The effects of altering milking frequency and/or diet in early lactation on the energy balance, production and reproduction of dairy cows

End of Project Report
Project Number 4997

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1. Summary

It has been suggested that negative energy balance (NEB) in the immediate post-partum period is potentially an important factor in the association between increasing milk output and declining reproductive performance. The objective of this project was to design an experimental model that could be used to impose different degrees of NEB immediately after calving and to examine the effect of this model on dry matter intake (DMI), milk production, energy balance (EB), metabolic and reproductive hormonal profiles, the onset and pattern of post-partum ovarian cyclicity and reproductive physiology around AI.

Two experiments were carried out to evaluate the effects of milking frequency and diet on DMI, production, energy balance and blood metabolites and hormones in the first 4 weeks after calving and subsequent reproduction. Reducing milking frequency from either thrice or twice daily to once daily reduced DMI but also reduced milk production. This resulted in a better EB in once daily milked cows in both experiments, the reduction being significant in the first. Milk production during the 4-week treatment period was reduced by 23 and 20 percent by reducing milking frequency from thrice to once daily in experiments 1 and 2, respectively. There was a reduction of approximately 10 percent in the cumulative yield up to week 20 of lactation in experiment 1 and of approximately 9 percent in total lactation yield in experiment 2. Reducing milking frequency resulted in increased plasma glucose, insulin and IGF-1 concentrations and reduced non-esterified fatty acid (NEFA) and beta hydroxybutyrate (BHB) concentrations. Conception rates to first service or overall pregnancy rates were not different between milking frequency treatments but once daily milking resulted in a shorter interval to first ovulation than thrice daily milking, due to a higher proportion of cows on this treatment ovulating the first post-partum dominant follicle. Increasing the energy density

of the diet increased DMI and milk production with no consequent effect on energy balance.

Logistic regression on the combined data from the two experiments showed that lower energy intake, greater NEB and lower milk protein content and were significantly associated with poorer conception to first service. Lower plasma IGF-1 concentrations in experiment 2 were also associated with a lower conception rate to first service.

A third experiment which investigated protein concentration in the concentrate combined with concentrate feeding level post calving (for two groups of cows in different body condition score at calving) showed no effect of post calving diet on BCS change.

Overall the results suggest that reducing milking frequency to once per day during the first 4 weeks of lactation reduces NEB and appears to be a suitable strategy for altering energy balance at this time. However, the short-term reduction in milking frequency immediately post partum reduces total lactation yields. Blood metabolite and hormonal concentrations indicate better energy balance for cows milked once daily. Increasing dietary energy density or reducing the protein content of the diet does not appear to be effective in changing energy balance in early lactation. Decreased NEB in the first 4 weeks post-partum is associated with an improved conception rate to first service.

2. Introduction

Irish data show that there was a significant decline in calving rate to first service between 1991 and 1998 of approximately 0.9% per annum (Mee 2004). A recent Teagasc survey of 74 farms, incorporating records from over 6,000 cows, showed that conception rate to first service was 49% (26-87% by herd), (Buckley et al., 2003). This survey also indicated that cows having a body condition score (BCS) of 2.75 or greater at the start of the breeding season had higher conception rates to first service than those with a lower BCS. Horan et al. (2004) reported an infertility rate of 21% with high genetic merit high-yielding cows in a recent Teagasc study. Both in Ireland and internationally this decline in fertility has occurred simultaneous with an increase in milk production.

Energy balance is defined as the difference between the net energy intake of the animal and the net energy required for maintenance and milk production. It has been suggested that negative energy balance (NEB) is the most important causative factor in the association between increased milk output and declining reproductive performance (Nebel and McGilliard, 1993). The high producing cow cannot consume enough energy in early lactation to meet her maintenance and production demands. Britt (1991) proposed an elegant hypothesis as to how early lactation NEB might affect reproductive performance. The essential elements are that the pre-antral follicle, which is developing in the early post-partum period and is subjected to the period of greatest negative energy, is the follicle that ovulates 60-80 days later and produces the ovum that is to be fertilised at the first insemination. Exposing this pre-antral follicle to an adverse environment due to NEB may result in it having altered gene expression leading to impaired development. This would result in a dysfunctional mature follicle that would produce a poorer quality oocyte and result in the formation of a sub functional corpus luteum. This hypothesis however, has not been proven in experiments using dairy cows, though supporting data have been published (Fahey et al. 2005)

Milk production and dry matter intake (DMI) in dairy cows increase after calving but at different rates and maximum DMI occurs some time after maximum milk production. The result of this disparity in time between peak milk production and DMI is that cows experience NEB that can persist for 4 to 12 weeks of lactation (Butler *et al*., 1981). The NEB during the first 3-4 weeks post partum is highly correlated with the interval to first ovulation in dairy cows and a delay in the onset of normal ovarian activity caused by NEB limits the number of oestrous cycles before breeding and may account for some of the observed decrease in fertility (Butler and Smith, 1989).

Thus EB in the early postpartum period is an area of investigation that could potentially explain the increasingly poor reproductive performance of Irish dairy cows. Changing milking frequency and diet quality were the strategies investigated in this project to change EB. The main objective of the project was to examine the effect of these strategies in the immediate post-partum period on DMI, milk production, EB, metabolic and reproductive hormonal profiles, the onset and pattern of postpartum ovarian cyclicity, reproductive physiology around AI and pregnancy rates.

3. Experiments

3.1 Experiment 1. The effect of different milking frequencies in early lactation on intake, production, energy balance, blood metabolites and conception rate of dairy cows

Introduction

Because EB is the difference between energy input and energy output in milk plus energy required for maintenance, changing either intake or output will change EB. Thus reducing milk output should potentially improve EB provided intake is not reduced proportionally. Remond *et al*. (1999) successfully improved EB in an experiment where they examined once a day milking for the first three or six weeks of lactation in comparison to a standard twice a day milking during the entire lactation. Frajblat *et al*. (1998) used 3 milking frequencies (no milking, conventional twice daily milking or three times per day milking) for 3 weeks post partum. This resulted in 3 significantly different EB levels in 3 groups of cows. Therefore, the specific objective of this experiment was to examine the effect of milking frequency on EB, blood metabolites and reproductive performance with Irish Holstein-Friesian cows.

Materials and Methods

Sixty-three pluriparous Holstein-Friesian cows were split into 3 groups by two week expected calving intervals, and allocated from within groups according to parity (lactation number = 2 and lactation number > 2) BCS and body weight (BW) into blocks of three. Cows were assigned at random from within blocks to one of three treatments in a randomised block design:

1. Once daily milking for the first 4 weeks of lactation and then twice daily milking for the rest of the lactation (X1)
2. Twice daily milking which continued for the entire lactation (X2)
3. Thrice daily milking for the first 4 weeks of lactation and then twice daily milking for the rest of the lactation (X3)

Milking times were 07:30, 15:30 and 22:30, with the 1XS cows milked at 07:30. Treatments began on the morning after the second or third milking post-calving. Animals were individually offered *ad-lib* a complete diet pre-mixed in a diet feeder (Keenan Ltd., Borris, Ireland) once daily at 10.30 a.m. The diet consisted of maize silage, grass silage and concentrate in the proportion 25:25:50 on a DM basis. On day 29 post-calving cows returned to conventional twice a day milking with continued access to the complete diet, offered *ad-lib*. Cows were turned out to pasture on day 42 post-calving and were offered high quality grass plus 4 kg/day of concentrate, decreasing to 2 kg/day on day 21 after first service.

DMI was calculated on a daily basis. Milk yield (kg) was recorded daily at the morning, evening and night milking. Milk composition (fat, protein and lactose) was determined thrice weekly from successive morning, evening and night milk samples during the treatment period and on a weekly basis thereafter (from one successive morning and evening milk sample). Cow BW (kg) was recorded within 24h of calving and on one day per week subsequently. The dry cows were weighed before feeding in the morning and the lactating cows were weighed before feeding after morning milking. The BCS (Lowman *et al*.,

1976) of the cows was determined at drying off, at the time of allocation to treatment, at 4 DIM and every 2 weeks thereafter.

Energy balance was calculated as the difference between energy intake and the sum of energy for maintenance and milk production. The French NE system was used (Jarrige, 1989). The NE content of the concentrates offered was determined using the NE values (UFL) of ingredients (INRAtion, 1999, version 2.7). One UFL is the NE content of 1 kg of air-dry standard barley for milk production (Jarrige, 1989). The NE value of silage was related to its *in-vitro* DMD concentration (O'Mara *et al.,* 1997). The following equations were used to determine the energy required for maintenance and the energy output in milk:

Energy requirement for maintenance: (UFL/day) = 1.4 + 0.6 BW/100

UFL requirements for milk: (UFL/kg of milk) = 0.0054FC+0.0031PC+0.0028LC-0.015 where BW = body weight, FC = fat content, PC = protein content and LC = lactose content all in g/kg.

