Strategies to alleviate reproductive wastage in dairy cows

End of Project Report

Project 5397

Author: S.T. Butler

Contents

1. Summary 3

2. Introduction 4

3. Experiments
   3.1 Experiment 1
   Characterization of the physiological differences between North American and New Zealand strains of dairy cow under a grass based system of production. 6

3.2 Experiment 2
   The effect of dry period duration and dietary energy density on milk production, bioenergetic status and postpartum ovarian function in Holstein-Friesian dairy cows. 21

3.3 Experiment 3
   Extended lactations in a seasonal-calving pastoral system of production to modulate the effects of reproductive failure 29

3.3 Experiment 4
   Effects of CLA supplementation on milk production and reproductive performance of dairy cows 44

4. Overall summary 54

4. References 56

5. Publications from this project 60

Summary
Reproductive performance plays a key role in determining the efficiency of seasonal grass-based systems of production. A series of studies were carried out to (i) improve our understanding of the physiological basis of poor reproductive performance; (ii) examine management and nutritional strategies to improve fertility; and (iii) examine the potential role of extended lactation to mitigate the effects of poor reproductive performance.
A comprehensive characterization of the North American and New Zealand strains of Holstein-Friesian cow was carried out. North American cows produce a greater volume of milk, but yield a similar amount of fat and protein on a grass-based diet. Dry matter intake was greater for the larger NA strain, but energy balance did not differ during the first 20 weeks of lactation. Circulating concentrations of metabolic hormones and metabolites during the early lactation period were indicative of lesser bioenergetic status in the NZ strain compared to the NA strain. During established lactation, circulating concentrations of insulin-like growth factor-I (IGF-I) were greater in the NZ strain. Liver biopsies collected at day 35 and day 150 postpartum indicated that the greater circulating concentration of IGF-I was due to greater hepatic mRNA abundance of IGF-I and acid labile subunit (ALS). Embryos were collected from both strains after superovulation. A greater proportion of the embryos recovered were transferable in the NZ strain compared with the NA strain, indicating that the previously reported differences in reproductive performance were manifest as early as 7 days post-insemination. Collectively, the results of the study indicate that the NZ strain are genetically better equipped to survive on a pasture-based seasonal calving system.
A study was carried out to examine the effect of dry period duration and dietary energy density on milk production, bioenergetic status and postpartum ovarian function. Omitting the dry period and increasing dietary energy density both resulted in improved energy balance and metabolic status, but omitting the dry period reduced the postpartum interval to resumption of cyclicity whereas increasing dietary energy density had no effect. Omitting the dry period reduced the inherent drive to produce milk, and allowed the cow to fully meet nutritional requirements from voluntary dry matter intake. Increased dietary energy density also allowed the cow to more closely meet nutritional requirements from a higher energy density diet, albeit at a greater milk yield. The results suggest that the mechanism by which a cow arrives at a particular energy balance status may be as important as energy balance *per se*.
One of the main energy costs associated with lactation is milk fat. Trans 10, cis 12 conjugated linoleic acid (CLA) is a geometric and positional isomer of linoleic acid that reduces milk fat synthesis in a dose dependent manner. Supplementing cows with CLA resulted in improved energy balance status during the transition period and reduced postpartum body condition score loss. Some indices of reproductive performance were also improved.
In seasonal systems, cows that fail to become pregnant by the end of the breeding season are typically culled and replaced. When reproductive performance is poor, this represents a major cost on dairy farms. A study was carried out to examine the feasibility of extending the lactation to 22 months, resulting in a calving interval of 24 months instead of 12 months. High yielding cows produced the equivalent of 2 normal lactations in an extended lactation system. An economic analysis indicated that an efficient spring calving system with a compact calving pattern and a 12 month calving interval is still the most profitable, but with high yielding cows extending the lactation of non-pregnant cows is more profitable than culling and replacing.

Introduction

The advent of artificial insemination has resulted in remarkable genetic gain for milk production in dairy cattle, with progress currently continuing in a linear fashion (Foote, 1996). This increase in milk production has been associated with opposite changes in reproductive performance (Butler, 2003), with substantial economic impact. Genetic selection for increased milk yield over the last half century was associated with a decline in genetic merit for fertility traits. Most countries, including Ireland, now have fertility traits incorporated into the selection index (Veerkamp et al., 2002). Research carried out in Moorepark has clearly indicated marked differences in reproductive performance between cows of North American and New Zealand ancestry under grass-based systems of production (Horan et al., 2005b, Horan et al., 2004, McCarthy et al., 2007a). In response to genetic selection for milk yield, little variation is observed among cows in digestion and nutrient absorption, maintenance requirements, and the efficiency with which metabolizable energy is used for milk synthesis. There is, however, substantial individual variation in feed intake and partitioning of nutrients for productive purposes (Bauman et al., 1985). It appears that the continued preferential partitioning of nutrients to the mammary gland at the expense of body reserves and other biological processes (e.g., reproduction) is one of the distinguishing features of the NA strain of Holstein-Friesian cow. The 'dialogue' between body reserves and the hypothalamic-pituitary-ovarian axis is known to regulate reproductive efficiency in many species. The modern NA Holstein has been selected so heavily for increased milk production that she cannot meet nutrient requirements on a grass-based diet. Failure to partition nutrients towards body reserves during lactation appears to play a key role in determining the suboptimal fertility performance in NA Holstein cattle.

It is generally accepted that energy balance (calories consumed minus calories expended), rather than intake of any specific class of nutrient (carbohydrate, lipid, protein), is the main regulator of reproductive status (Butler and Smith, 1989; I'Anson et al., 1991). To date, the precise mechanisms by which nutritional status interacts with the reproductive axis have not been delineated. Dietary strategies to increase periparturient dry matter intake, thereby improving EB, could have beneficial effects on reproductive performance. Additionally, management strategies to improve energy balance could benefit reproductive performance. One management strategy that could reduce postpartum negative energy balance is omitting the dry period. Recent studies have indicated that continuous milking during the dry period results in greater prepartum DMI, improved EB and reduced BCS loss postpartum, and modest reductions in solids-corrected milk yield (Annen et al., 2004b, Rastani et al., 2005). One report has suggested improvements in fertility indices (Gumen et al., 2005). Another strategy of improving postpartum EB is to reduce mammary milk fat output. From a pure energy perspective, fat is the mostly costly milk component for the cow to produce. Trans 10, cis 12 conjugated linoleic acid (CLA) can reduce mammary fat synthesis (Baumgard et al., 2000, de Veth et al., 2004). The effects of CLA induced milk fat depression in early lactation on bioenergetic status and fertility performance has not been fully explored.

In a seasonal grass based system of milk production, a compact calving pattern during the spring is desirable such that increasing demand for feed occurs in tandem with increasing grass supply. However, the adverse effects of increased milk production on reproductive performance result in a significant proportion of the herd

not pregnant at the end of the breeding season. This results in a high proportion of the herd being involuntarily culled for reproductive problems. Many cows are still producing significant quantities of milk at the typical time of drying-off (305 days in milk). In a pasture-based system, little is known about the duration that modern high producing cows can continue to produce milk when not pregnant, or how they respond to plane of nutrition. Furthermore, the effect of extended lactation on reproductive performance during a second breeding season is unknown. The period of negative energy balance — and associated loss of body condition — typically encountered during early lactation would be avoided, and it can reasonably be expected that most cows would be in zero to positive EB for several months prior to breeding. Economic analysis of extended lactation data is necessary to determine if it may be suitable for Irish seasonal calving systems of production.

3. Experiments

3.1. Experiment 1. Characterization of the physiological differences between North American and New Zealand strains of dairy cow under a grass based system of production.

3.1.1 Introduction

Dairy cows typically enter a state of negative energy balance (NEB) *post partum*, when the combined energy requirements for maintenance and milk production exceed dietary energy intake. This energy deficit arises because cows generally achieve peak milk production at an earlier stage than maximal feed intake. The shortfall in dietary intake is met by increased mobilization of body reserves in support of lactation, which occurs through coordinated adaptation of metabolism across several body tissues (Bauman, 2000). The magnitude and duration of NEB is dependent on the direct and interactive effects of numerous factors including genotype, plane of nutrition, and body condition score (BCS) at calving. Genetic selection for increased milk yield has resulted in cows that are predisposed to more severe NEB, as the correlated response in feed intake to selection accounts for only approximately 45 to 65 percent of the increase in milk yield (Veerkamp, 1998). A negative genetic correlation consequently exists between BCS and genetic merit for milk yield (Berry et al., 2003b).

There is compelling evidence of a negative genetic correlation between milk production and fertility performance (Hansen, 2000). Though the precise mechanisms remain unresolved, increasing negative energy balance (NEB) and altered partitioning of dietary energy have been cited as being detrimental to reproductive efficiency (Butler, 2003). This is further intimated by negative genetic correlations identified between body condition score (BCS) and fertility performance (Pryce et al., 2001). Strain comparison studies in New Zealand and Ireland have reported lower milk volume, higher BCS throughout lactation and superior reproductive performance for the New Zealand (NZ) Holstein Friesian compared to North American (NA) Holstein Friesian (Harris and Kolver, 2001, Horan et al., 2004). The NA strain has been selected for increased milk yield, body size and angularity in a production system based on year-round calving and high levels of concentrate supplementation, with little emphasis on traits such as fertility. The NZ strain has been selected for increased milk solids yield and improved fertility and survival in a pasture-based production system. The strain comparison model provides a framework for examining the effects of divergent genetic selection programmes within the Holstein Friesian on key physiological variables that are linked with dairy cow fertility. The objective of this experiment was to characterize the temporal changes in whole body bioenergetics, responsiveness to homeostatic challenges, hepatic gene expression, and reproductive hormone profiles and embryo quality in the NA and NZ strains, which differ in genetic merit for milk production and fertility traits.

3.1.2 Materials and Methods

Animals and experimental design

Two groups of 10 spring-calving, multiparous Holstein-Friesian cows were selected from the NA and NZ groups of the Moorepark strain comparison study. The origins and establishment of the experimental groups from which the cows were selected have been previously described by (Horan et al., 2004). The experimental

animals used in the current study were selected from the existing NA and NZ treatment groups involved in the Moorepark strain comparison study (Table 3.1). Mean calving dates were 25th February (s.d. 18 days) for the NA group and 2nd March (s.d. 17 days) for the NZ group.

Table 3.1 *Genetic merit of the North American and New Zealand strains of Holstein Friesian based on predicted differences[2] and standard deviations (SD) for milk production, calving interval and survival*

	Strain[1]	
Trait	NA	NZ
Milk (kg)	+ 210 (117)	+ 1 (157)
Fat (kg)	+ 6.2 (3.5)	+ 6.5 (5.0)
Protein (kg)	+ 7.4 (4.4)	+ 3.7 (4.0)
Fat (g/kg)	+ 0.10 (1.4)	+ 1.13 (0.62)
Protein (g/kg)	+ 0.40 (0.32)	+ 0.75 (0.43)
Calving interval (days)	+ 0.99 (1.98)	- 2.86 (1.53)
Survival (%)	+ 0.04 (0.29)	+ 1.14 (0.48)

[1]NA = North American Holstein Friesian; NZ = New Zealand Holstein Friesian

[2]All predicted differences obtained from the February 2004 international evaluations of the INTERBULL Animal Centre (Uppsala, Sweden).

The cows were housed in a free-stall barn from 3 weeks prior to the expected calving date, with the treatment groups sharing common accommodation space. The cows were trained to use the Griffith Elder feeding system (Griffith Elder Ltd, Bury St Edmunds, Suffolk, UK). The *pre partum* diet comprised *ad libitum* grass silage, with 2 kg per day of the lactating concentrate introduced from 2 weeks prior to the expected calving date. The *post partum* diet consisted of *ad libitum* grass silage and 8 kg of concentrate. From March 20th, all lactating cows were offered zero-grazed grass (*L. perenne spp)* supplemented with 4kg concentrate. Grass was harvested and fed each morning. Cows were turned out to pasture on July 30th and were offered high quality grazed grass (*L. perenne spp.)* plus 4 kg/day of concentrate.

