

The aim of this study was to determine the effect of once-daily (1X) milking during a feed deficit on energy status of grazing dairy cows. Multiparous cows (n = 120), grazing to residuals of 1,600 kg DM/ha, and milked twice-daily (2X) from calving until approximately 35 days-in-milk, were allocated to one of four treatments in a 2 x 2 factorial arrangement. Cows were adequately fed (14 kg DM/cow/d) or underfed (8 kg DM/cow/d) and milked 2X or 1X for three weeks, after which all cows were milked 2X and grazed to residuals of 1,600 kg DM/ha for 20 weeks. During the three-week treatment period, plasma concentrations of glucose, insulin and insulin-like growth factor-I (IGF-I) were less in underfed compared with adequately cows, while non-esterified fatty acids (NEFA), β -hydroxybutyrate (BHBA) and liver enzymes were greater. An interaction existed and the increase in liver enzymes with underfeeding only occurred in cows milked 1X. Cows milked 1X had greater plasma glucose, insulin and IGF-I and less NEFA and BHBA than those milked 2X. Data indicate that milking cows 1X during an acute feed deficit improves energy status.

Keywords: once-daily milking; nutrition; energy status

Introduction

In pasture-based dairy systems, adverse weather events such as floods, heavy snowfall, storms or droughts can result in severe feed deficits. Reports indicate that the New Zealand dairy industry will face more extreme weather events and rising temperatures in the future (Renwick et al. 2010). These conditions threaten low-input systems and increase the importance of understanding the impact of feed deficits on energy balance during and after these events. Once-daily milking (1X) is a management strategy proposed to alleviate nutritional stress; however, more information is required on the effect of 1X milking on the energetic status of the dairy cow both during and after a temporary feed deficit.

We recently reported that cows milked 1X during a three week pasture deficit in early lactation produced 14% less milk than those milked twice-daily (2X) with both groups losing 0.2 units of body condition score (BCS) (1 – 10 scale; Roche et al. 2004) (Kay et al. 2011). The lack of a difference in BCS loss may be because BCS is only an indirect measure of energy balance. Previous research (Auldist & Prosser 1998; Remond et al. 2002; Guinard-Flament et al. 2007) indicated energy status, as estimated from individual energy input less energy output and/or plasma hormone and metabolite data, was improved when milking frequency was reduced during periods of restricted energy intake. However, these latter experiments are limited in their application to pasture-based systems, as they either milked cows 1X for only two-days (Auldist & Prosser 1998), used small numbers of cows fed a total mixed ration in confined housing (Remond et al. 2002; Guinard-Flament et al. 2007), or had only a small difference in energy intake (Remond et al.

2002). Additionally, none of these studies measured the carry-over effects of milking 1X during a temporary feed deficit.

In this experiment, the hypothesis was that 1X milking during a feed deficit would improve cow energy balance as measured by plasma hormones and metabolite concentrations, both during and after the feed deficit.

Materials and methods

Experimental research was conducted at the Westpac Taranaki Agricultural Research Station, Hawera, New Zealand between July 2009 and April 2010. All procedures involving animals were approved by the Ruakura Animal Ethics Committee, Hamilton, New Zealand.

Experimental design

Cow selection, treatments, intake and milk measurements have been reported previously (Kay et al. 2011). Briefly, 120 multiparous Holstein-Friesian (n = 112) and Holstein-Friesian x Jersey cows (n = 8) were milked 2X and grazed to residuals of 1,600 kg DM/ha as one herd for the first 34 \pm 6 (mean \pm standard deviation) days in milk (DIM). Cows were then allocated to one of four treatments in a 2 x 2 factorial arrangement. Treatments consisted of two milking frequencies (2X or 1X) and two feeding levels, adequately fed (14 kg DM/cow/d) or underfed (8 kg DM/cow/d) for three weeks. To achieve the different pasture intakes for the adequately fed and underfed groups different sized areas were allotted to each treatments based on pre-grazing pasture mass and the desired allowance per cow. All cows were offered a fresh pasture break, twice daily at approx. 0800 h and 1600 h. Daily milking times were 0700 h