Blood samples were collected from the coccygeal vessels into lithium heparin vaccutainers from all cows after morning milking and before feeding between 24 and 48 hours after calving and twice weekly on Mondays and Thursdays until day 42 after calving. Plasma was analysed for glucose, triglycerides, non-esterified fatty acids (NEFA), beta hydroxy butyrate (BHB), glutamic acid dehydrogenase (GLDH), and bile acids using appropriate kits and an ABX Mira autoanalyzer (ABX Mira, Cedex 4, France). Plasma insulin concentration was measured using the DELFIA kit (EG&G Wallac, Turku, Finland). Blood samples were also collected from the coccygeal vessels into lithium heparin vaccutainers on the day of AI and on days 4 to 8 inclusively post AI for plasma progesterone analysis. Plasma progesterone concentrations were measured using the DELFIA kit (EG&G Wallac, Turku, Finland).

Oestrous detection was carried out thrice daily using tail paint (which was first applied 10 days post partum) before and after the voluntary waiting period. Each cow had a voluntary waiting period of 65 days post partum. Oestrus was induced on day 65 post partum in cows not seen in oestrus using a CIDR (Controlled Internal Drug Release; InterAg. Te Rapa Road, Hamilton, NZ) which contained 1.94g of progesterone, and an injection of 2mg of Oestradiol Benzoate, (5mg/ml) (Intervet Ireland Ltd. Cookstown, Tallaght, Dublin 24). The CIDRs were removed on day 8 and an injection of prostaglandin-$F_{2\alpha}$ ($PGF_{2\alpha}$; 2ml Estrumate^{TM}, equivalent to 562μg of Cloprostenol Sodium, BP (Vet) Coopers, Berkhamsted, England) was administered intramuscularly. Cows received 1mg of Oestradiol Benzoate 24 hours after CIDR removal. Semen from a single bull was used to serve the cows by one AI technician.

Onset of cyclicity was recorded as the first observed standing oestrus post partum. The pattern of luteal activity (milk progesterone concentration) was recorded from day 10 after calving until first service by thrice-weekly milk sampling. Pregnancy examinations were carried out by ultrasound using a 5 MHz rectal transducer (Aloka SSD-500V, Japan), at 30 to 40 days post insemination and one month after the end of the breeding season.

Statistical analysis

Five cows were removed from the experiment for reasons not connected with the treatments (two, two and one cow from treatments X1, X2, and X3 respectively). A smoothing spline was fitted in Genstat 5 (Genstat, 1997), to facilitate the estimation of daily BW records prior to analysis. Repeated measures analysis of variance for the effects of the milking frequency on DMI, BW change, and BCS change, milk yield, milk constituent yield, milk composition, blood metabolites, blood progesterone, milk progesterone and EB data was carried out using the GLM procedure of SAS (SAS Institute, 1991). Treatment, time, and treatment by time interactions were tested. The reproduction data were analysed using analysis of variance for continuous variables and the chi-square procedure for pregnancy rate data. Differences between treatments were tested for significance (separately) using Students t-test. Correlation coefficients between EB and some of the blood metabolites were obtained using the PROC CORR procedure of SAS (SAS Institute, 1991). Post-hoc analysis was carried out by grouping cows that conceived or failed to conceive to first service and analysing their EB, BCS, blood progesterone and blood metabolite data by analysis of variance using the GLM procedure of SAS (SAS Institute, 1991).

Results

Dry matter intake

Treatment X1 had lower (P < 0.05) DMI (1.4 kg/day) in the first four and six weeks of lactation compared to X2 but not compared to X3 (Table 1). There was no significant difference in DMI between X2 and X3 for the first four weeks of lactation (Table 1 and Figure 1). In weeks 5 to 6 there were no significant differences in DMI between any of the treatments.

Table 1 Effect of milking frequency on average DMI (kg DM/cow per day).

Week of lactation	X1[1]	X2	X3	s.e.d. [2]
1 to 4	15.7^{a3}	17.1^{b}	16.1^{ab}	0.43
5 to 6	17.6	18.8	18.4	0.74
1 to 6	16.4^{a}	17.7^{b}	16.9^{ab}	0.60

[1] X1 = once a day milking for the first 4 weeks of lactation; X2 = twice a day milking ; X3 = three times a day milking for the first 4 weeks of lactation: [2]s.e.d. = standard error of the difference: [3] means within rows having different superscripts differ significantly (*P* < 0.05)

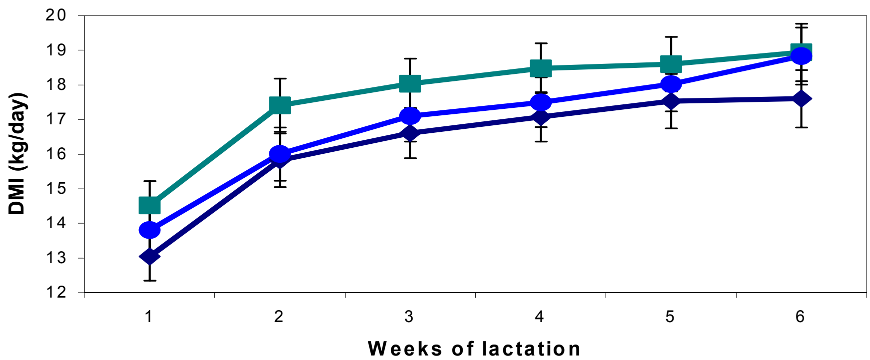


Figure 1 Effect of milking frequency on dry matter intake (◆ X1; ■ X2; ● X3)

Milk yield and composition

Milk yield, fat yield, protein yield and lactose yield were reduced (*P* < 0.001) on X1 compared to X2 for the first four weeks of lactation (Table 2). Milk yield was reduced by 24.5% on X1 compared to X2. Consequently, yield of milk constituents was also reduced on X1. The residual effect on milk yield of once a day milking was clearly evident in weeks 5 to 6 as it was still lower (P < 0.01) on X1 than on X2. Milk fat concentration was not affected by milking frequency in the first four weeks of lactation. Milk protein concentration tended to be greater (P = 0.083) on X1 than on X2. Milk lactose concentration was greater (P = 0.017) on X1 than on X2 for the first four weeks of lactation. The residual effect on milk yield of once a day milking was still significant (P < 0.05) at week 10 of lactation with a 3 kg/day differential between X1 and X2 cows and it was approaching significance at week 15 (P = 0.06) (Figure 2). At week 20 of lactation the difference was not significant (P > 0.05). However, cumulative milk yield at week 20 was significantly greater (P < 0.01) on X2 (4026 kg) and X3 (3973 kg) than on X1 (3585 kg) (s.e.d of 144.5 kg). There was no difference between X2 and X3 in the first 4 weeks of lactation. When milking frequency changed to twice a day, milk yield on X3 tended to be lower (P = 0.085) in weeks 5 to 6 post calving compared to X2. Milk constituent yields or milk composition was not significantly different between X2 and X3.

Table 2 Effect of milking frequency on milk production

	Week of lactation	X1[1]	X2	X3	**s.e.d.[2]**
Milk yield (kg/cow per day)	1-4	24.1[a][3]	31.9[b]	31.4[b]	1.21
	5-6	28.3[a]	33.4[b]	30.9[a]	1.39
Milk fat (kg/cow per day)	1-4	1.18[a]	1.51[b]	1.49[b]	0.072
	5-6	1.18[a]	1.37[b]	1.28[ab]	0.072

	Week of lactation	X1[1]	X2	X3	s.e.d.[2]
			b	ab	
Milk protein (kg/cow per day)	1-4	0.84[a]	1.07[b]	1.08[b]	0.045
	5-6	0.83[a]	0.95[b]	0.88[ab]	0.041
Milk lactose (kg/cow per day)	1-4	1.12[a]	1.41[b]	1.44[b]	0.058
	5-6	1.28[a]	1.50[b]	1.38[a]	0.059
Milk fat (g/kg)	1-4	47.0	47.4	47.6	1.61
	5-6	41.7	41.0	41.4	1.51
Milk protein (g/kg)	1-4	34.9	33.5	34.6	0.74
	5-6	29.6	28.4	28.5	0.61
Milk lactose (g/kg)	1-4	46.4[a]	44.4[b]	45.9[ab]	0.76
	5-6	45.2	45.1	44.7	0.81

[1] X1 = once a day milking for the first 4 weeks of lactation; X2 = twice a day milking ; X3 = three times a day milking for the first 4 weeks of lactation: [2]s.e.d. = standard error of the difference; [3] means within rows having different superscripts differ significantly ($P < 0.05$)