Samples and animal measurements

Milk yield (kg) was recorded daily and milk composition (fat, protein and lactose) was determined weekly. Solids-corrected milk (SCM) yield was calculated using the equation of (Tyrrell and Reid, 1965). Cow body weight (kg) and BCS were measured once weekly throughout lactation. Blood samples were collected three times weekly (Monday, Wednesday, Friday) for 2 weeks before expected calving date, daily from day of calving until day 14 *post partum*, and twice weekly (Monday, Thursday) from day 15 to day 100 *post partum*. The plasma was decanted and stored at -20 °C until analysis for insulin, IGF-I, glucose, NEFA and BHBA. Energy balance was estimated as the difference between energy intake and the sum of energy for maintenance and milk production.

Homeostatic challenges

Homeostatic challenges were carried out at two time periods during lactation. The first time period (T1) was approximately 35 days in milk, and the second time period (T2) was approximately 140 days in milk. At both time periods, glucose tolerance tests, insulin tolerance tests and epinephrine challenges were carried out using standard protocols.

Hepatic mRNA abundance of genes in the somatotropic axis.
Liver biopsies were collected on the day after the final homeostatic challenge at both time periods, immediately snap-frozen in liquid nitrogen, and stored at –80 °C. Total RNA was isolated from 100 mg of the frozen liver biopsy using TRIzol reagent, and appropriate quality tests were conducted. Absolute real-time RT-PCR was performed on 20 genes involved in the GH-IGF system to quantify gene transcripts using the ABI 7500 Fast Real-Time PCR System with Power SYBR® Master Mix (Applied Biosystems, Warrington, UK).

3.1.2.5 Superovulation and embryo collection, transrectal ultrasound, and blood collection for reproductive hormones.

All cows were submitted to a superovulation protocol on three occasions between July and November 2005. All cows were inseminated with frozen-thawed semen collected from a single ejaculate of a Holstein-Friesian bull (Dairygold A.I., Mallow, Co. Cork, Ireland.) at 36 and 48 hrs after the final injection of FSH. Uteri were non-surgically flushed on day 7 post AI by an experienced technician using standard techniques. Each uterine horn was flushed with 500 ml of phosphate buffer saline (PBS). Recovered structures were isolated and graded according to the criteria of the International Embryo Transfer Society (IETS).

Data handling and statistical analysis

Daily milk yield and DMI data were collapsed into weekly means, and EB values were similarly calculated as weekly means. Repeated measures analyses of genotype effects on DMI, milk yield, milk composition, plasma metabolites, insulin and IGF-I, energy balance, BCS and bodyweight were carried out using the MIXED procedure of SAS (SAS Institute, 1991). A first order autoregressive covariance structure was used. Genotype, time, and the interaction of genotype and time were included as fixed effects. Cow within genotype was included as a random effect.

For the analysis of the homeostatic challenges data, metabolite and hormone responses to each homeostatic challenge were calculated as area under the response curve (AUC), corrected for differences in baseline value. Area under the curve was calculated using the EXPAND procedure in SAS (SAS Institute, 1991). The half life ($t_{½}$) and clearance rate (CR) of glucose and insulin were calculated using the NLIN procedure in SAS (SAS Institute, 1991). Data from T1 and T2 were analyzed as repeated measures using the MIXED procedure of SAS (SAS Institute, 1991). Treatment, time and a treatment by time interaction term were included in the models as fixed effects; cow was treated as a random variable nested within treatment, and an autoregressive covariance structure was used.

Liver gene expression data were log transformed for normalization of variances and were analysed using the PROC MIXED procedure of SAS (SAS, 2003). Cow was treated as a random effect with terms for day and strain and their interaction included as fixed effects in the model.

For the analysis of the embryo data, the proportion of recovered structures that were transferable (morulae and blastocysts), the proportion of recovered structures that were morulae and the proportion of recovered structures that were blastocysts were calculated. For all flushes yielding transferable embryos the proportions at the morula and blastocyst stages were also calculated. This data was then analysed using the Mann-Whitney non-parametric test with Wilcoxon scores, and Fishers exact test was used to compare differences between strains.

3.1.3 Results

All the results of the studies with the NA and NZ strains of cows are described in detail in published scientific papers. Only the main points of interest are discussed in this end of project report.

Milk production, DMI, EB, and feed efficiency.

These results are described in detail by (Patton et al., 2008). The NA strain had greater milk yield during week 1-20 of lactation, and tended to have greater SCM yield compared to the NZ strain (Table 3.2). The NA strain had greater peak milk yield, but peak SCM yield did not differ between the strains. Milk fat concentration over the full lactation was greater for NZ cows, while milk protein concentration did not differ between the strains. Total combined yield of milk fat and protein over the full lactation was 12.7% greater for the NA strain. The NA strain produced 20.4% greater volume of milk over the full lactation compared to the NZ strain; total lactation SCM yield was 12.7% greater for the NA strain.

Table 3.2 *Effect of strain[1] of Holstein Friesian on milk production and composition*

Variable	NA	NZ	s.e.d.[2]	P-value
Week 1-20 of Lactation				
Milk yield (kg/day)	30.9	26.8	1.1	<0.01
Solids corrected milk[3] (SCM) yield (kg/day)	29.6	27.7	1.0	0.06
Milk fat content (g/kg)	42.0	47.7	1.8	<0.01
Milk protein content (g/kg)	32.4	32.5	0.6	0.97
Peak Milk yield (kg)	37.6	32.7	1.1	<0.001
Peak SCM yield (kg)	38.0	36.6	1.6	0.39
Total Lactation[4]				
Milk yield (kg)	7280	6045	362	<0.01
SCM (kg)	6816	6048	342	0.04
Milk fat content (g/kg)	40.2	43.9	1.2	<0.01
Milk protein content (g/kg)	33.5	34.1	0.6	0.33
Total fat + protein yield (kg)	533	473	27	0.03

[1] NA= North American Holstein Friesian; NZ= New Zealand Holstein Friesian
[2] SED = Standard error of difference
[3] Calculated as described by Tyrell and Reid (1965)
[4] Mean lactation length was 287d for NZ and 290d for NA strain

Mean DMI and net energy intake tended to be greater for NA compared to NZ cows during wk 1-20 of lactation. When expressed as a percentage of metabolic bodyweight, however, the strains had similar mean daily DMI over the same time period (Table 3.3). The NA and NZ strains had similar mean daily calculated energy balance (EB) during week 1-20 of lactation. The strains also had a similar magnitude of EB nadir, timing of EB nadir, and interval to neutral EB. The NA and NZ strains had similar milk yield per kg of DMI, and similar output of milk energy per unit of net energy intake for week 1-20 of lactation. Solids corrected milk yield as a proportion of metabolic bodyweight did not differ between the strains.

Table 3.3 *Effect of strain on Energy Balance and Feed Intake*

Variable	NA[1]	NZ[1]	s.e.d.[2]	P-Value
Dry Matter and Energy Intake wk1-20				
Dry matter intake (DMI) (kg / d)	17.2	15.7	0.78	0.07
Net energy intake (UFL /d)	16.8	15.5	0.70	0.08
DMI as proportion of MBW[3] (%)	14.3	14.1	0.71	0.78
Energy Balance (EB)				
EB wk 1-20 (UFL[4] / d)	-1.80	-1.84	0.66	0.95
Nadir EB (UFL / d)	-6.88	-7.31	1.20	0.72
Interval to nadir EB (days)	10.3	10.6	1.84	0.77
Interval to neutral EB (days)	72	73	9.5	0.87
Milk Production Efficiency wk1-20				
Milk yield per kg DMI (kg)	1.86	1.75	0.08	0.22
UFL milk per UFL intake (UFL)	0.84	0.85	0.12	0.91
SCM[5] as proportion of MBW (%)	17.7	18.1	0.71	0.57

[1] NA= North American Holstein Friesian; NZ= New Zealand Holstein Friesian
[2] s.e.d. = Standard error of difference
[3]MBW = Metabolic bodyweight, calculated as $B^{0.75}$, where B=bodyweight (kg)
[4]1 UFL = Net energy for lactation equivalent of 1 kg standard air-dry barley (Jarrige, 1989)
[5]SCM = Solids Corrected Milk

The NA and NZ strains had similar BCS at the beginning of lactation (3.17 vs. 3.22). Both strains had lost a similar amount of BCS by week 20 of lactation (0.65 vs. 0.55). Thereafter, the NZ strain began to increase in BCS whereas the NA strain did not, resulting in a greater BCS for NZ compared to NA by the end of lactation (2.85 vs. 2.43). Mean bodyweight across the full lactation was greater for NA compared to NZ cows (596 vs. 544 kg respectively; Figure 3.1).

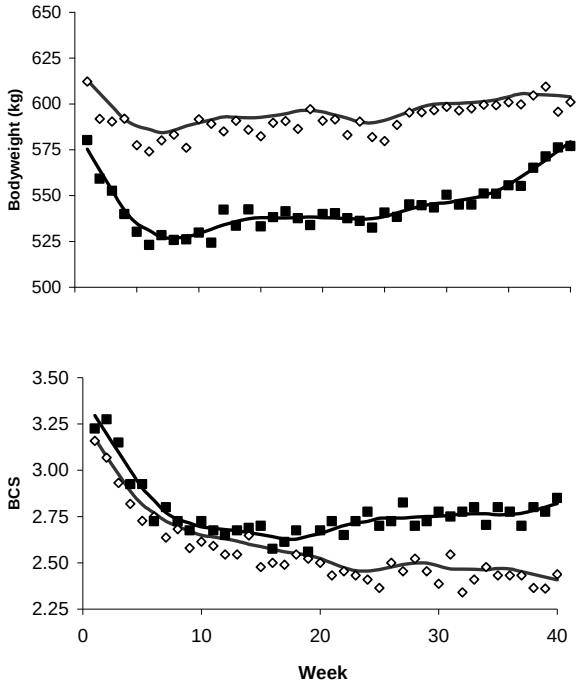

Figure 3.1 Effect of strain of Holstein-Friesian on body condition score (BCS) and bodyweight (◊ = North American Holstein Friesian; ■ = New Zealand Holstein Friesian).

Plasma insulin, IGF-I and metabolites

These results are described in detail by (Patton et al., 2008). Mean plasma insulin concentration was higher for the NA strain during the transition period (Table 3.4). The NZ strain had greater plasma IGF-I concentrations from d 29 to d 100 of lactation. Plasma glucose concentrations were greater for the NA strain during the transition period, but differences were not observed in the post-transition period.