Table 1 Plasma metabolite, hormone and liver enzyme content from grazing cows (34 ± 6 days in milk) milked twice- (2X) or once-daily (1X) and adequately fed (AF) at 14 kg DM/cow/d or underfed (UF) at 8 kg DM/cow/d for three weeks. After the treatment period, all cows grazed to residuals $> 1,600$ kg DM/ha and were milked twice daily for 20 weeks. Week 1 to 3 = Treatment period; Week 4 to 12 = First half of post-treatment period; Week 13 to 23 = Second half of post-treatment period; SED = Standard error of difference; MF = Milking frequency effect; FL = Feeding level effect; MF x FL = Milking frequency by feeding level interaction; NEFA = Non-esterified fatty acids, BHBA = β -hydroxybutyrate, AST = Aspartate aminotransferase, GDH = Glutamate dehydrogenase, IGF-I = Insulin-like growth factor I, GH = Growth hormone. P values in bold indicate significance at $P < 0.05$. P values in italics indicate approaching significance with P value between 0.05 and 0.10.

Variable	Week of trial	Treatment				SED	P value		
		2XAF	1XAF	2XUF	1XUF		MF	FL	MFxFL
NEFA (mmol/L)	1 to 3	0.57	0.34	1.44	1.15	0.07	<0.001	<0.001	0.56
	4 to 12	0.34	0.34	0.28	0.28	0.02	0.98	<0.001	0.88
	13 to 23	0.24	0.25	0.21	0.22	0.02	0.73	0.04	0.83
BHBA (mmol/L)	1 to 3	0.59	0.44	1.61	0.82	0.11	<0.001	<0.001	<0.001
	4 to 12	0.55	0.54	0.54	0.50	0.02	0.10	0.16	0.24
	13 to 23	0.50	0.47	0.50	0.49	0.02	0.28	0.61	0.85
AST (IU/L)	1 to 3	78	75	87	99	7	0.45	0.001	0.12
	4 to 12	83	81	82	80	4	0.52	0.76	0.90
	13 to 23	80	75	81	81	3	0.24	0.17	0.29
GDH (IU/L)	1 to 3	28	20	28	38	6	0.73	0.03	0.03
	4 to 12	26	30	26	28	7	0.54	0.70	0.84
	13 to 23	20	16	20	21	2	0.47	0.16	0.19
Glucose (mmol/L)	1 to 3	4.16	4.40	3.57	3.96	0.07	<0.001	<0.001	0.13
	4 to 12	4.08	4.19	4.08	4.09	0.04	<i>0.06</i>	<i>0.06</i>	0.10
	13 to 23	4.10	4.11	4.06	4.09	0.06	0.58	0.50	0.79
Insulin (μ U/mL)	1 to 3	3.4	4.0	2.4	2.9	0.3	0.02	<0.001	0.83
	4 to 12	3.21	3.80	3.54	3.76	0.31	<i>0.08</i>	0.52	0.39
	13 to 23	2.80	3.07	3.15	3.17	0.30	0.51	0.30	0.56
IGF-I (ng/mL)	1 to 3	10.88	15.29	7.24	10.39	0.93	<0.001	<0.001	0.34
	4 to 12	11.70	13.94	11.27	13.12	0.84	<0.001	0.28	0.74
	13 to 23	17.01	19.03	17.27	19.22	1.44	<i>0.06</i>	0.83	0.97
GH (ng/mL)	1 to 3	2.34	2.36	2.46	2.09	0.20	0.21	0.56	0.17
	4 to 12	2.62	2.71	3.05	2.75	0.20	0.43	0.11	0.17
	13 to 23	2.45	2.40	2.67	2.39	0.23	0.31	0.52	0.48

for 1X and 0700 h and 1500 h for 2X. After the treatment period, all cows were milked 2X and grazed to residuals of 1,600 kg DM/ha for 20 weeks.