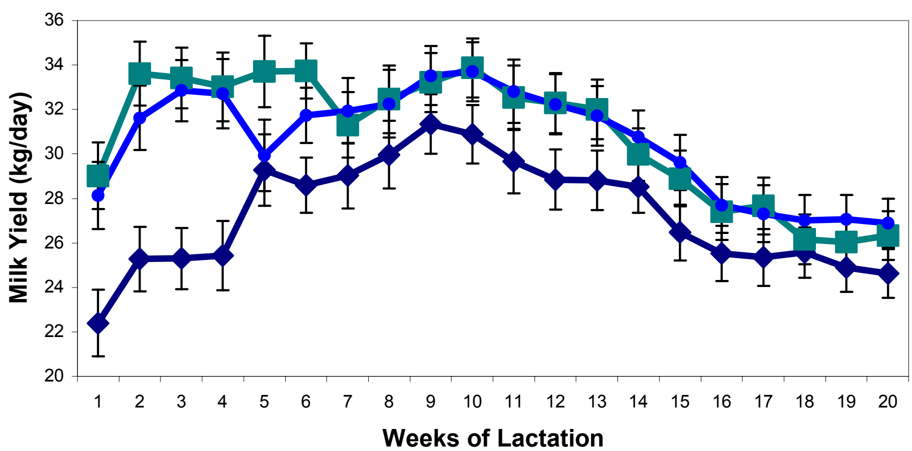


Figure 2 **Effect of milking frequency on milk yield (◆ X1; ■ X2; ● X3)**

Energy balance

Weekly EB tended to be less negative (P = 0.1) on X1 than on X2 in the first week of lactation and was less negative (P < 0.02) in weeks 2 to 4 (Figure 3). Weekly EB was also less negative (P < 0.01) on X1 than on X3 in the first 4 weeks of lactation, when the milking treatments were imposed. There was no significant difference between X2 and X3 in mean EB in the first four weeks of lactation, weeks 5 to 6 of lactation or weeks 1 to 6 of lactation (Table 3). Cows on X1 were in less (P < 0.05) NEB than cows on X3 in the first 4 weeks of lactation. Cows on X1, X2 and X3 treatments reached positive EB by weeks 4, 5 and 6 of lactation, respectively (Figure 3). Mean EB was significantly better on X1 than on X2 and X3 when averaged over weeks 1 to 4 and weeks 1 to 6 (Table 3).

Table 3 Effect of milking frequency on mean energy balance (UFL/cow per day).

Weeks of lactation	X1[1]	X2	X3	s.e.d.[2]
1 to 4	-1.20[a3]	-3.30[b]	-4.36[b]	0.679
5 to 6	0.21	-0.63	0.04	0.664
1 to 6	-0.69[a]	-2.54[b]	-2.74[b]	0.562

[1] X1 = once a day milking for the first 4 weeks of lactation; X2 = twice a day milking ; X3 = three times a day milking for the first 4 weeks of lactation : s.e.d. = standard error of the difference: [3] means within rows having different superscripts differ significantly (*P* < 0.05)

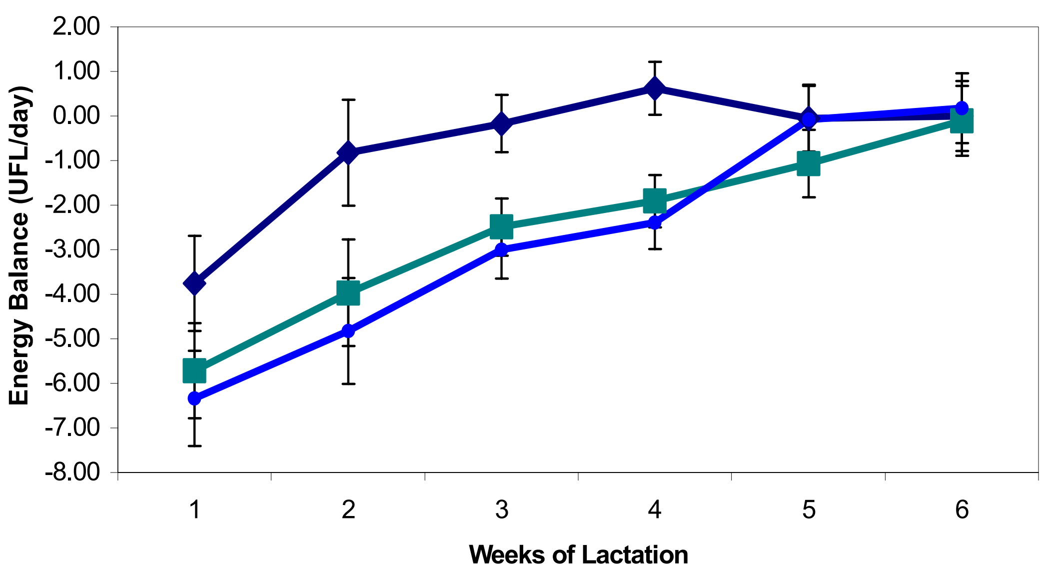


Figure 3 Effect of milking frequency on energy balance(◆ X1; ■ X2; ● X3)

Body weight (BW)

At 4 days in milk (DIM) there was no significant difference in BW between the three treatments. Loss in BW tended to be greater (P = 0.06 to 0.08) on X2 and X3 than on X1 between 1 DIM and 28 DIM and was greater (P < 0.05) on

X3 than on X1 between 1 and 56 DIM (Table 4). There was no difference between treatments in BW loss between 28 and 56 DIM.

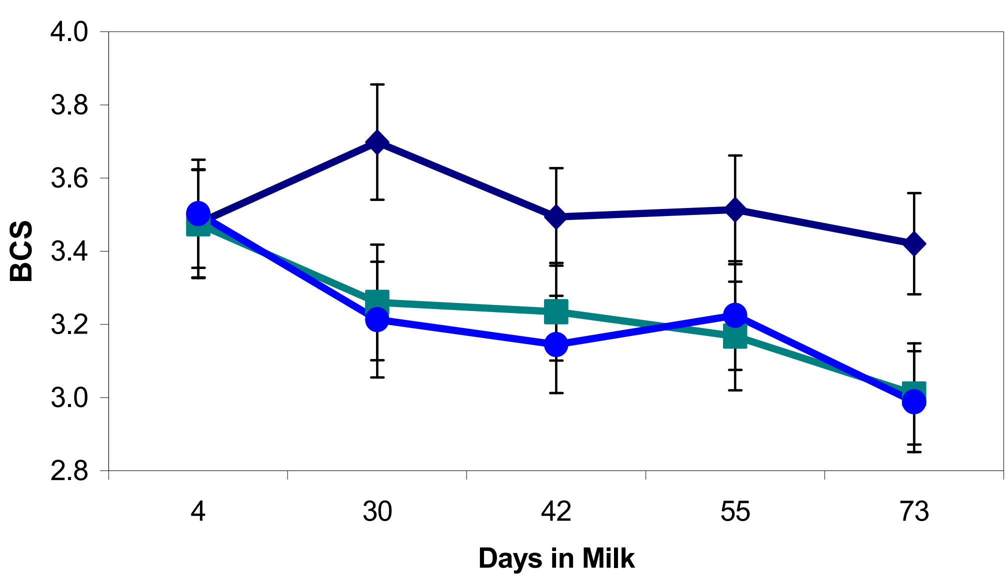
Table 4 Effect of milking frequency on body weight (kg) and body weight change (kg/day)

Days in Milk	**X1**[1]	**X2**	**X3**	s.e.d.[2]
4	576	600	594	13.3
1 – 28	-0.59	-1.01	-1.01	0.224
1 – 42	-0.58	-0.96	-0.95	0.193
1 – 56	-0.57[a3]	-0.85[ab]	-0.88[b]	0.156
28 – 56	-0.55	-0.70	-0.75	0.178

[1] X1 = once a day milking for the first 4 weeks of lactation; X2 = twice a day milking ; X3 = three times a day milking for the first 4 weeks of lactation: [2]s.e.d. = standard error of the difference: [3] means within rows having different superscripts differ significantly ($P < 0.05$)

Body condition score

There was no difference in BCS between treatments at 4 DIM. The BCS change from 4 to 30 DIM was less ($P < 0.05$) on X1 than on the other two treatments (Table 5). Cows on X1 gained 0.22 of a BCS unit while those on X2 and X3 lost 0.22 and 0.29 of a BCS unit, respectively. The BCS loss between 4 and 73 DIM was higher (P < 0.05) on X2 and X3 than on X1. There was no significant difference ($P > 0.05$) in BCS change from 30 to 73 DIM between the treatments groups, at a time when all cows were milked twice a day. The absolute BCS at 30 and 73 DIM was higher ($P < 0.05$) on X1 than on X2 and X3 (Figure 4) and was close to being significantly higher at 42 and 55



DIM.

Figure 4. Effect of milking frequency on body condition score (◆ X1; ■ X2; ● X3)

Table 5 Effect of milking frequency on BCS change.