Table 3.4 *Effect of strain on plasma concentrations[1] of insulin, IGF-I and metabolites*

Variable	NA[2]	NZ[2]	Mean ratio[3]	P-value
Transition Period[4]				
Insulin (uIU/mL)	4.39 (3.82, 5.16)	3.32 (2.86, 3.82)	1.33 (1.08, 1.65)	0.01
IGF-I (ng/mL)	56.8 (49.4, 66.7)	59.2 (51.4, 68.7)	0.96 (0.78, 1.19)	0.71
Glucose (Mmol/L)	3.50 (3.39, 3.63)	3.29 (3.16, 3.39)	1.07 (1.01, 1.12)	0.01
NEFA (Mmol/L)	0.34 (0.28, 0.41)	0.39 (0.33, 0.47)	0.85 (0.66, 1.11)	0.29
BHB (Mmol/L)	0.63 (0.57, 0.70)	0.77 (0.69, 0.87)	0.82 (0.70, 0.96)	0.02
Post Transition[4]				
Insulin (uIU/mL)	4.66 (4.06, 5.42)	3.86 (3.35, 4.44)	1.21 (0.99, 1.48)	0.06
IGF-I (ng/mL)	77.5 (67.4, 90.0)	97.5 (83.9, 112.2)	0.80 (0.65, 0.99)	0.04
Glucose (Mmol/L)	3.25 (3.19, 3.35)	3.32 (3.25, 3.42)	0.98 (0.95, 1.01)	0.21
NEFA (Mmol/L)	0.17 (0.14, 0.20)	0.17 (0.14, 0.20)	1.00 (0.78, 1.29)	0.99
BHB (Mmol/L)	0.46 (0.41, 0.51)	0.44 (0.39, 0.48)	1.05 (0.92, 1.21)	0.45

[1] Geometric Means (95% Confidence interval in parentheses)
[2] NA = North American Holstein Friesian; NZ = New Zealand Holstein Friesian
[3] Ratio of geometric means (95% Confidence interval in parentheses)
[4] Transition = d 15 *pre partum* to d 28 *post partum*; Post transition = d 29 to 100 *post partum*

Glucose tolerance test

These results are described in detail by (Patton et al., 2009). Intravenous infusion of glucose resulted in an acute increase in plasma insulin concentrations, and a reduction in plasma NEFA concentrations (Table 3.5; Figure 3.2). The NA and NZ strains had similar glucose AUC, insulin AUC, NEFA AUC, glucose CR, and glucose t½ at T1 (early lactation). The CR of glucose was greater for NZ compared to NA cows at T2, while t½ for glucose tended to be greater for NA cows at that time. There were no differences between the strains at T2 in insulin AUC, glucose AUC or NEFA AUC. A significant effect of time was observed, where the insulin response to glucose infusion was greater at T2 versus T1

Table 3.5 *Effect of cow strain on responses to intravenous glucose tolerance tests in early and mid-lactation*

	T1[1]		T2[1]			*P -values*		
	NA[2]	NZ[2]	NA	NZ	S.E.D[3]	S[4]	T[4]	S x T
Glucose AUC[5]	254	262	258	227	22.1	0.52	0.27	0.18
Insulin AUC	1617[A]	2195[A]	3289[B]	3368[B]	412	0.42	<0.01	0.55
NEFA AUC	-4.62	-4.88	-3.17	-2.45	1.52	0.81	0.11	0.67
t ½ glucose[6]	36.9	36.6	41.1	34.4	3.59	0.19	0.71	0.22
CR glucose[7]	1.78	1.93	1.66[a]	2.04[b]	0.17	0.02	0.96	0.38

[1] T1 = 32 ± 0.48 (mean ± s.e.m) days in milk; T2 = 137 ± 2.44 days in milk
[2] NA = North American Holstein Friesian; NZ= New Zealand Holstein Friesian
[3] SED = Standard error of difference
[4] S = Strain, T = time period
[5] AUC = Area under the response curve. Expressed in units of Mmol*min/L for glucose and NEFA and µIU*min/mL for insulin
[6] t ½ = glucose half-life (min)
[7] CR = clearance rate (%/min)
[ABab] Means having different upper case superscripts differ significantly within strain across time period ($P < 0.05$). Means having different lower case superscripts differ significantly within time period across strain ($P < 0.05$)

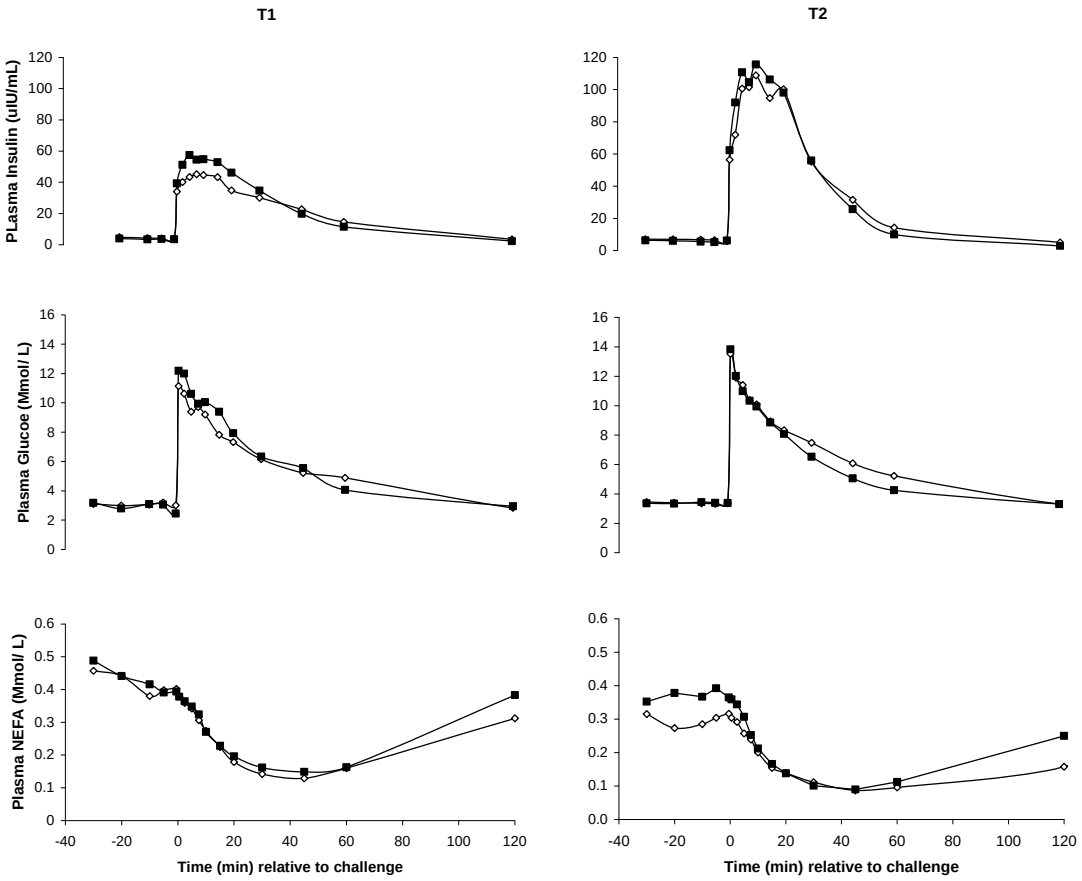

Figure 3.2 Responses of the NA (◊) and NZ (■) strains of Holstein-Friesian cattle to intravenous glucose tolerance tests at 2 stages of lactation (T1 = 32 ± 0.48 (mean ± s.e.m) days in milk; T2 = 137 ± 2.44 days in milk). Cows were infused with 1.5g glucose (50% wt/vol)/kg of $BW^{0.75}$ via a jugular catheter. Areas under the response curve and statistical analysis are outlined in Table 3.5.

Epinephrine Challenge

Plasma concentrations of glucose were acutely elevated by intravenous infusion of epinephrine at T1 and T2 (Table 3.6; Figure 3.3). Across the two time-points, the NA strain had a greater glucose response to epinephrine.

Table 3.6 *Effect of cow strain on responses to intravenous epinephrine challenges in early and mid-lactation*

	T1[1]		T2[1]			*P-values*		
	NA[2]	NZ[2]	NA	NZ	S.E.D[3]	S[5]	T[5]	S x T
Insulin AUC[4]	585	339	753	463	172	0.07	0.17	0.83
Glucose AUC	43.7[A]	36.8[A]	32.2[B]	26.1[B]	4.11	0.04	<0.01	0.88
NEFA AUC	6.11	5.03	5.14	5.44	0.93	0.56	0.65	0.27

[1] T1 = 32 ± 0.48 (mean ± s.e.m) days in milk; T2 = 137 ± 2.44 days in milk

[2] NA = North American Holstein Friesian; NZ= New Zealand Holstein Friesian

[3] SED = Standard error of difference

[4] AUC = Area under the response curve. Expressed in units of Mmol*min/L for glucose and NEFA and µIU*min/mL for insulin

[5] S = Strain, T = time period

[AB][ab] Means having different upper case superscripts differ significantly within strain across time period ($P < 0.05$). Means having different lower case superscripts differ significantly within time period across strain ($P < 0.05$)

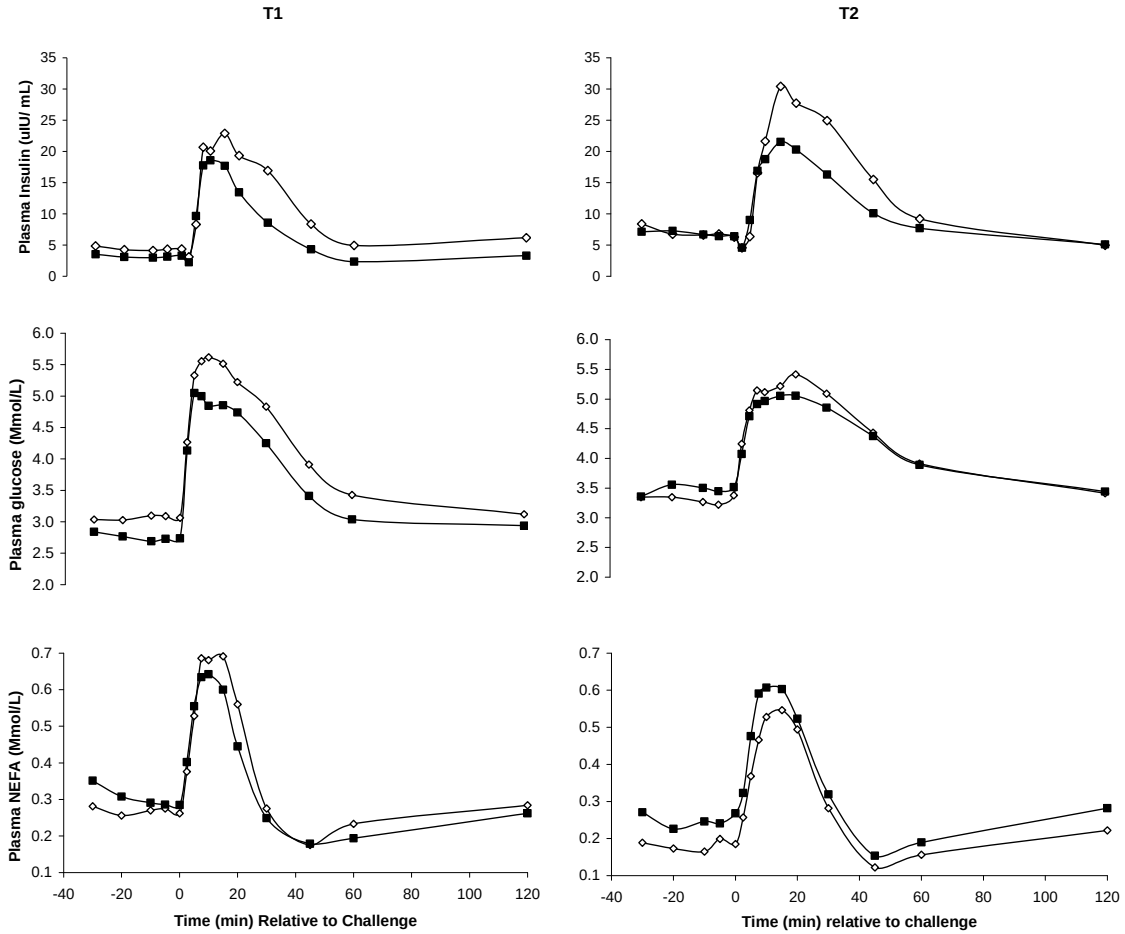

Figure 3.3 Responses of the NA (◊) and NZ (■) strains of Holstein-Friesian cattle to intravenous epinephrine challenges at 2 stages of lactation (T1 = 32 ± 0.48 (mean ± s.e.m) days in milk; T2 = 137 ± 2.44 days in milk). Epinephrine acid tartrate (1.4 µg/kg BW) was administered via a jugular catheter. Areas under the response curve and statistical analysis are outlined in Table 3.6.

Insulin Challenge

The NA cows had a greater NEFA AUC compared to NZ cows at T1 (P = 0.02), whereas the strains had a similar (P = 0.51) AUC at T2 (Figure 3.4; Table 3.7). The NEFA AUC in response to the insulin challenge was greater in both strains at T1 versus T2 (Time effect, P < 0.01).