Plasma measurements

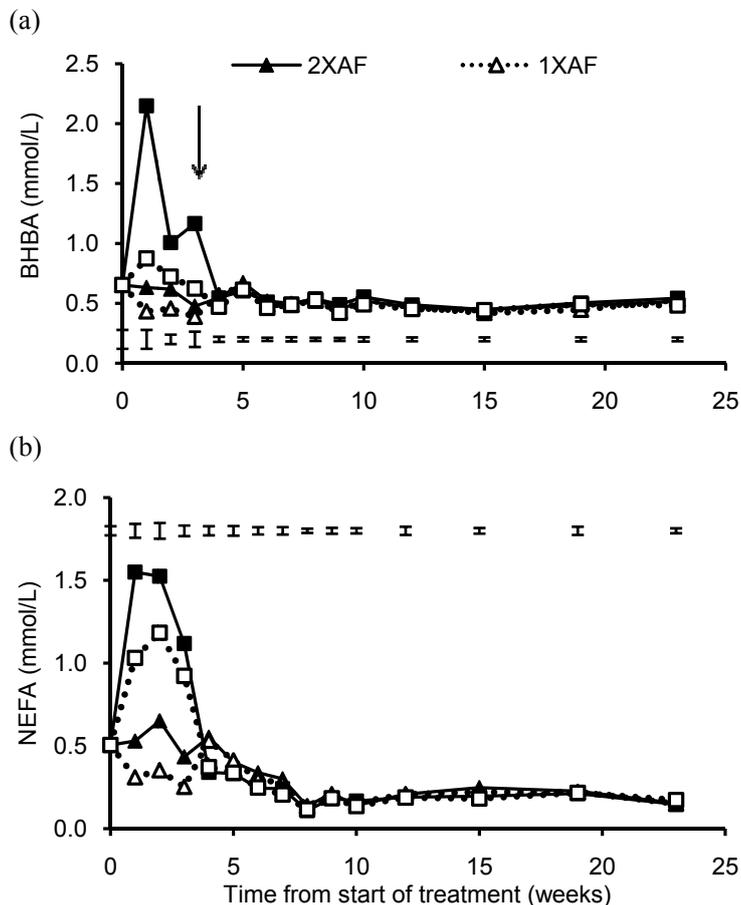
Blood samples were collected from the coccygeal vein of individual cows after the morning milking on one day each week for two weeks pre- until 10 weeks post-treatment initiation, then fortnightly for a further four weeks and monthly for eight weeks. Samples were placed on ice immediately and centrifuged at 1,120 g for 12 minutes at 4°C. Plasma were harvested and stored at -20°C before subsequent analyses for glucose, insulin, insulin-like growth factor I (IGF-I), growth hormone (GH), β -hydroxybutyrate (BHBA), non-esterified fatty acids (NEFA), glutamate dehydrogenase (GDH) and aspartate aminotransferase (AST). Insulin (Hales & Randle 1963), IGF-I (Gluckman *et al.* 1983), and GH (Downing *et al.* 1995) were measured in duplicate by double-antibody RIA with inter and intra-assay

coefficients of variation (CV) $< 6\%$. The NEFA, glucose, BHBA, GDH and AST analyses were performed at 37°C on a Modular P800 analyser (Roche, Basel, Switzerland) by Gribbles Veterinary Pathology Ltd. (Hamilton, New Zealand). The inter- and intra-assay CV were $< 2\%$ for all assays except GDH which had an inter-assay CV of 5%.

Statistical analysis

Pre-treatment measurements were used as a covariate, and means of data for each week and means for each time period of Week 1 to 3, Week 4 to 12 and Week 13 to 23, were calculated for individual cows. These data were analysed using mixed models fitted with REML in GenStat (Payne *et al.* 2009), including fixed effects for feeding level, milking frequency and the interaction of feeding level and milking frequency with cow as a random effect. Data were also \log_{10} transformed and analysed; however, for ease of interpretation, raw data are presented, as statistical and biological conclusions

Figure 1 (a) Plasma β -hydroxybutyrate (BHBA) and (b) non-esterified fatty acids (NEFA) from grazing cows (34 ± 6 days in milk) milked twice- (2X) or once-daily (1X) and adequately fed (AF) 14 kg DM/cow/d or underfed (UF) 8 kg DM/cow/d for three weeks. After the treatment period, all cows grazed to residuals > 1600 kg DM/ha and were milked 2X for 20 weeks. The end of the treatment period is denoted by an arrow. Vertical bars represent the standard error of the difference.



were similar. Differences were considered significant at $P < 0.05$ and a trend declared at $P < 0.10$.