Days in milk	**X1**[1]	**X2**	**X3**	s.e.d.[2]
4 – 30	0.22^{a}[3]	-0.21^{b}	-0.29^{b}	0.167
4 – 42	0.02^{a}	-0.24^{ab}	-0.36^{b}	0.173
4 – 55	0.01	-0.30	-0.28	0.207
4 – 73	-0.06^{a}	-0.46^{b}	-0.51^{b}	0.156
30 – 73	-0.28	-0.25	-0.22	0.124

[1] X1 = once a day milking for the first 4 weeks of lactation; X2 = twice a day milking ; X3 = three times a day milking for the first 4 weeks of lactation: [2]s.e.d. = standard error of the difference: [3] means within rows having different superscripts differ significantly (*P* < 0.05)

Blood metabolites

Blood metabolites, for which significant differences or a tendency towards significant differences due to treatment were observed, are shown in Figure 5. Plasma NEFA concentrations were consistently lower on X1 than on X2 or X3 over the first 4 weeks of lactation. The differences were significant (P < 0.05) between X1 and X3. Significant differences occurred in plasma NEFA concentrations between X1 and X2 cows in the first and the fourth week of lactation. By week 6 of lactation there was no residual effect on NEFA from the different milking frequencies. The correlation between average EB in the first 4 weeks of lactation and plasma NEFA was r = -0.55 (P < 0.001).

Plasma glucose concentrations followed the opposite trend to that of plasma NEFA. They were higher (P < 0.05) in almost all samplings on X1 cows than on X2 or X3 over the first 4 weeks of lactation. By week 6 there was no residual effect of milking frequency on plasma glucose concentration. A positive correlation was found between average EB in the first 4 weeks of lactation and plasma glucose (r = 0.45; P < 0.01). Plasma BHB concentrations tended to be lower, but not significantly, on X1 than on X2 or X3. As cows resumed twice daily milking the magnitude of any differences in plasma BHB between treatments decreased. The correlation between BHB and average EB in the first 4 weeks of lactation was r = -0.30 (P < 0.05).

Plasma insulin concentrations were significantly greater or close to being greater on X1 than on X2 or X3 in the first 2 weeks of lactation. Treatment X1 tended to have the highest plasma insulin concentrations up to week 5 of lactation, at which time values were similar for the treatments. At no point were there any significant differences between X2 and X3 over the 6-week sampling period.

Milking frequency had no significant effect on plasma progesterone concentrations at AI or on days 4 to 8 post AI.

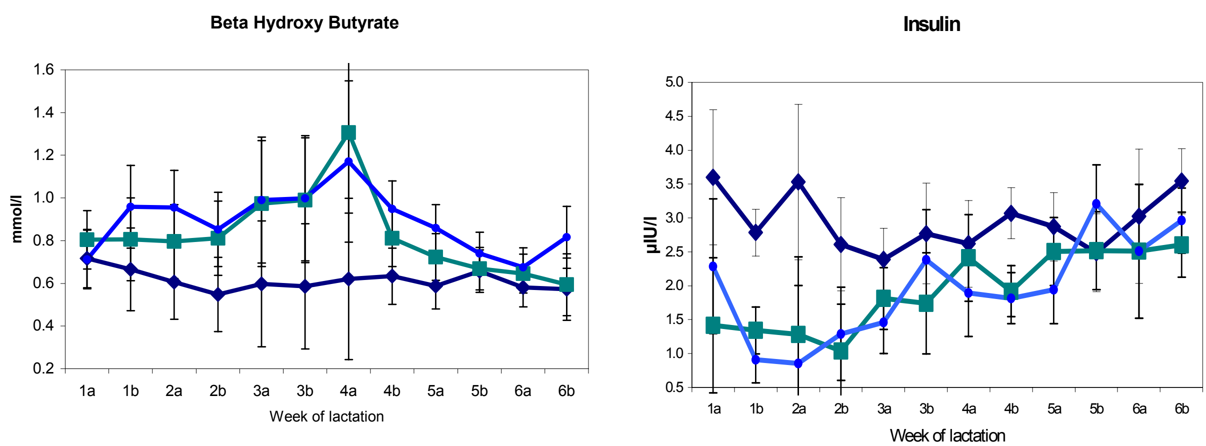


Figure 6 Plasma concentrations of NEFA, glucose, beta hydroxy butyrate and insulin in the first 6 weeks of lactation. The numbers 1 to 6 refer to week of lactation, and a and b signify the first and second sampling within particular weeks. (◆ X1; ■ X2; ● X3)

Reproductive performance

Reproductive performance was not significantly affected by milking frequency. There were no significant differences between treatments for calving to first observed oestrus (30, 32 and 29 days for X1, X2 and X3, respectively), calving to service interval (74, 76 and 75 days for X1, X2 and X3, respectively), calving to conception interval (78, 78 and 80 days for X1, X2 and X3, respectively), conception rate to first service (68, 74 and 65% for X1, X2 and X3, respectively), conception rate to second service (40, 40 and 57% for X1, X2 and X3, respectively), or overall pregnancy rate (84, 84 and 85% for X1, X2 and X3, respectively).

Comparison of cows that conceived and failed to conceive to first service

Retrospective analysis was carried out on reproductive variables, EB, BCS and blood metabolite data by comparing animals that conceived (n = 40) or failed to conceive (n = 18) to first service. There was no significant difference ($P > 0.05$) in any of the reproductive variables measured between those that conceived and those that failed to conceive to first service. Calving to first observed oestrous interval tended to be longer ($P = 0.11$) in the cows that failed to conceive to first service compared to those that conceived and calving to service interval also tended to be longer ($P = 0.08$) in the cows that failed to conceive to first service. Cows that conceived to first service tended to be in more positive EB through the first 6 weeks of lactation. The differences were significant ($P < 0.05$) at weeks 2 and 3 of lactation. The only significant difference in BCS occurred at day 73 of lactation, when it was significantly higher for cows that conceived compared to those that failed to conceive to first service. Plasma NEFA concentrations tended to be lower in the first 4 weeks of lactation in cows that conceived to first service compared to those that failed to conceive but the differences were only significant in week 2 of lactation. Plasma glucose concentrations tended to be higher (significantly ($P < 0.05$) at week 2) in cows that conceived compared to those that failed to conceive over the first 4 weeks of lactation. Plasma BHB concentrations also tended to be lower (significantly ($P < 0.05$) at week 2) in those cows that conceived to first service compared to those that failed to conceive.

Conclusions

Results suggest that reducing milking frequency to once per day can reduce NEB in the early post-partum period and this could be a useful model to study the effects of reduced NEB on reproduction. Imposing the reduced milking frequency results in lower milk production during the treatment period and also over the first 20 weeks of lactation. The reduced NEB was reflected in changes in BCS and in the concentration of certain blood metabolites. While the number of animals per treatment was too small to show significant effects on reproduction, post hoc analysis comparing those that conceived with those that failed to conceive to first service showed trends towards greater NEB during the first 6 weeks of lactation in those that failed to conceive, with the difference being significant in weeks 2 and 3.

3.2 Experiment 2. Effect of milking frequency and nutrition on energy balance and concentrations of blood metabolites and hormones associated with fertility in dairy cows

Introduction

Results from experiment 1 demonstrated that changing daily milking frequency could change EB in the first 4 weeks of lactation and this resulted in effects on blood metabolites. It was important to determine if these observations were repeatable and also to determine if EB could be changed at this early stage of lactation by nutrition. Therefore, thrice versus once daily milking was compared on a typical post-calving diet fed in Ireland and this typical diet and a high specification diet were compared with cows milked thrice daily.

Materials and methods

Pluriparous spring-calving Holstein-Friesian cows were blocked, two weeks prior to expected calving date, according to parity (lactation number = 2 and lactation number > 2), BCS, previous milk yield and expected calving date and randomly allocated post-partum to one of three treatments:

1. Once daily milking on a diet of grass silage ad-lib + 8 kg/cow per day of concentrate supplement (1XS)
2. Thrice daily milking on a diet of grass silage ad-lib + 8 kg/cow per day of concentrate supplement (3XS)
3. Thrice daily milking on a diet of grass silage/maize silage ad-lib + 12 kg/cow per day of concentrate supplement (3XHE)

Milking times were 07:00, 15:00 and 22:00, with the 1XS cows milked at 07:00. There were 23, 21 and 22 cows per treatment, respectively and the treatments were imposed for 28 days post-calving. All cows were then put on twice daily milking and a diet of grass silage ad-lib and 8 kg/cow per day of concentrates was offered.

The methodology employed was similar to experiment 1. Milk yields, milk composition, DMI, BW and BCS were measured and EB calculated. Blood samples were collected two weeks before expected calving date, twice weekly on Mondays and Fridays, for the first 28 days post partum and once weekly from day 29 to day 70 post partum. Plasma was analysed for glucose, beta-hydroxy butyrate (BHB), non-esterified fatty acids (NEFA), triglycerides, total protein, urea, cholesterol, insulin and insulin like growth factor 1 (IGF-1).

Body fat cell size was determined in this experiment by obtaining subcutaneous adipose tissue by biopsy on days 1 and 29 post partum. Samples were fixed using 4% osmium tetroxide solution, filtered through a 400µm membrane, dispersed using a non-ionic surfactant, and then re-filtered onto a 0.45 µm membrane filter for photographing. Samples were photographed at 4X magnification using a standard light microscope and a Nikon Coolpix 4500 camera fitted with C-mount adapter. Images were processed and measured using Adobe Photoshop. A minimum of 100 cells per sample was measured.