Table 3.7. *Effect of cow strain on responses to intravenous insulin tolerance tests in early and mid-lactation*

	T1[1]		T2[1]		*P-values*			
	NA[2]	NZ[2]	NA	NZ	S.E.D[3]	S[4]	T[4]	S x T
NEFA AUC[5]	-2.29^{Aa}	-1.38^{Ab}	-0.69^{B}	-0.42^{B}	0.40	0.01	<0.01	0.33
t ½ insulin	6.27	5.84	5.99	5.04	0.65	0.20	0.18	0.51
CR insulin	6.53	6.82	6.27	5.50	0.64	0.59	0.08	0.24
Glucose AUC	-17.0	-12.7^{A}	-17.7	-21.8^{B}	2.68	0.95	0.02	0.04

[1] T1 = 32 ± 0.48 (mean ± s.e.m) days in milk; T2 = 137 ± 2.44 days in milk

[2] NA = North American Holstein Friesian; NZ= New Zealand Holstein Friesian

[3] SED= Standard error of difference

[4] S = Strain, T = time period

[5]AUC= Area under the response curve. Expressed in units of Mmol*min/L

^{ABab} Means having different upper case superscripts differ significantly within strain across time period (P < 0.05). Means having different lower case superscripts differ significantly within time period across strain (P < 0.05).

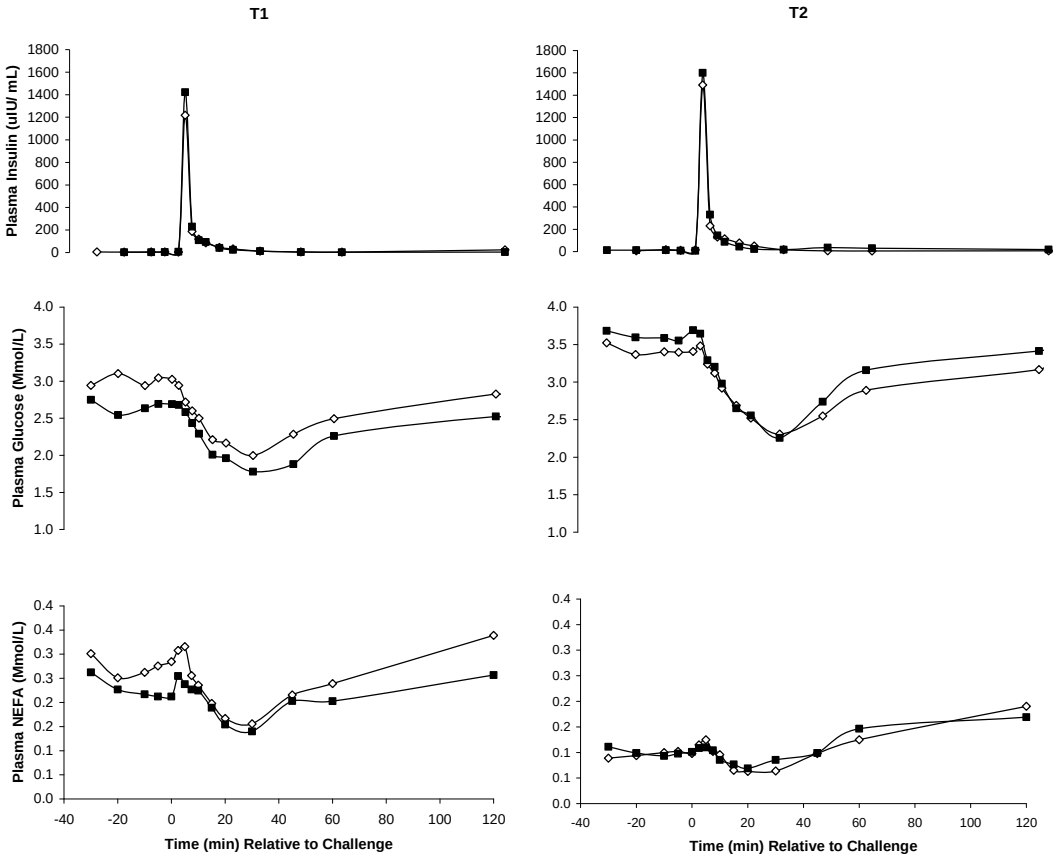

Figure 3.4 Responses of the NA (◊) and NZ (■) strains of Holstein-Friesian cattle to intravenous insulin challenges at 2 stages of lactation (T1 = 32 ± 0.48 (mean ± s.e.m) days in milk; T2 = 137 ± 2.44 days in milk). Cows were infused with 1.0 µg/kg BW of bovine pancreatic insulin, administered via a jugular catheter. Areas under the response curve and statistical analysis are outlined in Table 3.7.

Effect of cow strain on liver somatotropic axis gene expression

These results are described in detail by (McCarthy et al., 2009). The results of the real-time RT-PCR analysis for independent time and strain effects are summarized in Table 3.8. IGF-1 gene expression was affected by cow genotype, with mRNA abundance 1.6 times greater in the NZHF strain. The mRNA abundance of SOCS-3 was also 1.6 times greater for the NZ strain, and abundance of ALS mRNA tended to be greater for the NZ strain.

Across strains, a number of genes were upregulated or downregulated at d 150 compared to d 35 postpartum. Upregulated genes included IGF-1R, ERα, and suppressor of cytokine signalling-3 (SOCS3), and trends for increased mRNA abundance were observed for ALS and IGF-1. Downregulated genes included GHR1A, IGF-2R, IGFBP-1, IGFBP-2, IGFBP-3, IR-A, IR-B, hepatocyte nuclear factor-4 alpha (HNF-4α), and a trend for reduced mRNA abundance for GHRtot was observed.

Significant ($P < 0.05$) strain by time interactions, were observed for IGFBP-4, IR-A and SOCS3 genes (Table 3.9). IGFBP-4 mRNA abundance increased between d 35 and d 150 postpartum in NA cows, but declined during this interval in NZ cows. IR-A mRNA abundance declined in NA cows between d 35 and d 150, but remained stable in NZ cows. SOCS3 mRNA abundance increased in both strains between d 35 and d 150, but the relative increase was much greater in the NA strain compared to the NZ strain. A trend for a strain by time interaction was observed for IGFBP-6, where mRNA abundance increased between d 35 and d 150 for NZ cows, but remained constant in NA cows. Conversely, IGFBP3 tended to decrease in the NA cows between d 35 and d 150, but remained stable in NZ cows.

Table 3.8. Real-time RT-PCR analysis for independent strain and time effects. Real Time RT-PCR Values are back-transformed least square means followed by the 95% confidence limits and are expressed as pg per μg of reversed transcribed RNA.

				DAYS		
Gene	**NAHF**	**NZHF**	**P**	**35**	**150**	**P**
IGF-1	0.32 (.23-	0.51 (.37-	<	0.32 (.22-.47)	0.51 (.34-.76)	0.15
IGF-1R	0.004 (.004-	0.005	0.30	0.004 (.004-.005)	0.006 (.005-	<
GHR (tot)	0.51 (.37-	0.47 (.33-	0.71	0.6 (.42-.88)	0.4 (.27-.58)	0.15
GHR1A	0.03 (.02-	0.03 (.02-	0.65	0.04 (.03-.05)	0.02 (.02-.03)	<
IGFBPALS	0.06 (.04-	0.1 (.07-	0.60	0.06 (.04-.09)	0.1 (.07-.16)	0.11
IGF-2	15.0 (11.9-	14.6 (11.5-	0.85	14.9 (11.7-18.9)	14.7 (11.5-	0.95
IGF-2R	0.14 (.13-	0.14 (.13-	0.97	0.16 (.15-.18)	0.13 (.12-.14)	<
IGFBP-1	4.3 (2.8-6.8)	4.3 (2.7-	0.98	7.7 (4.9-12.3)	2.4 (1.5-3.8)	<
IGFBP-2	3.4 (2.6-4.4)	3.1 (2.3-	0.62	5.5 (4.0-7.5)	1.9 (1.4-2.6)	<
IGFBP-3	0.48 (.37-	0.5 (.39 -	0.82	0.64 (.49 -.84)	0.38 (.29 -.5)	<
IGFBP-4	0.25 (.21-	0.24 (.2-	0.85	0.25 (.2-.3)	0.24 (.2-.3)	0.93
IGFBP-5	0.23 (.2-.27)	0.23 (.2-	0.98	0.2 (.18-.25)	0.25 (.21-.3)	0.21
IGFBP-6	0.003 (.002-	0.003	0.79	.003 (.003-.005)	0.004 (.003-	0.20
ER-α	0.41 (.37-	0.46 (.41-	0.22	0.36 (.31-.4)	0.54 (.47-.61)	<
IR-A	0.03 (.03-	0.02 (0.02-	0.18	0.031 (.03-.04)	0.025 (.02-	<
IR-B	0.008 (.006-	0.009	0.37	0.011 (.008-.015)	0.006(.004-	<
HNF4-α	0.3 (.26-.35)	0.32 (.27-	0.56	0.47 (.4-.54)	0.2 (.18-.24)	<
SOCS3	0.5 (.41-.6)	0.8 (.66-	<	0.4 (.33-.49)	0.99 (.8-1.23)	<
JAK2	0.0004	0.0005	0.28	0.0005 (.0003-	0.0004	0.45
STAT5b	0.1 (.095-	0.1 (.09-	0.51	0.11 (.1-.12)	0.1 (.09-1.1)	0.29

Abbreviations: Insulin-like growth factor 1 and 2 (IGF-1 and 2); Insulin like growth factor binding proteins 1-6 (IGFBP-1/2/3/4/5/6) and acid labile subunit (IGFBP-ALS); growth hormone receptor (GH-R_{tot}) and 1A variant (GH-R1A); estrogen receptor α (ERα); insulin receptor type A/B (IR-A/B); janus activated kinase 2 (JAK2); hepatocyte nuclear factor 4α (HNF4α); suppressor of cytokine signalling-3 (SOCS3); signal transducer and activator of transcription 5b (STAT5b).

Table 3.9. Strain by time (S*T) interaction effects with respective P-values, where NA 35 and NA 150 represent NAHF strain at d 35 and d 150 respectively and similarly NZ 35 and NZ 150 denote NZHF at d 35 and d 150 respectively. Values are back-transformed least square means followed by the 95% confidence limits and are expressed as pg per µg of reversed transcribed RNA.

Gene	S*T NA 35	S*T NA 150	S*T NZ 35	S*T NZ 150	P value
IGF-1	0.25 (.14-.42)	0.41 (.24-.71)	0.42 (.26-.69)	0.62 (.38-1.04)	0.79
IGF-1R	0.003 (.003-.005)	0.006 (.004-.008)	0.004 (.003-.005)	0.007 (.006-.01)	0.52
GHR (tot)	0.61 (.36-1.03)	0.43 (.25-.72)	0.59 (.37-.95)	0.37 (.23-.6)	0.80
GHR1A	0.03 (.02-.05)	0.02 (.02-.03)	0.04 (.02-.05)	0.02 (.02-.03)	0.58
IGFBPALS	0.05 (.03-.09)	0.08 (.04-.13)	0.07 (.04-.12)	0.14 (.08-.24)	0.58
IGF-2	17.2 (11.9-24.7)	13.1 (9.2-18.6)	12.9 (9.3-17.9)	16.5 (11.6-23.3)	0.15
IGF-2R	0.16 (.14-.19)	0.12 (.11-.14)	0.16 (.14-.18)	0.13 (.11-.15)	0.50
IGFBP-1	6.4 (3.4-12.2)	2.9 (1.5-5.6)	9.4 (5.3-16.7)	2.0 (1.1-3.6)	0.12
IGFBP-2	6.0 (3.8-9.2)	1.9-(1.2-3.0)	5.1- (3.4-7.4)	1.9 (1.3-2.8)	0.68
IGFBP-3	0.74 (.5-1.1)	0.32 (.22-.46)	0.56 (.39-.8)	0.45 (.31-.66)	0.11
IGFBP-4	0.3 (.22-.4)	0.21 (.16-.27)	0.2 (.16-.27)	0.29 (.22-.38)	< 0.05
IGFBP-5	0.2 (.16-.27)	0.26 (.2-.33)	0.22 (.17-.27)	0.25 (.19-.32)	0.74
IGFBP-6	0.003 (.002-.005)	0.003 (.002-.004)	0.002 (.002-.003)	0.004 (.003-.006)	0.06
ER-α	0.34 (.28-.41)	0.51 (.42-.61)	0.37 (.31-.45)	0.57 (.47-.68)	0.93
IR-A	0.04 (.03-.06)	0.02 (.02-.03)	0.03 (.02-.03)	0.02 (.02-.03)	< 0.05
IR-B	0.01 (.007-.016)	0.006 (.004-.008)	0.011 (.008-.017)	0.007 (.005-.01)	0.71
HNF4-α	0.25 (.19-.34)	0.2 (.16-.25)	0.49 (.4-.6)	0.2 (.17-.25)	0.62
SOCS3	0.25 (.19-.34)	0.97 (.72-1.3)	0.64 (.48-.84)	1.0 (.75-1.4)	< 0.01
JAK2	0.0003 (.0002-.0006)	0.0004 (.0002-.0007)	0.0006 (.0004-.001)	0.0004 (.0002-.0007)	0.34
STAT5b	0.12 (.095-.13)	0.1 (.09-.11)	0.1 (.09-.12)	0.1 (.09-.11)	0.64

Abbreviations: Insulin-like growth factor 1 and 2 (IGF-1 and 2); Insulin like growth factor binding proteins 1-6 (IGFBP-1/2/3/4/5/6) and acid labile subunit (IGFBP-ALS); growth hormone receptor (GH-R_{tot}) and 1A variant (GH-R1A); estrogen receptor α (ERα); insulin receptor type A/B (IR-A/B); janus activated kinase 2 (JAK2); hepatocyte nuclear factor 4α (HNF4α); suppressor of cytokine signalling-3 (SOCS3); signal transducer and activator of transcription 5b (STAT5b).