Results

During the treatment period, plasma BHBA and NEFA were greater ($P < 0.001$) in underfed and less in 1X cows compared with adequately fed and 2X cows, respectively (Table 1; Figs. 1a, 1b). There was an interaction ($P < 0.001$) such that the decrease in BHBA due to 1X milking was greater in underfed compared with adequately fed cows. There was no carry-over effect of milking frequency or feeding level on BHBA content; however, plasma NEFA content was less in underfed cows post treatment (Weeks 4 to 12, $P < 0.001$; and Weeks 13 to 23, $P < 0.05$; Table 1; Figs. 1a,1b).

During the treatment period, plasma AST was greater ($P < 0.001$) in underfed compared with adequately fed cows, but was not affected by 1X

milking (Table 1). Plasma GDH was also greater ($P < 0.05$) in underfed relative to adequately fed cows, however an interaction existed ($P < 0.05$) such that the increase with underfeeding only occurred in cows milked 1X (Table 1). Post-treatment there was no effect of milking frequency or feeding level on these liver enzymes (Table 1).

During the treatment period, plasma glucose, insulin and IGF-I content were greater ($P < 0.001$; $P < 0.05$; and $P < 0.001$, respectively) in cows milked 1X compared with 2X and less ($P < 0.001$) in underfed compared with adequately fed cows (Table 1). Immediately post-treatment (Weeks 4 to 12), IGF-I was greater ($P < 0.001$) and glucose and insulin tended ($P = 0.06$ and $P = 0.08$, respectively) to be greater in cows milked 1X relative to 2X, while glucose tended ($P = 0.06$) to be less in underfed compared with adequately fed cows. During Weeks 13 to 23, there was no effect of milking frequency or feeding level on glucose, insulin or IGF-I content. There was no effect of milking frequency or feeding level on plasma GH content during the treatment or post-treatment periods (Table 1).

Discussion

During a period of underfeeding, cows enter a state of negative energy balance, as energy output associated with milk production does not decrease to the same extent, or at the same rate, as energy intake. In the present study, during the first week of underfeeding, concentrations of plasma BHBA and NEFA peaked in cows milked 2X, with BHBA reaching levels indicative of clinical ketosis (> 1.5 mmol/L) (Oetzel 2004). During this first week, intake was restricted by approximately 40%, whereas milk production only decreased by 15%. This situation mimics what is known as the third stage of fuel homeostasis or “early starvation phase” (Newsholme & Leech 1983), where NEFA from increased lipolysis, and ketone bodies are oxidised to fuel peripheral tissues, and support milk production. These homeorhetic changes ensure glucose and or ketone bodies are available for use by vital organs, such as the brain which cannot oxidise NEFA for energy (Newsholme & Leech 1983). As the treatment period continued, milk production decreased further in underfed cows to compensate for the reduced intake, and by Week 3, underfed cows were producing approximately 32% less milk compared with adequately fed cows. At this stage, although plasma NEFA and BHBA content were still greater than in adequately fed cows, they were substantially less than during Week 1, indicating

NEFA and ketone body oxidation were reduced and cows were in an improved energetic status.

The negative energy balance associated with a period of acute underfeeding, was partially alleviated by 1X milking as indicated by the lower NEFA and BHBA content in 1X cows. This improvement is consistent with previous research based on NEFA and BHBA content combined with calculated individual energy balance (Auldish & Prosser 1998, Remond *et al.* 2002 and Guinard-Flament *et al.* 2007). The average circulating content of BHBA in cows milked 1X did not exceed 1.0 mmol/L, even during the first week of the feed deficit, indicating these animals were at less risk of being ketotic (Oetzel 2004).