Results

Dry matter Intake

Mean daily DMI was increased in 3XHE compared to 3XS ($P < 0.05$) during the 4-week treatment period (Figure 7; Table 6). Milking frequency did not significantly alter DMI averaged over the 4-week treatment period ($P = 0.46$). However, 1XS cows had significantly lower DMI than 3XS cows in week 4 ($P < 0.05$), suggesting that a milking frequency effect was emerging. There were no significant residual milking frequency ($P = 0.76$) or diet ($P = 0.13$) effects in the two-week post treatment period.

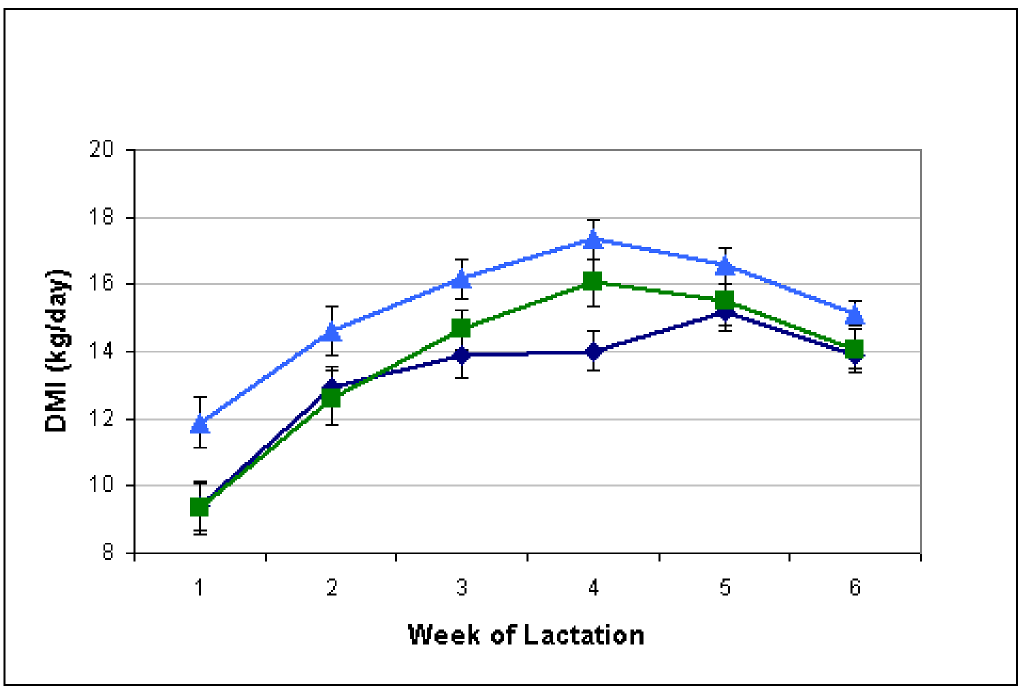


Figure 7 Effect of milking frequency and diet on mean daily dry matter intake (1XS ♦; 3XS ■; 3XHE ▲)

The increased energy density of the HE diet, coupled with the higher DMI, resulted in significantly higher NEI for 3XHE compared to 3XS ($P < 0.001$). Differences due to milking frequency reflected DMI, with no significant differences in NEI evident between 1XS and 3XS ($P = 0.61$).

Table 6 Effect of milking frequency and diet on daily DMI and NEI

	Week of Lactation	1XS[1]	3XS	3XHE	s.e.d[2]
DMI (kg/d)	1-4	12.6[a3]	13.3[a]	15.1[b]	0.81
	5-6	15.1[a]	15.3[a]	16.4[a]	0.70
Net Energy Intake (UFL/d)	1-4	11.9[a]	12.2[a]	14.7[b]	0.71

5-6	14.1[a]	14.1[a]	15.4[b]	
0.61				

[1] 1XS = once a day milking for the first 4 weeks of lactation on standard diet; 3XS = three times a day milking for the first 4 weeks of lactation on standard diet: 3XHE = three times a day milking for the first 4 weeks of lactation on a high energy diet: [2]s.e.d. = standard error of the difference: [3]means within rows having different superscripts differ significantly (*P* < 0.05)

Milk yield and composition

Milk yield was significantly affected by both diet and milking frequency (P < 0.01) during the treatment period (Figure 8; Table 7). Milk yield was 19.6% lower on 1XS than on 3XS. Milk yield was 17.3% greater on 3XHE than on 3XS over the same time. There was a decline in milk yield for 3XS and 3XHE in the two weeks post treatment, simultaneous with an accelerated increase in yield for 1XS. This reflected the commencement of conventional twice a day milking on all treatments. Consequently, there was no significant difference (P = 0.93) in milk yield between treatments 1XS and 3XS in these weeks. Average daily milk yield on 3XHE during weeks 5 and 6 remained higher (P < 0.01) than on 3XS, however, indicating a significant carryover effect for the HE diet fed during the treatment period.

Total 305-day lactation yields were 6198kg, 6813kg and 7132kg for treatments 1XS, 3XS, and 3XHE respectively. The 615kg difference due to milking frequency treatment was significant (P < 0.05), while the 319kg difference due to dietary treatment was not (P = 0.31).

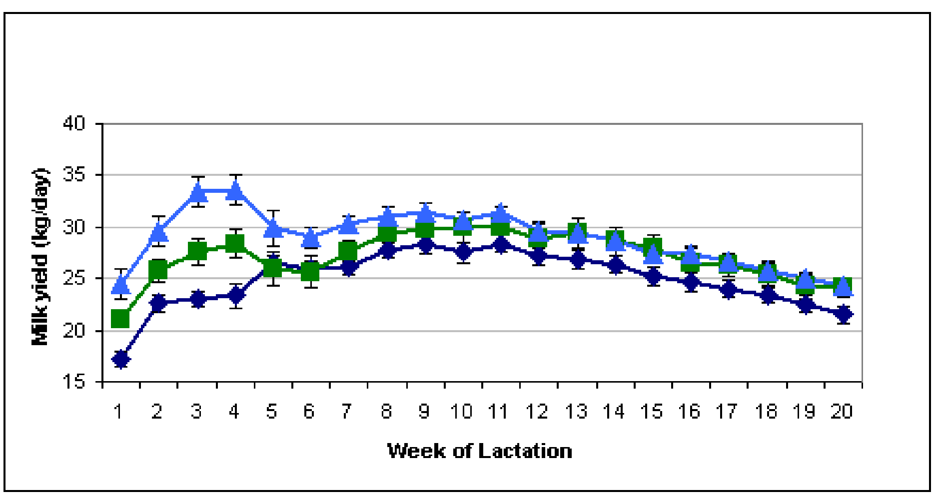


Figure 8 Effect of milking frequency and diet on milk yield (1XS ♦; 3XS ■; 3XHE ▲)

Treatment 1XS resulted in a marked increase (P < 0.01) in milk fat concentration during the treatment period, compared to 3XS milking (Table 7). This concentration effect offset the reduced milk volume on 1XS, resulting in no difference (P = 0.23) in daily fat yield due to milking frequency treatment. Diet did not significantly affect milk fat concentration (P = 0.36) over the treatment period. However, 3XHE increased mean daily fat yield by 21% compared to 3XS (P < 0.001).

Milk protein concentration was increased both by once daily milking (P < 0.001) and feeding the HE diet (P < 0.05) during the treatment period. Mean daily protein yield was increased (P < 0.001) on 3XHE compared to 3XS, but reduced milk volume in 1XS resulted in lower (P < 0.05) daily protein yield compared to 3XS. Milk protein concentration declined across all treatments in the two weeks post treatment.

Lactose concentration was not affected by milking frequency (P = 0.27) or diet (P = 0.67). Hence, the significant differences observed for mean daily lactose yield (P < 0.001) during the treatment period were primarily due to milk volume differences.

Daily milk energy output was lower (P < 0.05) in 1XS than in 3XS and higher (P < 0.001) in 3XHE than in 3XS. Milking frequency had no significant effect (P = 0.28) on milk energy output in the two weeks post-treatment. Diet had a significant residual effect however, with 3XHE having higher milk energy output than 3XS (P < 0.05).