Embryo quality

These results are described in detail by (de Feu et al., 2008). The total number of CL and structures recovered are summarized in Table 3.10. The proportion of transferable embryos (morula and blastocyst) and the proportion of blastocysts recovered were higher for the NZ cows compared to the NA cows.

Table 3.10. The effect of strain of Holstein-Friesian cow on embryo recovery, quality and stage of development on Day 7 post AI[1]

	NZ	NA	P-value
No. of cows	10	10	
No. of flushes recorded	15	14	
No. of corpora lutea (CL)	180	141	
No. of structures recovered	72	59	
Recovery rate[2]	0.40	0.42	
	Proportion (total no.)	Proportion (total no.)	
Transferable embryos	0.91 (63)	0.58 (42)	<0.01
Blastocysts	0.53 (45)	0.17 (10)	0.01
Morulae	0.37 (18)	0.41 (32)	0.7
Transferable-Blastocysts[3]	0.58	0.29	0.099
Transferable-Morula[4]	0.42	0.71	0.099
Non transferable structures	0.09 (9)	0.42 (17)	0.01
Degenerative embryos	(1)	(5)	
Unfertilised oocytes	(8)	(9)	
Empty zona's	(0)	(3)	

[1]NZ = New Zealand Holstein-Friesian; NA = North American Holstein-Friesian
[2]Recovery rate = no. of structures recovered/no. of CL
[3]The proportion of transferable embryos that were at the blastocyst stage
[4]The proportion of transferable embryos that were at the morula stage

3.1.4 Discussion and Conclusions

This study clearly identified underlying physiological differences between the NA and NZ strains of Holstein Friesian that are linked to the observed differences in milk production, nutrient partitioning, and reproductive performance. Peak daily milk yield was higher for the NA strain as had been reported previously (Horan et al., 2005a), however peak yield did not differ between the groups when expressed as SCM. Interestingly, milk solids production per kg DMI or per kg BW were not different between the strains. It can therefore be concluded that the strains experienced a comparable magnitude of milk energy demand at peak SCM production. The NA strain had approximately 1.5 kg per day greater DMI, equivalent to 1.26 UFL of NE intake per day, compared to the NZ strain from wk 1-20 *post partum*. However, the daily energy requirements for milk and maintenance during this time were approximately 1.0 UFL and 0.30 UFL greater for the NA strain respectively, resulting in similar EB profiles for the strains. Consistent with the EB

results, the BCS profiles of the strains were not different for weeks 1-20 of lactation. The profiles subsequently diverged however, as the NZ cows began to increase BCS while the NA cows failed to gain BCS. Similarly (McCarthy et al., 2007b) reported no difference in the rate of BCS change between NA and NZ cows during early lactation, but a greater rate of BCS accretion post nadir for NZ cows. The SCM yield of the NA cows was greater than NZ cows from approximately wk 20 until the end of lactation, coincident with the divergence of the BCS profiles of the strains.

The NA cows had increased plasma insulin concentrations during the transition period, despite the similar calculated EB of the strains at this time. The increased plasma glucose concentration for the NA cows was consistent with the observed differences in insulin concentrations. The temporal patterns of plasma IGF-I concentration observed were similar to previous reports, with a decline at parturition and a gradual increase thereafter (Bell et al., 2000). While the strains had similar plasma IGF-I profiles during the transition period, plasma IGF-I was higher for NZ cows from approximately d 30 of lactation. This occurred despite the similar EB profiles between the strains, and the higher plasma insulin concentrations in the NA cows.

Intravenous infusion of glucose resulted in an acute increase in plasma insulin concentrations. The insulin response was similar for both strains at each time period. It is well documented that peripheral tissue responses to insulin are attenuated during early lactation. These tissue-specific adaptations are collectively described as '*insulin resistance*', and include reduced stimulation of lipogenesis in adipose tissue and whole-body oxidation of glucose (Bauman, 2000). The net effect is to increase the availability of glucose in support of mammary glucose requirements. Glucose response to the glucose challenge was measured as area under the response curve (AUC), half-life (t½) and clearance rate (CR). While no strain differences were apparent for measures of glucose response at T1, it was found that glucose had a greater CR and shorter t½ in NZ cows at T2. This indicates greater insulin responsiveness in the NZ cows compared to NA cows in mid-lactation, as insulin resistance is associated with slower CR, longer t½, and a greater AUC for glucose at similar insulin concentrations (Mertz, 1993). Greater insulin responsiveness in the NZ cows in mid-lactation is consistent with accumulation of more body reserves from mid-lactation to the end of lactation.

There were significant interactions between strain and time period for glucose AUC in response to the insulin challenge, and also for basal glucose concentration during the insulin challenge. The strain with the greater basal glucose concentration had the greater AUC in response to insulin at both time periods, indicating that the magnitude of the response to insulin was dependent on basal concentration.

The effect of epinephrine treatment on adipose tissue mobilization may be determined from the plasma NEFA response profile. The elevated concentrations of plasma NEFA after an epinephrine challenge represents mobilization of fatty acids, the net effect of β-adrenergic induced lipolysis minus fatty acid reesterification. The NEFA response (i.e. mobilization) to the epinephrine challenges was not affected by strain or stage of lactation. Epinephrine stimulated an acute increase in circulating glucose concentrations, presumably reflecting increased hepatic glycogenolysis and reduced glycogenesis in both strains. The glucose response to epinephrine infusion was greater in NA cows compared to NZ cows in the current study.

A marked increase in the liver mRNA abundance of IGF-1 was observed in the NZHF compared to the NAHF, and this coincided with a numerical but non-significant increase in ALS mRNA abundance in the NZHF strain. Interestingly,

signalling molecules associated with activating IGF-1 gene transcription were not increased in the NZHF strain, but the negative feedback molecule SOCS3 was increased in the NZHF strain compared to the NAHF strain. Across strains, altered mRNA abundance of IGFBP-1, IGFBP-2, GHR1A as well as IGF-1 and the ALS were observed between d 35 and d 150. The mRNA abundance of transcriptional regulators and signalling genes were also affected by stage of lactation. These findings are critical to the advancement of our understanding of the biological mechanisms regulating altered nutrient partitioning during NEB and how this, in turn, may be influenced by genetic selection.

A greater proportion of the embryos recovered from the NZ cows were transferable compared to the NA cows. Furthermore, of the transferable embryos recovered the proportion at the blastocyst stage was higher in the NZ cows. This indicates that the factors responsible for the previously reported differences in conception rate between these strains are manifest as early as 7 days after insemination. It is generally accepted that morulae and blastocysts are equally likely to establish a pregnancy in multiple ovulation and embryo transfer programmes. However, it was previously reported that the transition from morula to blastocyst represents a major area of embryo loss in sub-fertile repeat breeder dairy cows (Ayalon, 1978). The results of the current study indicate that the NZ strain makes the transition from morula to blastocyst earlier than their NA counterparts. Previous studies have examined early embryo mortality in lactating dairy cows, and concluded that embryo mortality could be detected as early as day 5 after oestrus (Wiebold, 1988). Our results indicate that marked differences between NZ and NA cows in the proportion of transferable embryos could be detected by day 7 after oestrus. The greater proportion of transferable embryos yielded by the NZ cows is consistent with reports of superior pregnancy rates and reduced numbers of non-pregnant cows at the end of the breeding period (Horan et al., 2005b, Horan et al., 2004).

Collectively, the results observed in the current study indicate a divergence in the prioritisation of nutrient use between the strains from the time of peak SCM yield through the end of the study period; the NA cows continued to preferentially partition nutrients to mammary milk synthesis, whereas the NZ cows commenced partitioning energy to replenishing body reserves. This long-term type of physiological regulation is consistent with the concept of homeorhesis, defined as the coordinated control in metabolism of body tissues necessary to support a physiological state (Bauman and Currie, 1980). It is well established that greater BCS improves likelihood of establishing a successful pregnancy in lactating dairy cows (Berry et al., 2003b), and thus it is plausible that the inherent genetic drive of the NZ strain to commence partitioning energy to BCS gain after peak SCM yield could be responsible for the superior reproductive performance of this strain compared to their NA counterparts on pasture-based systems of production. Identification of specific causative mutations associated with compromised reproductive performance (e.g., fertilization failure, early embryo mortality, late embryo mortality etc.) is an important area of research; incorporation of favourable mutations into progeny testing and genetic improvement programmes could have beneficial effects on dairy cow reproductive performance.

3.2 Experiment 2. Examining the effect of dry period duration and dietary energy density during the early postpartum period on energy balance and fertility.

3.2.1 Introduction

The onset of lactation in dairy cattle causes a dramatic increase in mammary glucose requirements, and marked changes in whole body metabolism are required to accommodate these needs. Following parturition, high-producing dairy cows typically experience a variable period of negative energy balance (NEB), as DMI is inadequate to fully meet the rising energetic requirements of milk production. The severity and duration of NEB experienced in early lactation affects the postpartum interval to first ovulation and has a detrimental effect on subsequent likelihood of conception (Butler and Smith, 1989, Villa-Godoy et al., 1988). A delay in the onset of ovulatory ovarian activity limits the number of oestrous cycles prior to breeding, reducing the likelihood of conception and increasing the calving to conception interval (Butler, 2003). Nutritional approaches to overcome early lactation NEB have been largely unsuccessful. This is primarily due to the inherent drive to produce additional milk in response to additional nutrient intake — the hallmark of the modern Holstein-Friesian dairy cow. The metabolic and endocrine milieu that ensues during NEB is antagonistic to resumption of ovulatory ovarian activity (Butler et al., 2006), resulting in anoestrus and reduced conception rates.

There has been substantial interest recently in decreasing the duration of the dry period. Most literature indicates that a dry period of 40-60 days is necessary to achieve maximum milk production during the following lactation (Bauman and Currie, 1980). Decreasing the length of the dry period reduces the capacity of the mammary gland to secrete milk in the subsequent lactation (Rastani et al., 2005). Cows that were assigned to a treatment with no planned dry period (actual mean dry period = 6.3 ± 1.7 days) had less severe NEB postpartum compared to cows with a traditional dry period (actual mean dry period = 62 ± 1.9 days); the improvement in postpartum energy balance was achieved by a combination of reduced milk production and greater dry matter intake (Rastani et al., 2005). In addition, the onset of postpartum cyclicity was advanced and indicators of reproductive performance were improved in the cows on the short dry period treatment compared to cows on the traditional dry period treatment (Gumen et al., 2005). The current study was carried out to examine the effect of dry period duration and dietary energy density on milk production, DMI, energy balance, metabolic status and indicators of reproductive efficiency. Specifically, postpartum follicular dynamics and reproductive hormones profiles were examined to assess the effects of dry period duration and feeding level on resumption of cyclicity.