Plasma NEFA and BHBA content were also less with 1X milking in adequately fed cows, most probably due to the approximately 20% reduction in milk energy output with no detectable difference in feed intake (Kay *et al.* 2011). Such an improvement in energy status with 1X milking indicates adequately fed cows milked 2X were in a state of negative energy balance and undergoing lipolysis during early lactation to meet the demands of milk production.

After treatment, when all cows had returned to 2X milking and normalised intakes, BHBA content was not affected by underfeeding or milking frequency. However, plasma NEFA content was less in underfed compared with adequately fed cows. This is probably due to cows still producing approximately 7% less milk (Kay *et al.* 2011) during the carry-over period, and may reflect a greater partitioning of energy towards tissue reserves to recover from the BCS loss.

In the present study, underfed cows had greater plasma AST content relative to adequately fed cows. Hepatic enzymes such as AST and GDH are indicators of acute metabolic stress in the dairy cow (Bobe *et al.* 2004). Circulating concentrations of hepatic enzymes increase with hepatic lipidosis, a disorder commonly referred to as fatty liver syndrome. Fatty liver, which is closely associated with ketosis, is characterised by triglyceride accumulation in the liver and occurs when hepatic NEFA uptake exceeds β -oxidation and triglyceride secretion (Bobe *et al.* 2004). Fatty liver occurs when cows are mobilising and oxidising large quantities of NEFA, and is associated with decreased health status, productivity and reproductive performance (Bobe *et al.* 2004). The greater AST and GDH content is consistent with the greater NEFA and BHBA content and indicates a degree of fatty liver in underfed cows. Interestingly, although other plasma hormones and metabolites indicate that 1X milking improves energy balance, a milking frequency \times feeding level interaction existed, such that the increase in GDH with underfed, only occurred in cows milked 1X, and although not significant ($P = 0.12$), this pattern was also evident for AST. A reason for this is not clear, but indicates increased stress on the liver when underfed cows are milked 1X. Further analyses of hepatic genes involved in lipid metabolism may

clarify the effect of 1X milking on triglyceride accumulation in the liver.

During the treatment period, plasma glucose content was greater in cows milked 1X. Guinard-Flament *et al.* (2007) also found a greater arterial glucose content when milking frequency was reduced from 2X to 1X. However, in contrast to the present study, where glucose content was less in underfed cows, Guinard-Flament *et al.* (2007) reported no effect of feed restriction on arterial glucose content. This different response may be due to the less severe feed restriction of approximately 21% imposed by Guinard-Flament *et al.* (2007). Plasma insulin is one of the primary hormones responsible for regulating glucose content (Bauman & Currie 1980). Insulin followed a similar pattern to glucose, being greater in cows milked 1X relative to 2X and in adequately fed compared with underfed cows. In early lactation, another homeorhetic mechanism that occurs is uncoupling of the somatotrophic axis which is reflected by increased plasma GH and decreased IGF-I content (Lucy *et al.* 2001). Factors such as cow genetic strain and/or nutrition alter the timing, with earlier re-coupling occurring in cows with a more positive energy balance (Lucy *et al.* 2009; Grala *et al.* 2011). Although 1X milking did not affect GH content in the present study, the increase in plasma insulin and IGF-I content with 1X milking, likely reflect an earlier re-coupling of the somatotrophic axis due to the improved energy status.

The combination of greater plasma glucose, insulin, and IGF-I, and less BHBA and NEFA, in cows milked 1X, supports the hypothesis that 1X milking improves the cow's energetic status during an energy deficit, and could potentially improve animal health. Furthermore, the improvement in energy balance indicates that 1X milking may improve reproductive performance if there was a temporary feed deficit before and/or during the mating period (Burke *et al.* 2010). Further research involving larger cow numbers is required to investigate the effect of 1X milking on reproduction.

In summary, reducing milking frequency from 2X to 1X partially alleviates the negative energy balance caused by an acute feed deficit; however, the carry-over benefits after cows returned to 2X milking and were adequately fed were minimal. These results need to be considered in conjunction with the decreased milk production, when developing management strategies to optimise profitability during an energy deficit.

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