Table 7 Effect of milking frequency and diet on milk yield and composition

	Week of lactation	1XS[1]	3XS[1]	3XHE[1]	s.e.d.[2]
Milk yield (kg/day)	1-4	21.7[a3]	27.0[b]	31.6[c]	1.45
	5-6	26.8[a]	26.7[a]	30.7[b]	1.38
	Total Lactation Yield	6198[a]	6813[b]	7132[b]	304
Fat (g/kg)	1-4	47.1[a]	40.7[b]	42.2[b]	1.61
	5-6	42.0	40.0	39.0	1.67
Protein (g/kg)	1-4	35.5[a]	32.2[b]	34.0[a]	0.87
	5-6	30.8[a]	28.6[b]	28.5[b]	0.80
Lactose (g/kg)	1-4	47.0	46.2	46.5	0.85
	5-6	49.2[a]	47.0[b]	46.9[b]	1.00
Fat (kg/day)	1-4	1.02[a]	1.09[a]	1.33[b]	0.06
	5-6	1.13	1.06	1.20	0.07
Protein (kg/day)	1-4	0.77[a]	0.87[b]	1.07[c]	0.05
	5-6	0.82[ab]	0.76[a]	0.87[b]	0.04
Lactose (kg/day)	1-4	1.02[a]	1.25[b]	1.47[c]	0.07
	5-6	1.32[ab]	1.26[a]	1.43[b]	0.07
Milk energy (UFL/day)	1-4	10.4[a]	11.7[b]	14.1[c]	0.62
	5-6	11.9[ab]	11.2[a]	12.7[b]	0.66

[1] 1XS = once a day milking for the first 4 weeks of lactation on standard diet; 3XS = three times a day milking for the first 4 weeks of lactation on standard diet; 3XHE = three times a day milking for the first 4 weeks of lactation on a high energy diet

[2]s.e.d. = standard error of the difference; [3] means within rows having different superscripts differ significantly ($P < 0.05$)

Energy balance

The mean daily EB over the treatment period did not significantly differ between any of the treatment groups (P = 0.17) (Figure 9). Treatment 1XS tended to improve EB by approximately 1UFL/day compared to 3XS (P = 0.13), while treatment 3XHE did not improve EB relative to 3XS (P = 0.88). Daily EB on 1XS was more positive than on 3XS during week 2 (P < 0.05). Dietary treatment had no significant within-week effects on EB, though 3XHE tended to have lower EB than 3XS in week 4 (P = 0.068). There were no residual treatment effects in the two weeks post treatment. None of the groups achieved positive EB by week 6 post partum.

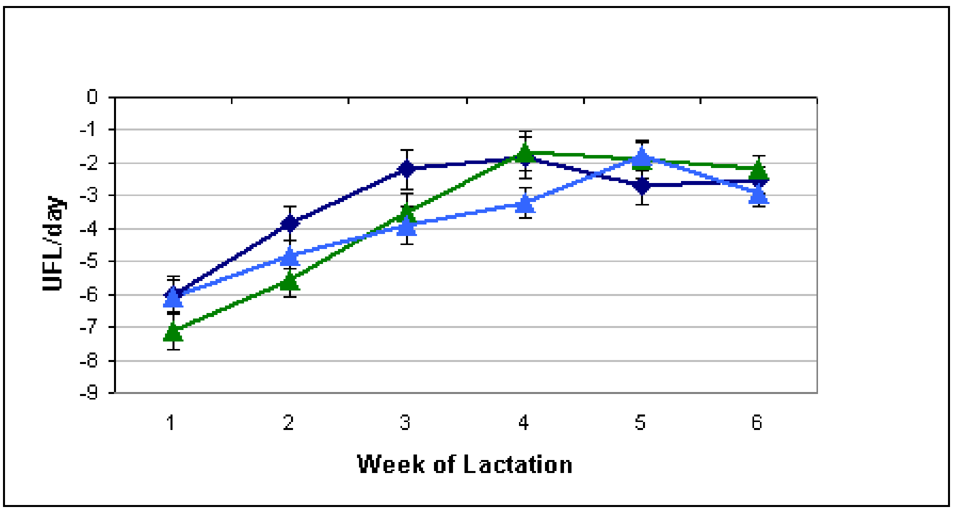


Figure 9 Effect of milking frequency and diet on mean daily EB (1XS ♦; 3XS ■; 3XHE ▲)

Body weight

Neither milking frequency (P = 0.15) nor dietary treatment (P = 0.74) had a significant effect on the degree of BW change during the treatment period (Table 8). However, cows on 1XS began to gain weight earlier post partum (Figure 10). Consequently, they had lost less (P < 0.01) weight than 3XS or 3XHE cows by day 60 of lactation. Dietary treatment did not result in any such residual effect.

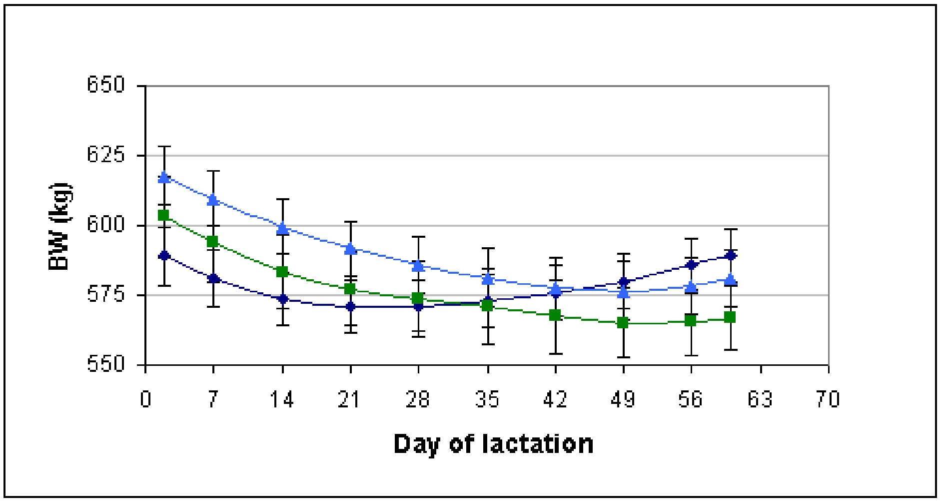


Figure 10 Effect of milking frequency and diet on BW change (1XS ♦; 3XS ■; 3XHE ▲)

Table 8 Effect of milking frequency and diet on BW change (kg)

Days in milk	1XS[1]	3XS	3XHE	s.e.d.[2]
0-28	-17.8	-29.3	-32.0	8.1
0-42	-16.9[a3]	-35.7[ab]	-39.9[b]	10.6
0-60	+0.1[a]	-36.3[b]	-36.6[b]	9.8

[1] 1XS = once a day milking for the first 4 weeks of lactation on standard diet; 3XS = three times a day milking for the first 4 weeks of lactation on standard diet; 3XHE = three times a day milking for the first 4 weeks of lactation on a high energy diet

[2]s.e.d. = standard error of the difference; [3] means within rows having different superscripts differ significantly ($P < 0.05$)

Body condition score

Neither milking frequency (P = 0.60) nor dietary treatment (P = 0.64) affected the degree of BCS loss during the treatment period (Table 9; Figure 11). The mean BCS loss for all groups during the treatment period was approximately 0.25 units. By the start of the breeding season, the mean BCS loss was approximately 0.5 units for all groups, again unaffected by milking frequency or dietary treatments. The rate of BCS loss had decreased for all treatment groups by day 120 of lactation

Table 9 Effect of milking frequency and diet on BCS change

Days in milk	1XS[1]	3XS	3XHE	S.E.D[2]
0-28	-0.30	-0.29	-0.24	0.11
0-42	-0.42	-0.34	-0.46	0.13
0-80	-0.52	-0.49	-0.54	0.16
0-120	-0.57	-0.53	-0.69	0.14

[1] 1XS = once a day milking for the first 4 weeks of lactation on standard diet; 3XS = three times a day milking for the first 4 weeks of lactation on standard diet: 3XHE = three times a day milking for the first 4 weeks of lactation on a high energy diet; [2]s.e.d. = standard error of the difference

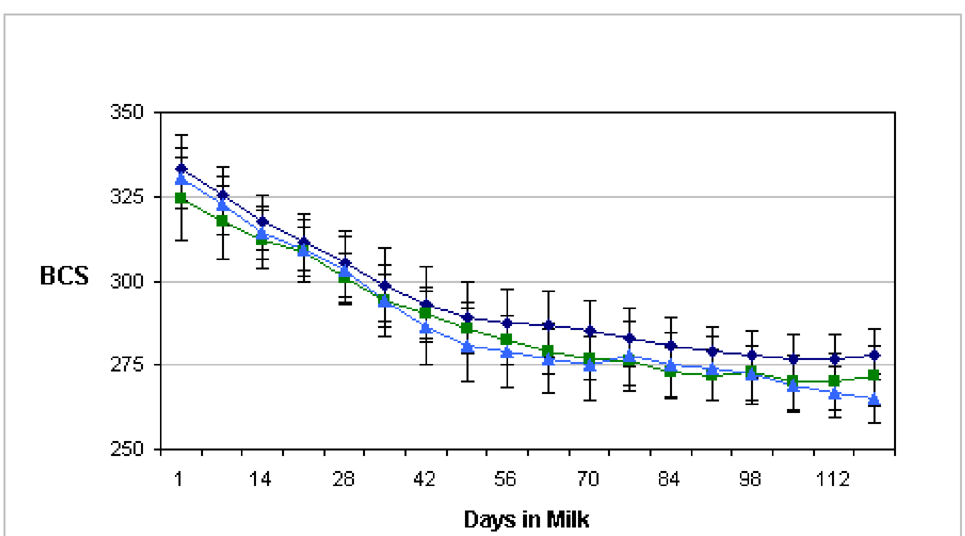


Figure 11 Effect of milking frequency and diet on BCS (X100) (1XS ♦; 3XS ■; 3XHE ▲)

Adipocyte diameter

Adipocyte diameter is correlated with body fat content and was used in this study along with BCS as a more objective measure of cow condition. The degree of reduction in mean diameter of subcutaneous adipocytes (MCD), from d1-28 of lactation, tended to be lower (P = 0.07) on 1XS compared to 3XS. The HE diet had no effect on change in mean adipocyte diameter (P = 0.63).