3.2.2 Materials and Methods

This experiment was a completely randomized block design with a 2 × 2 factorial arrangement of treatments. Forty mature Holstein-Friesian cows were blocked on the basis of expected calving date, previous lactation yield, bodyweight and body condition score (BCS), and were assigned to one of two dry period treatments (standard 8 week dry period (**SDP**) or no planned dry period (**NDP**)) and one of two dietary energy density treatments (standard TMR (**STMR**) or high quality TMR (**HTMR**). Cows assigned to SDP were fed ad libitum grass silage prepartum, and either the STMR or HTMR postpartum. Cows assigned to NDP were fed their

assigned STMR or HTMR both pre- and postpartum. Actual dry period lengths (mean ± SEM) were 62.1 ± 1.9 days and 6.3 ± 1.7 days for cows on the SDP and NDP treatments, respectively. Two cows were dropped from the SDP treatment and two cows were dropped from the NDP treatment due to their dry periods being too short (SDP), too long (NDP), or illnesses unrelated to the study. Cows were housed in free stall housing from 4 weeks before expected calving until 12 weeks postpartum. If prepartum daily milk yield dropped below 2 kg/day for cows on the NDP treatments, milking was discontinued for the remainder of the prepartum period. Bodyweight (BW) and body condition score (BCS) were measured weekly by the same technician, and health records were recorded for each animal pre and postpartum.

Daily measurements of dry matter intake were recorded using the Griffith-Elder MealMaster system (Griffith Elder & Co Ltd, Suffolk, UK). Energy Balance (EB) was calculated as previously described. The ingredient and nutrient composition of the TMR diets is indicated in Table 3.11.

Table 3.11: Ingredients and nutrient compositions of Standard TMR and High TMR feed.

Diet ingredients	Standard TMR (%)	High TMR (%)
Grass Silage	50	20
Barley	35	24
Brewers grains and beet pulp mix	-	30
Soya bean meal	13	10
Soya hulls	-	15
Vitamins and minerals[1]	2	1
Nutrient composition (DM basis)		
DM (g/kg)	892	904
Net energy (UFL/kg DM)	0.96	1.02
Ash (g/kg DM)	71	65
Crude protein (g/kg DM)	164	178
NDF (g/kg DM)	385	415
Oil (Acid Hydrolysis) %	2.9	3.3

[1]Vitamin and mineral mix: 15g/kg DiCa P, 8g/kg Limestone Flour, 5g/kg Salt, 2.5 g/kg Cal-Mag, 80gm Manganous Oxide, 200gm Copper Sulphate, 125gm Zinc Oxide, 18gm Potassium Iodate, 20gm Soduim Selenite (4.6%), 10gm Cobalt Sulphate, 8MIU/t vitamin A, 2MIU/t vitamin D3, 15,000iu/t vitamin E.

Blood Sampling

Blood samples were collected 3 times/week (M, W, F) for the final 3 weeks prepartum, daily for the first 28 days postpartum and once per fortnight thereafter until day 84 of lactation. Plasma samples were analysed for glucose, non-esterified fatty acids (NEFA), urea and beta-hydroxybutyrate (BHBA), insulin, and IGF-I.

Circulating FSH concentrations were analysed in daily plasma samples collected from the day of parturition until 10 days in milk.

Postpartum Ultrasound Evaluation and Reproductive Management

Ovarian follicular activity was examined by linear array ultrasonography (Aloka 900; 7.5-MHz transrectal transducer, Aloka Ltd., Tokyo, Japan.) thrice weekly (M, W, F) beginning on day 8-10 postpartum and continuing until first ovulation. Initiation of breeding commenced on a calendar mating start date (27th of November 2005). Pregnancy status was determined using transrectal ultrasonography on day 30 - 36 and day 60 - 66 post AI. Visualization of a fluid filled uterine horn with the presence of a viable embryo was used as positive indication of pregnancy.

Statistical Analysis

Milk yield, milk composition, SCM, bodyweight, DMI, EB and FSH data were analysed as repeated measures using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with an autoregressive covariance structure. The fixed effects included in the model were dry period, feeding level, time (day or week), and all possible interactions. Block was included as a random effect. Conception and pregnancy rate data were analysed using Fisher's exact test. The metabolite and insulin data for each cow were divided into two time points; the transition period lasted from 3 weeks before parturition to 3 weeks postpartum and the post-transition period lasted from 4 weeks to 12 weeks postpartum. The peak circulating concentration of FSH during the first 10 days postpartum was analysed using the MIXED procedure of SAS. Fixed effects included in the model were dry period, feeding level and the interaction between dry period and feeding level. Block was included as a random effect.

3.2.3 Results

The results of this study are described in detail by de Feu et al. (2009), and only the major finding will be reported here. There was no interaction between dry period duration and feeding level for most variables, so both factors are presented separately. Milk production data are summarized in Table 3.12. Solids corrected milk yield was reduced by 19.5% in cows assigned to the NDP treatment during the first 12 weeks of lactation compared to cows assigned to the SDP ($P = 0.004$). The yield of fat and protein was reduced by 11.3% in the NDP cows compared with the SDP cows ($P = 0.02$). Cows assigned to the NDP treatment had greater ($P = 0.001$) milk protein concentration compared to cows assigned to the SDP.

Milk yield was 16.9% greater ($P = 0.02$), and the yield of fat and protein was increased by 12.7% ($P = 0.02$), in cows fed HTMR compared to those fed the STMR. Milk fat concentration was significantly reduced ($P < 0.001$) for cows on the HTMR diet, resulting in an increase in SCM yield of only 3.1% compared to cows on the STMR diet.

Table 3.12. *The effect of dry period length and dietary energy density on milk production and composition for weeks 1 to 12 of lactation*

	Dry Period		Feeding Level		SEM	*P - value*		
	SDP	NDP	STMR	HTMR		DP[1]	FL[2]	DP×FL
SCM[3] (kg/d)	28.7	23.1	25.5	26.3	1.3	0.004	0.67	0.6
Milk Yield(kg/d)	29.4	24.6	24.9	29.1	1.50	0.01	0.02	0.4
Fat (g/kg)	40.0	41.4	42.8	38.6	1.10	0.2	<0.001	0.8
Protein (g/kg)	33.7	37.0	34.5	36.2	0.67	0.001	0.081	0.06
Lactose (g/kg)	46.8	47.0	47.0	46.8	0.33	0.5	0.5	0.3
F&P[4] (kg/d)	2.13	1.89	1.89	2.13	0.10	0.02	0.02	0.4
Energy[5](UFL/d)	12.8	10.4	11.2	12.0	0.57	<0.001	0.15	0.9

[1]DP = dry period duration
[2]FL = feeding level
[3]SCM = solid corrected milk yield
[4]F&P = fat and protein yield
[5]Milk energy output

Dry Matter Intake, Energy Balance and Body Condition Score

The DMI, EB and BCS data are summarized in Table 3.13. Cows assigned to the NDP treatment had greater pre-partum DMI compared to cows assigned to the SDP treatment, but there was no difference in postpartum DMI. Mean daily energy balance was greater in cows assigned to the NDP treatment compared to cows on the SDP treatment during the postpartum period. The energy balance nadir was lower and the mean duration from parturition to return to zero energy balance was longer for cows on the SDP treatment compared to the NDP treatment. Cows assigned to the NDP treatment had greater postpartum BCS compared to SDP treatment cows.

There was no difference in DMI between cows assigned to STMR compared to those assigned to HTMR during weeks 1 to 4 (P = 0.8), but during weeks 5 to 12 cows assigned to the HTMR had increased DMI compared to those on the STMR diet (P = 0.01). There was no difference in calculated EB between cows on the HTMR and STMR diets during either weeks 1 – 4 or weeks 5 – 12. The EB nadir tended to be lower (P = 0.07) and the duration from parturition to return to zero EB tended to be longer (P = 0.08) for cows on the STMR diet compared to cows on the HTMR diet. There was no effect of diet on BCS during weeks 1 – 4 postpartum, but cows fed the HTMR had greater BCS during weeks 5 – 12 compared to cows fed the STMR (P = 0.01).

Table 3.13. *The effect of dry period length and dietary energy density on dry matter intake, body condition score and energy balance for weeks 1 to 4 and 5 to 12 of lactation.*

	Dry Period		Feeding Level		SEM	*P - value*		
	SDP	NDP	STMR	HTMR		DP	FL	DP×FL
EB wks 1 to 4 (UFL)	1.92	1.61	0.83	0.53	0.80	<0.001	0.15	0.3
EB wks 5 to12 (UFL	0.74	2.41	1.23	1.91	0.53	0.02	0.3	0.9
DMI wk -6 to 0	9.9	15.5	13.0	12.4	0.59	<0.001	0.54	0.4
DMI wk 1 to 4	16.0	16.9	16.3	16.6	0.63	0.3	0.8	0.9
DMI wk 5 to 12	19.6	18.6	18.6	19.8	0.43	0.1	0.01	0.6
BCS wk 1 to 4	2.96	3.25	3.06	3.16	0.12	0.07	0.5	0.3
BCS wk 5 to 12	2.74	3.34	2.83	3.25	0.11	<0.001	0.01	0.6

Plasma Insulin, IGF-I and Metabolites

The cows on the NDP treatment had greater circulating concentrations of glucose (P <0.0001), insulin (P = 0.001) and IGF-I (P = 0.004) concentrations during the transition period compared to cows on the SDP treatment, whereas cows assigned to the SDP treatment had greater (P = 0.009) NEFA concentrations compared to cows on the NDP treatment (Table 3.14). During the post-transition period, cows assigned to the NDP treatment had increased circulating IGF-I concentrations compared to cows assigned to the SDP treatment (P = 0.02), whereas cows on the SDP treatment had greater circulating NEFA concentrations (P = 0.02).

During the transition period, cows fed the HTMR diet had increased concentrations of glucose compared to cows fed the STMR diet (P < 0.001) whereas cows on the STMR had significantly greater concentrations of BHBA (P < 0.001). There was no effect of dietary energy density on insulin or IGF-I during the transition period. During the post-transition period cows fed the HTMR diet had greater circulating concentrations of insulin (P = 0.015), glucose (P < 0.001) and urea (P < 0.001) compared to cows on the STMR diet. Cows on the STMR diet had increased circulating concentrations of NEFA (P = 0.04) and BHBA (P < 0.001) compared to cows fed the HTMR diet during the post-transition period. Cows on the HTMR diet had greater circulating IGF-I concentrations (P = 0.006) compared to cows fed the STMR diet for weeks 4 to 9 relative to parturition (Table 3.14).

Table 3.14. *The effect of dry period length and dietary energy density on the circulating metabolic hormones and metabolites from weeks -3 to 3 and from 4 to 12 relative to parturition*

	Dry Period		Feeding Level		SEM	*P - value*		
Transition period	SDP	NDP	STMR	HTMR		DP	FL	DP×FL
Glucose	3.35	3.69	3.43	3.61	0.04	<0.001	0.001	0.43
Insulin	4.37	7.94	5.35	6.97	0.62	<0.001	0.04	0.14
IGF-I[1]	110	165	131	144	13.15	0.004	0.49	0.63
NEFA	0.25	0.16	0.22	0.19	0.03	0.009	0.32	0.003
BHBA	0.49	0.47	0.58	0.38	0.03	0.5	<0.001	0.018
Urea	4.80	5.36	4.88	5.28	0.19	0.02	0.09	0.5
Post transition period								
Glucose	3.52	3.60	3.42	3.70	0.04	0.2	<0.001	0.55
Insulin	5.44	7.21	4.69	7.97	0.58	0.02	<0.001	0.75
IGF-I[2]	114	150	109	155	13.24	0.02	0.006	0.67
NEFA	0.14	0.09	0.14	0.09	0.02	0.02	0.05	0.046
BHBA	0.48	0.41	0.54	0.35	0.04	0.19	<0.001	0.12
Urea	6.09	5.89	5.38	6.59	0.18	0.36	<0.001	0.74

[1] IGF-I was measured from weeks -2 to 3 relative to parturition
[2] IGF-I was measured from weeks 4 to 9 relative to parturition

Reproductive hormones and follicular dynamics

Cows assigned to the SDP treatment had greater mean FSH concentrations during days 1 to 10 postpartum (P = 0.006) and greater peak FSH concentrations (P=0.008) compared to those assigned to the NDP treatment (Figure 3.5). Dry period duration did not affect the interval from calving until peak circulating FSH concentrations (5.1 ± 0.5 *vs.* 4.6 ± 0.4 DIM; P = 0.4, NDP *vs.* SDP, respectively). There was a weak, but statistically significant, negative correlation between the diameter of the dominant follicle at first postpartum ultrasound examination and the

days in milk when peak FSH occurred (r = -0.34; P = 0.04). Ovulation occurred later in cows assigned to the SDP treatment compared to cows assigned to the NDP treatment (16.9 ± 2.5 vs. 24.8 ± 2.6 days in milk; NDP *vs.* SDP, respectively; P = 0.02). There was no significant effect of dry period duration on calving to service interval, conception rate to first service, calving to conception interval or overall pregnancy rate (Table 3.15).

There were no differences in FSH concentrations during days 1 to 10 postpartum (0.27 *vs.* 0.27 ng/ml; P = 0.9) nor was there a difference in peak FSH concentration (0.46 *vs.* 0.45 ng/ml; P = 0.7) between cows assigned to the HTMR and STMR diets. Dietary energy density did not affect the number of days from calving until peak circulating FSH concentration (4.9 ± 0.4 *vs*.4.8 ± 0.49; HTMR *vs.* STMR, respectively). Dietary energy density did not affect the timing of the first postpartum ovulation (20.8 ± 2.7 *vs.* 20.9 ± 2.5 DIM, HTMR *vs.* STMR, respectively; P = 0.9). Cows assigned to the STMR diet tended to have a greater conception rate to first service compared to cows on the HTMR diet (50.0 vs. 21.1%; STMR *vs.* HTMR, respectively; P = 0.07) but there was no effect of feeding level on the overall pregnancy rate at the end of the breeding period (Table 3.15).

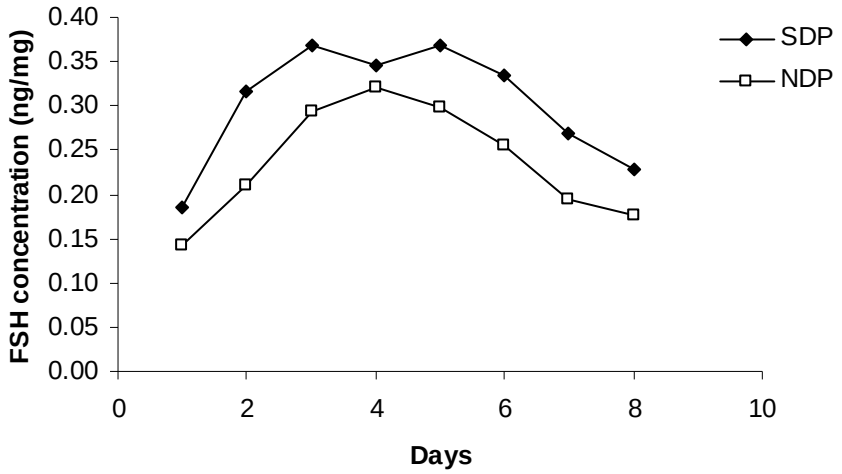

Figure 3.5. *Effect of dry period length on FSH concentration during the first 8 days postpartum. Cows assigned to the SDP treatment had significantly higher FSH concentrations than cows assigned to the NDP treatment (P = 0.007; pooled SEM was 0.034 ng/mg).*

Table 3.15. *The effect of dry period length and dietary energy density on the reproductive performance of Holstein-Friesian cows during the breeding season*

	Dry Period		Feeding Level		SEM	P value		
	SDP	NDP	STMR	HTMR		DP	FL	DP×FL
CSI (days)	78	83	77	85	7.1	0.5	0.3	0.8
CR1 (%)	29.4	38.9	50	21.1		0.6	0.07	
CCI (days)	126	112	117	120	11.1	0.3	0.8	0.9
Overall PR(%)	64.7	83.3	81.3	68.4		0.2	0.4	

CSI = calving to service interval
CR1 = conception rate to first service
CCI = calving to conception interval
PR = pregnancy rate

3.2.4 Discussion and Conclusions

The main findings from this study are that (i) omitting the dry period or feeding a higher energy density TMR resulted in improved energy balance and metabolic status, but the improvements were achieved via different mechanisms; (ii) postpartum plasma FSH concentrations and ovarian follicular development were affected by dry period duration; (iii) interval to first ovulation was reduced by omitting the dry period, but feeding a higher energy TMR had no effect. The results indicate that periparturient energy balance can be improved by altering management practices (dry period duration, feeding level). The results also suggest that improving energy balance/metabolic status *per se* will not necessarily result in an earlier onset of cyclicity.

Short dry periods reduce milk production in the subsequent lactation in a number of species including cattle, rats, and humans (Annen et al., 2004a); in cattle this occurs due to reduced mammary epithelial cell turnover and secretory capacity (Annen et al., 2007). In the current study, average daily milk production during the first 12 weeks of lactation was decreased by 16%. The cows assigned to the NDP did not enter NEB at any stage during the peripartum period and accordingly didn't lose BCS postpartum. In contrast, the cows on the SDP treatment were in NEB for an average duration of 7.2 weeks following parturition, and on average lost 0.5 units of body condition. The greater EB for the NDP cows was achieved via a reduction in milk energy output during the first 12 weeks of lactation (2.4 UFL/day) while having similar energy intake and maintenance requirements to cows on the SDP treatment. Combined, this had an overall effect of improving energy balance on the order of the reduction in milk energy output (i.e., ~2.4 UFL/day). Similar to the cows on the NDP treatment, cows assigned to the HTMR treatment didn't lose BCS during the postpartum period. However, in contrast to the NDP treatment, the HTMR diet resulted in a non-significant increase in total milk energy output, a significant increase in energy intake, with an overall effect of a non-significant improvement in calculated energy balance. Hence, reducing the duration of the dry period decreased the inherent drive to produce milk in the subsequent lactation, whereas increasing dietary energy density allowed dietary energy intake to more closely meet energy requirements, albeit at a higher daily milk yield.

In the current study, cows on the NDP treatment had higher circulating insulin, glucose and IGF-I concentrations, and lower circulating NEFA and BHBA concentrations, consistent with their superior energy balance status. (Beam and Butler, 1997) reported that circulating oestradiol concentrations during the first

postpartum follicle wave were greater and interval to first ovulation was shorter in cows with greater circulating IGF-I concentrations. The cows assigned to the NDP treatment ovulated earlier compared to cows on the SDP treatment, but there was no difference in interval to first ovulation between cows on the two dietary energy density treatments, despite the differences in plasma IGF-I concentrations.

Follicular growth and development is a result of the coordinated actions of LH and FSH on theca and granulosa cells. There is no evidence that either the timing or magnitude of the first post-partum elevation in circulating FSH concentrations are affected by energy balance or DMI. In the current study, dietary energy density had no effect on postpartum FSH concentrations, but cows on the SDP treatment had greater concentrations of FSH compared to the NDP treatment group. (Gumen et al., 2005) reported that cows assigned to the NDP treatment had lower postpartum circulating FSH concentrations compared to cows on a traditional dry period treatment on Day 6 postpartum. Those authors speculated that the cows on the NDP treatment had their postpartum FSH surge earlier than Day 6 postpartum. Our results in the current study do not support their hypothesis, as we observed no differences in the timing of the postpartum FSH surge between the NDP and SDP treatment groups. Prepartum circulating E2 concentrations are reduced by omitting the dry period (Gumen et al., 2005), and could potentially impact postpartum pituitary release of FSH.

A negative correlation was observed between the diameter of the dominant follicle at the first postpartum ultrasound examination and the day postpartum when peak FSH concentration occurred. Hence, the earlier the postpartum FSH surge occurred, the greater the size of the DF on day 8 – 10 postpartum. This is consistent with previous reports indicating that emergence of the first postpartum follicle wave is related to the timing of the postpartum FSH surge (Beam and Butler, 1997). Despite no differences being observed in the timing of peak FSH concentrations between either the dry period duration or dietary energy density treatments, the first postpartum ovulation occurred earlier for cows on the NDP treatment compared to cows on the SDP treatment, but dietary energy density had no significant effect.

Cows assigned to the NDP treatment did not enter NEB, had higher circulating insulin and IGF-I concentrations, and greater BCS compared with the cows on the SDP treatment, and accordingly the NDP treatment had an earlier onset of cyclicity compared to the SDP treatment. Interestingly, the HTMR treatment also resulted in greater circulating concentrations of insulin, IGF-I, improved BCS, and circulating metabolite concentrations indicative of superior EB. Despite this, feeding the HTMR diet did not advance the onset of cyclicity, and tended to have a negative effect on subsequent conception rate to first service.

The results indicate that omitting the dry period and feeding a higher energy density diet results in superior metabolic status. The improved bioenergetic status was achieved via contrasting mechanisms. Omitting the dry period reduced the drive to produce milk, whereas increasing dietary energy density allowed the feed consumed to more closely meet energy requirements, despite increased milk output. Omitting the dry period advanced the interval to first postpartum ovulation, whereas feeding a high energy TMR had no effect on onset of cyclicity. This study clearly shows that events during the dry period and early lactation critically affect nutrient partitioning, metabolism, milk production, and the reproductive axis.

3.3 Experiment 3. Evaluation of a role for extended lactation for cows failing to become pregnant during the breeding season.

A 305-day lactation is generally recognised as the optimum lactation length, allowing a 12 month calving interval, with 10 months of high milk production and a 2 month dry period. However, modern high producing cows continue to have high milk production at 305 days, the time of typical dry off. Recently, there has been interest in Australia and New Zealand in examining the potential role of extended lactations with a 24 month calving interval on pasture-based systems of production (Auldist et al., 2007, Kolver et al., 2007). A study was undertaken at Moorepark Research Centre to address the following questions: (i) can cows lactate for 22 months and calve every 2 years; (ii) is there a milk production response to greater concentrate supplementation during the winter period of indoor feeding; (iii) what is the milk potential of cows in the extended lactation compared to the first 305 days; (iv) would cows have good reproductive performance in the second year of lactation; and (v) would it be profitable?

MATERIALS AND METHODS

Study animals

Forty-six spring-calving cows that had failed to become pregnant during the preceding breeding season (average 2.8 services/cow; range = 1-6) were assembled from three Moorepark herds in November 2004 (average 264 days in milk; range 197-313). In Ireland, it is typical for non-pregnant spring-calving cows to be dried off and culled at this time of the year, but for the purposes of this study lactation was continued through the indoor winter feeding period and the following grazing season. Cows were milked twice daily, and milk yield was recorded at each milking during the entire lactation. Milk composition (fat, protein, lactose) was determined once per week. Body condition score (BCS) was recorded every two weeks. Cows were dried off when daily milk yield went below 5 kg or at 2 months before calving, whichever occurred first. For all cows used on the study, the mean (± SEM) proportion of Holstein genetics was 81 ± 2.4%, mean parity was 2.9 ± 0.3, mean predicted difference for milk production was +225 ± 31 kg, mean overall Economic Breeding Index (EBI) value was €42 ± 4.8, and the mean EBI subindex value for milk solids production was €37.5 ± 2.1.