Table 10 Effect of milking frequency and diet on mean subcutaneous adipocyte diameter (µm)

Days in milk	1XS[1]	3XS	3XHE	S.E.D[2]
1	134	138	139	7.1
28	124	112	109	7.9
change 1-28	-10	-26	-30	8.6

[1] 1XS = once a day milking for the first 4 weeks of lactation on standard diet; 3XS = three times a day milking for the first 4 weeks of lactation on standard diet; 3XHE = three times a day milking for the first 4 weeks of lactation on a high energy diet ;

[2]s.e.d. = standard error of the difference

Blood metabolites

Of the blood metabolites measured, treatments had significant effects on glucose, BHB, NEFA, insulin and IGF-1 (Figure 12).

Treatment 1XS tended to have an increased plasma glucose concentration during the treatment period compared to 3XS (P = 0.075). The effect was significant in week 2 (P < 0.01). Diet had no effect on plasma glucose concentration, during or after the treatment period. Plasma glucose concentration was weakly positively associated with daily EB during the treatment period (r = 0.11; P <0.05).

Treatment 1XS tended to reduce mean plasma BHB concentration (P = 0.063) compared to 3XS during the treatment period, while 3XHE and 3XS did not differ significantly (P = 0.53). Treatment 1XS reduced (P < 0.05) plasma BHB in week 2 and tended to do so in week 3 (P = 0.076). Plasma BHB concentration was weakly negatively associated with daily EB during the treatment period (r = -0.18; P < 0.001)

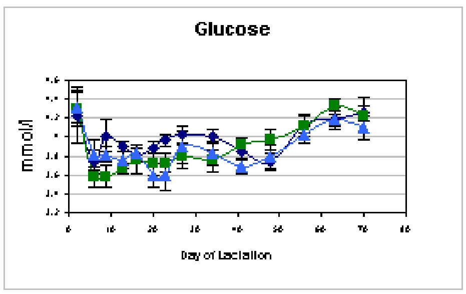
Mean plasma NEFA concentrations were lower (P < 0.05) in 1XS than in 3XS during the treatment period. Plasma NEFA concentrations were not significantly affected by diet. Plasma NEFA concentrations were negatively associated with daily EB during the treatment period (r = -0.45; P < 0.001).

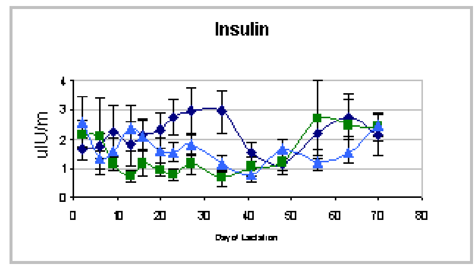
Treatment 1XS tended to have an increased plasma insulin concentration compared to 3XS over the treatment period (P = 0.088) with an increase close to significance in week 3 (P = 0.067), and a significant increase in week 4 (P < 0.01). Plasma insulin concentration on 1XS remained higher (P < 0.05) for one week post treatment but then declined considerably. Consequently, no residual effects of milking frequency were apparent after day 35 of lactation (P = 0.96).

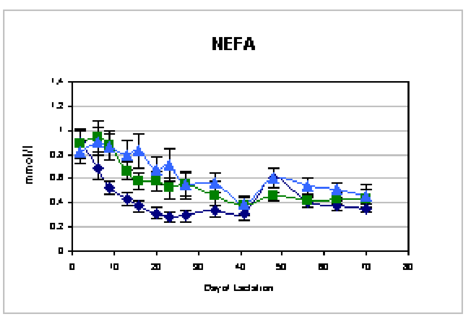
Treatment 1XS tended to increase plasma IGF-1 compared to 3XS during the treatment period (P = 0.082). There were no significant treatment effects on plasma IGF-1 in the post treatment period.

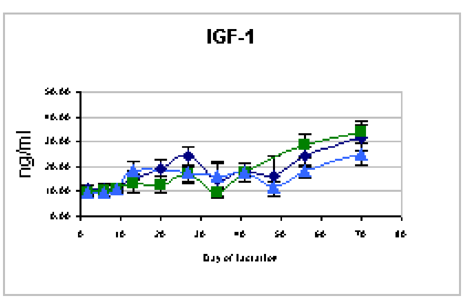
Reproductive Performance

Neither milking frequency nor diet had any effect on interval to service (77, 77 and 79 days for 1XS, 3XS and 3XHE, respectively), interval to conception (96, 96 and 98 days for 1XS, 3XS and 3XHE, respectively), conception rates to first service (50, 65 and 65% for 1XS, 3XS and 3XHE, respectively), second service (54, 50 and 71% for 1XS, 3XS and 3XHE, respectively), or overall pregnancy rate (86, 94 and 90% for 1XS, 3XS and 3XHE, respectively). Treatment 1XS had a reduced interval to first ovulation compared to 3XS (18.3 vs 28.6 days; $P < 0.001$), with a higher proportion of cows ovulating the first postpartum dominant follicle (DF) (82% vs 47%; $P < 0.05$). Dietary treatment did not affect interval to first ovulation ($P = 0.77$) or the proportion of cows ovulating the first postpartum DF ($P = 0.83$).









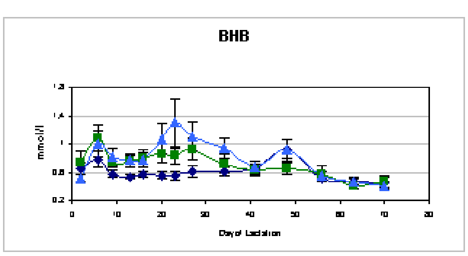


Figure 12 Effect of milking frequency and diet on plasma glucose, BHB, NEFA, insulin and IGF-1 concentrations (1XS ♦; 3XS ■; 3XHE ▲)

Conclusions

Reducing milking frequency to once per day again reduced the degree of NEB but the effect did not reach statistical significance in this study. This may be as a result of lower intakes being achieved in this study compared to the first, as the energy density of the diet was lower. Increasing the energy density of the diet on a thrice-daily milking regime did not affect energy balance because the increased intake was used to increase milk output rather than being partitioned towards body condition. The trends in blood metabolite concentrations were similar to the first study. Reducing milking frequency *per se* reduced the interval to first ovulation by 10 days but dietary energy density had no significant effect and conception performance was not changed.

3.3 Logistic Regression of Data from Experiments 1 and 2

Introduction

Experiments 1 and 2 outlined above were based on milking frequency comparisons and as there were similar treatments in both experiments it was possible to combine the datasets and subject the data to logistic regression. The objective of this analysis was to determine if there was a relationship between production variables or EB and conception rate to first service.

Materials and methods

Cows on twice-daily milking treatment in Year 1, having no corresponding milking frequency group in Year 2, were removed from the analysis. Two cows on the once daily milking treatment in Year 1 reappeared in Year 2. Data on these cows from Year 1 were excluded from the data set. Thus the data set consisted of cows in the X1 and X3 milking frequency groups from two experiments in two years. Following amendment of the data set, ninety-six cows remained with daily records for each variable over the first 28 days of lactation. Milk production, dietary intake and EB values included in the analysis were mean values for the first 28 days of lactation. The data set was numerically sorted by each individual continuous independent variable and then divided into quartiles.

Statistical Analysis

The dependent variable investigated was conception rate to first service (Con1), a binary variable (0,1). The statistical analysis performed was logistic regression, using the PROC LOGISTIC of SAS (SAS system, 1991). A reference quartile was designated for each variable, having and odds ratio of 1 (OR=1). An odds ratio of >1 implies an increased likelihood of Con1 while an odds ratio of <1 implies a reduced likelihood. Con1 for each category of independent variable was compared to Con1 of the reference group for that variable. Logistic regression models for the individual independent variables were constructed. The classifiable adjustment variables of year and milking frequency were included in each model. Univariate analysis was carried out to determine the associations between the adjustment variables and Con1.

Results

The means, maximum and minimum values, and standard deviations for each of the independent variables are given in Table 11. The associations between the adjustment variables and Con1 (determined by univariate analysis) are presented in Table 12. No significant associations were observed between any of the adjustment variables and Con1.

Table 11 Descriptive statistics for independent variables.

Variable	Mean	Std Dev	Min	Max
Milk Yield (kg/day)	27.6	6.26	13.0	40.7
Energy Intake (UFL/day)	14.57	2.93	7.04	20.1
Milk Energy (UFL/day)	12.6	2.62	5.9	18.3
Milk Fat %	4.50	0.55	3.44	5.74
Milk Protein %	3.42	0.30	2.90	4.63
Energy Balance (UFL)	-3.20	2.22	-8.77	3.69

Table 12 Associations between adjustment variables and conception rate to first service (Con1)

Variable OR 95% CI *P*-value

Milking Frequency
1
3
1
1.287

0.56-2.98

NS
Year
1
2
1
0.7

0.30-1.66

NS

Independent variables and conception rate to first service (Con1)

Table 13 contains the associations between the independent variables and Con1.

There was no association observed between mean daily milk yield, milk energy output, and mean milk fat concentration and Con1. Milk protein concentration was significantly associated with Con1, however. Cows in the highest milk protein concentration quartile (>35.5g/kg) had an increased likelihood of conception, compared to the two lowest quartiles. Odds ratios of 0.16 ($P < 0.05$) and 0.24 ($P < 0.05$) were observed for the two lowest milk protein quartiles, respectively.

The reference quartile for dietary NE intake (>16.1 UFL/day) had a significantly increased likelihood of conception compared to the other three quartiles. The middle quartiles both had an OR of 0.08 ($P < 0.01$ for both quartiles), while the lowest quartile had an OR of 0.05 ($P < 0.01$). There was a trend towards lower conception rates with increasing severity of NEB. Cows in the lowest quartile for EB (<-5.4 UFL/d) had a reduced likelihood of conception compared to the reference group (OR = 0.18, P < 0.05), while cows in quartile 3 (-3.7 to –5.4 UFL/d) showed a similar trend (OR = 0.29, P = 0.11)

Conclusions

The results of this study show a positive relationship between nutritional status in early lactation and subsequent conception rate to first service. Cows with greater energy intakes and those in more positive EB had an improved likelihood of conception. Thus the importance of maximising energy intake in early lactation for fertility performance is underlined. Mean daily milk yield over the first month of lactation was not associated with likelihood of conception but the results indicate that milk protein content, traditionally considered a benchmark of dietary energy adequacy, can be utilised to identify cows at risk of poor reproductive performance.

Table 13 Associations between milk, intake and EB variables and conception rate to first service (Con1)*

Variable	OR	95% CI	Max-likelihood P>Chisq
Milk Yield (kg/day)			
1 (>31.1)	1		
2 (27.8-31.1)	0.93	0.26-3.32	NS
3 (23.4-27.8)	0.99	0.24-4.20	NS
4 (<23.4)	1.372	0.27-7.07	NS
Milk Energy (UFL/day)			
1 (>14.6)	1		
2 (12.4-14.6)	1.482	0.41-5.34	NS
3 (11.1-12.4)	1.805	0.42-7.70	NS
4 (<11.1)	1.178	0.25-5.50	NS
Milk Fat %			
1 (>4.88)	1		
2 (4.46-4.88)	1.47	0.44-4.93	NS

Variable	OR	95% CI	Max-likelihood P>Chisq
3 (4.08-4.46)	2.04	0.56-7.41	NS
4 (<4.08)	1.57	0.45-5.57	NS
Milk Protein %			
1 (>3.55)	1		
2 (3.35-3.55)	1.01	0.25-4.09	NS
3 (3.22-3.35)	0.16	0.04-0.67	0.0125
4 (<3.22)	0.24	0.06-0.97	0.0451
Milk Fat/ Protein Ratio			
1 (<1.22)	1		
2 (1.22-1.30)	0.94	0.26-3.35	NS
3 (1.3-1.41)	1.08	0.31-3.77	NS
4 (1.41)	0.3	0.087-1.078	0.0652
Net energy intake (UFL/day)			
1 (>16.31)	1		
2 (14.3-16.3)	0.09	0.016-0.458	0.0044
3 (12.2-14.3)	0.08	0.012-0.534	0.0093
4 (<12.2)	0.08	0.010-0.582	0.013
Energy Balance (UFL/day)			
1 (>-1.94)	1		

Variable	OR	95% CI	Max-likelihood P>Chisq
2 (-1.94, -3.45)	0.47	0.12-1.86	NS
3 (-3.45, 5.37)	0.29	0.07-1.30	0.107
4 (<-5.37)	0.19	0.04-0.90	0.035

* All models include adjusted variables milking frequency and year

3.4 Experiment 3. Effect of altering the protein content in the concentrate supplement in early lactation on BCS change

Introduction

Dairy cows in general, and in particular high genetic merit cows, lose BCS and BW after calving, as shown in experiments 1 and 2 in this report. Analysis of the combined data from both experiments showed a negative relationship between the severity of NEB in the first 28 days post-calving and conception rate to first service. The question arises as to what other practical strategy, apart from milking frequency, can be employed in order to reduce the degree of NEB in early lactation. The objective of this study was to determine the effect of altering BCS at calving and diet post-calving on change in BCS, an indicator of EB, in the early postpartum period.

Materials and Methods

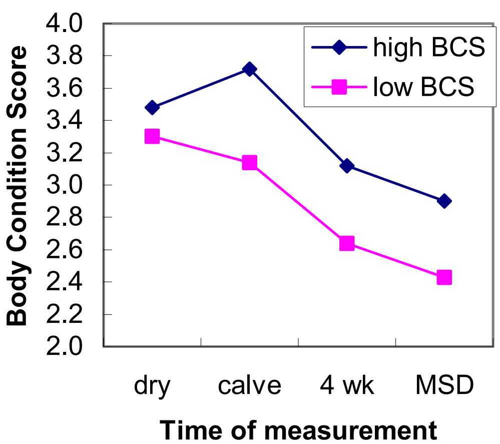
Seventy-six autumn-calving Holstein-Friesian cows were blocked onto pre-calving treatments at approximately 8 weeks before expected calving date (ECD) on the basis of ECD and BCS. Two pre-calving treatments were imposed: (1) Generous grass supply leaving a post grazing sward height of approximately 7 cm and (2) Restricted grass supply (grazed as followers after Treatment 1 cows or the milking cows) leaving a post grazing sward height of 4-5 cm. At calving cows were assigned alternately, from within these groups, to either (1) 6-7 kg/cow per day of a high crude protein (CP) concentrate (180-200g CP/kg) or (2) 9-10 kg/cow per day of a low CP concentrate (120g CP/kg). The mean calving date of the herd was October 12. Between early September and mid-October all calved cows were at pasture. Between mid-October and mid-December they grazed by day and had unrestricted access to grass silage (until early November) or a mixed forage diet consisting of 0.6 grass silage, 0.25 maize silage and 0.15 brewers grains on a DM basis (from early November to mid-December) by night. From mid December until early March cows were indoors fulltime and received the mixed forage diet plus their respective concentrates. Body weight and BCS were measured weekly before and after calving. Milk yield was measured daily and milk composition once weekly. Concentrates were fed in the milking parlour at each milking and forage intake was monitored on a group basis after calving (total consumed by the 12 % CP and 20 % CP treatment groups separately).

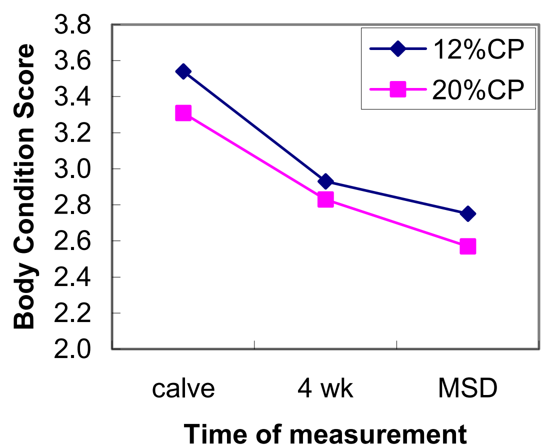
Results

There was no significant interaction between the pre-calving and post-calving treatments for BCS. Therefore, BCS for the pre-calving treatments and post-calving treatments are illustrated separately in Figures 13 and 14, respectively. The group offered the generous grass supply pre-calving gained 0.24 of a BCS unit between drying off and pre-calving whereas those receiving the restricted grass supply lost 0.21 of a BCS unit, a difference of 0.45 of a BCS unit ($P < 0.001$). Their mean BCS values at calving were 3.72 and 3.14, respectively. The pattern of BCS loss post-calving was similar and the average loss between calving and MSD for those on a generous or restricted grass supply before calving was not significantly different (0.80 vs 0.66, respectively).

The pattern of loss of BCS was similar on the post-calving treatments. The greatest loss of BCS occurred between calving and 4 weeks post-calving. Losses of BCS on the 12 % CP and 20 % CP treatments for that period were 0.62 and 0.46 of a unit, respectively ($P < 0.09$). The BCS losses on these treatments between calving and MSD were 0.75 and 0.72 of a unit, respectively ($P = 0.81$)

Conclusion





Results suggest that neither diet pre-calving, which alters BCS at calving, nor supplementing with a greater quantity of a lower CP concentrate post-calving, significantly affects the magnitude or pattern of BCS change in early lactation.

Figure 13. The effect of pre-calving **Figure 14.** The effect of crude protein treatment on BCS at drying off (dry), content of the post-calving supplement at calving (calve) at 4 weeks on BCS post-calving post- calving (4 wk) and at mating start date (MSD)

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