Winter feeding treatment

Cows were paired on the basis of parity, days in milk, previous milk production, and BCS. They were then randomly assigned to receive either low (3 kg/cow day^{-1}) or high (6 kg/cow day^{-1}) levels of concentrate supplementation over a 13 week winter feeding period commencing in December 2004. A basal diet of 0.5 grass silage and 0.5 maize silage (dry matter basis) was offered *ad libitum* to both treatments. Cows were turned out to *ad libitum* pasture on March 31, and from then until the end of lactation all cows were offered 1 kg of concentrate per day.

Ranking based on cumulative milk production

Large variation in milk yield between cows was observed throughout lactation. When all cows had finished milking, cumulative milk solids production (CMSP) from calving to dry-off was calculated for each cow, and cows were ranked on the basis of CMSP (regardless of previous winter feeding treatment). The cows were then separated into 3 Ranks: R1 = 15 highest CMSP; R2 = 15 intermediate CMSP; and R3

= 16 lowest CMSP. The mean predicted difference for milk production was 225 ± 31 kg, 242 ± 38 kg, and 125 ± 31 kg R1, R2, and R3, respectively.

Lactation persistency

A measure of lactation persistency was calculated for all cows during the normal lactation period and during the extended lactation period. During the normal lactation period, persistency was calculated by subtracting weekly milk yield at the end of the normal period from weekly milk yield at peak, and dividing by the number of weeks between peak yield and the end of the normal period. All cows had a characteristic "second peak" during the indoor winter feeding period. During the extended lactation period, persistency was calculated by subtracting weekly milk yield at the end of the extended lactation period from weekly milk yield at the second peak, and dividing by the number of weeks between the second peak yield and the end of the extended lactation period.

Reproductive measures

The second breeding season began on April 18 and finished on July 18 2005. On average, cows were 405 days in milk (range 336 – 452) on the mating start date. Pregnancy diagnosis was carried out using transrectal ultrasonography at 30-36 and 60-66 days post AI (Aloka 900, 7.5-MHz transrectal transducer; Tokyo, Japan).

Data Handling and Statistical Analysis

All data analysis was carried out using SAS (SAS Institute Inc., Cary, NC). The daily measurements of milk yield were collapsed into weekly means, and mean daily yields of milk fat, protein, and lactose were calculated for each week. For the analysis of the effect of the winter feeding treatment on milk production, high or low winter feeding was compared using repeated measures and the MIXED procedure from the beginning until the end of the period of differential feeding (13 weeks). The model contained treatment, week, and the interaction of treatment and week as fixed effects, and block as a random effect. Week was used in the repeated statement, and an autoregressive covariance structure was used. Cumulative milk production from the beginning of the feeding treatments until dry off was calculated for each cow, and the effect of winter concentrate feeding was analyzed using PROC MIXED with treatment as a fixed effect and lactation length, parity and previous milk production from calving until initiation of winter feeding treatment used as adjustment variables. Block was included as a random effect. The milk production and BCS of the different Ranks was compared using PROC MIXED with Rank as a fixed effect, and the data was adjusted for winter feeding treatment and parity. The effects of winter feeding treatment and Rank on lactation length were compared using survival analysis (PROC LIFETEST). Differences in lactation persistency were compared using PROC MIXED. The model for winter feeding treatment contained the fixed effects winter feeding treatment and parity, and block was included as a random variable. The model for milk production rank contained the fixed effect rank, parity and winter feeding treatment.

Pearson correlation coefficients (PROC CORR) were used to evaluate the relationship between animal factors (parity [1, 2, or >2], proportion of Holstein genetics, PTA for milk yield, EBI value for milk solids production) and various descriptors of the lactation curve profile (milk yield and milk solids yield from calving until 305 DIM, milk yield and milk solids yield from 305 DIM until dry off, milk yield at 305 DIM, lactation length, peak milk yield, week of peak milk yield, and persistency [100 day

cumulative milk yield as a proportion of 305 day cumulative milk yield]). Multiple linear regression (PROC REG) and the stepwise variable selection procedure were used to generate a model containing independent variables that were most effective at predicting cumulative milk yield and cumulative milk solids yield based on data available at 305 DIM. The significance level for entry (sle) and the significance level to stay (sls) in the model were both set at 0.05.

Economic Analysis

The Moorepark Dairy Systems Model (**MDSM**) (Shalloo et al., 2004), which is a stochastic budgetary simulation model, was used to simulate the economic effect of a number of strategies involving extending the lactation of dairy cows. The model integrates animal inventory and valuation, milk production, feed requirement, land and labour utilisation and economic analysis. Biological data recorded in the two years were included in the model to determine the effect on profitability of a 12 month or a 24 month calving interval.

Land area was treated as an opportunity cost with additional land rented when required, and leased out when not required, for on-farm feeding of animals. Variable costs (fertiliser, contractor charges, medical and veterinary costs, artificial insemination, silage harvesting, pasture reseeding), fixed costs (machinery maintenance and running costs, farm maintenance, car, telephone, electricity and insurance) and prices (calf, milk, cow) were based on 2008 prices (Teagasc, 2008). The quantity of feeds consumed (grass, grass silage and concentrate) were determined by the MDSM to meet the net energy requirement for maintenance, milk production, and live-weight change (Jarrige, 1989). The key herd default parameters used in the model farm are shown in Table 1. Milk output from the farm was maximized with a limitation on land of 40 Ha, and therefore a further increase in milk production would require the purchase of additional feed.

Table 1: Assumptions used in the model farm.

	Year 1	Extended Year 2
Farm size (ha)	40	40
Reference fat (g/kg)	36	36
Gross milk price (Low) (c/kg)	22.3	22.3
Gross milk price (High) (c/kg)	30.0	30.0
Price ratio protein to fat	2.00	2.00
Replacement Heifer price (€)	1550	1550
Reference cull cow price (€)	400	750
Reference male calf price (€)	108	-
Labour cost per unit (€)	22,800	22,800
Labour requirements per cow/year (hr)	42	35
Concentrate costs (€/tonne)	250	250
Opportunity cost of land (€/ha)	375	375

Replacement heifer costs were estimated at €1,550 (all costs including capital, land and labour). All male and fifty five percent of female calves were sold at one month of age. The proportion of cows removed from the herd in each Rank accounted for cows that failed to become pregnant by the end of the breeding season as well as voluntary culling and cow mortality. Based on the different cow live weights at the

end of a normal 305 day lactation or an extended 660 day lactation, it was assumed that cull cow values were €400 and €750, respectively.

Due to the impending removal of EU milk quotas and the current uncertain future for milk price, two economic scenarios were investigated. In scenario 1 it was assumed that land was fixed at 40 Ha with the only means of increasing milk output from the farm being through increased purchased feed input at a low milk price of 22.3 c/l. In scenario 2 it was also assumed that land was fixed at 40 Ha with the only means of increasing milk output from the farm being through increased purchased feed input, but the analysis was carried out at a milk price of 30.0 c/l. The milk prices computed were based on 33 g/kg protein content and 36 g/kg fat content with a price ratio of 2:1 for protein : fat.

The analysis compared R1 (highest CMSP), R3 (lowest CMSP) and an efficient grass based spring calving system with 365 day calving interval. For R1 and R3 animals it was assumed that there was an initial 305 day lactation followed by an extended lactation period. Genetic merit for milk yield and phenotypic milk yield were greater for R1 cows compared to R3 cows. It was therefore assumed that R1 cows would have poorer reproductive performance compared to R3. Rank 1 animals were assumed to have a 40% replacement rate if forced into a 12 month calving interval, whereas the R3 group was assumed to have a lower replacement rate of 17%. All data in relation to the efficient spring calving system originated from a Holstein Friesian genotype with a New Zealand origin under a stocking rate of 2.47 cows/Ha with 350 kg of bought in supplement based on data from (McCarthy et al., 2007c). This data was based on a 5 year study carried out between 2000 and 2005 where 3 genotypes were compared across three grass-based feed systems. Over the 5 years of the study, the New Zealand animals produced 6,335 kg of milk at 4.39% fat and 3.65% protein with an overall pregnancy rate of 93%, leading to replacement rate of 17%.. When analysing the data for R1 and R3 animals in an extended two year lactation, it was assumed that there would be a culling rate of 12% in the first 305 day period followed by an additional culling rate of 20% during or at the end of the extended period for both groups of animals. The culling rate of 12% during the first 305 days was included to account for normal losses during lactation and cows that would be deemed unsuitable for extended lactation (SCC, lameness, age, etc.). The 20% culling rate at the end of the extended lactation period is based on a proportion of cows failing to establish pregnancy, in addition to some voluntary culling for the same reasons as outlined above.

The analysis compares the three groups of animals individually, firstly in a system with a 12 month calving interval, and subsequently in a system where the annualised herd effects are captured when the lactation length is extended. In this scenario it was possible to achieve a direct comparison of milk production systems where animals are in a system with a 12 month calving interval versus a system where extended lactation is employed to maintain non-pregnant animals in the herd. In order to compare R1, R3 and an Efficient Spring group in a 12 month calving interval with systems that had extended lactations it was assumed that 30% of R1 and 10% of R3 animals would have to be recycled to maintain the system. Therefore R1, R3 and the Efficient Spring system with 12 month calving intervals were compared to R1 and R3 animals in a system where 30% and 10% of the animals had a 24 month calving interval, respectively.

RESULTS

Reproductive performance during the normal and extended lactation periods

During the breeding season of the normal lactation period (Year 1), the mean interval (± SEM) from calving to first insemination was 66.8 ± 4.6 days (range 13 – 165 days). The mean number of services per cow was 2.8 ± 0.2 (range 1 – 6 services/cow). Forty cows had at least 2 inseminations, and the mean return interval after first insemination for these cows was 33.8 ± 2.2 days (range 16 – 82 days). The proportion of cows that returned to oestrus during the 18-24 day interval after insemination was 30.0%, and the proportion of repeat inseminations occurring after day 24 post-insemination was 67.5%. There were no apparent differences between the different milk production ranks for any of the variables outlined above in Year 1. During the breeding season of the extended lactation period (Year 2), the mean interval from calving to first insemination was 415 ± 5.5 days (range 337 – 491). The submission rate in the first 3 weeks of the breeding season was 87%, the mean pregnancy rate to first service was 52%, the six-week in-calf rate was 65%, and during the 13-week breeding season, 85% of the cows became pregnant. The mean number of services per cows was 1.82 ± 0.17 (range 1 – 7), and the mean number of services per cow that conceived was 1.51 ± 0.11 (range 1 – 3). There were no apparent differences between the different winter feeding treatments or the different milk production ranks for any of the reproduction variables outlined above in Year 2.

Effect of winter feeding on milk production.

The level of concentrate supplementation during the indoor winter feeding period had a significant effect ($P < 0.001$) on milk production during the period of differential concentrate supplementation (19.9 ± 0.6 vs. 17.6 ± 0.6 kg/day for HIGH vs. LOW, respectively; Figure 1 and Figure 2), resulting in a total milk production response of 209 kg of milk during the 13 week period of indoor concentrate supplementation (0.77 kg milk/day per additional kg of concentrate supplement). Cumulative milk production from the beginning of the indoor feeding period until the end of lactation was increased by 462 kg (5355 ± 217 vs. 4912 ± 223 kg; $P = 0.04$). This increase in cumulative milk production equates to 1.69 kg milk per additional kg of concentrate supplement, indicating a carryover effect of 0.92 kg milk/kg additional concentrate from the end of the high level of winter feeding until dry off. The effect of winter feeding treatment on milk production and lactation length is summarized in Table 2 and Table 3, respectively. Winter feeding level did not affect mean lactation length (mean days in milk = 593 ± 12 vs. 593 ± 10 days for HIGH and LOW, respectively; $P > 0.5$). Weekly decline in milk yield was used as an index of lactation persistency. There was no difference in lactation persistency in the period prior to the winter feeding treatment, but cows on the HIGH winter feeding treatment had a greater ($P < 0.01$) weekly decline in milk yield during the extended lactation period.

Table 2. Effect of winter feeding treatment on milk production

	Low	High	SEM	*P*
Calving to end Nov `04				
Cumulative milk yield (kg)	6332	6176	260	0.7
Cumulative milk fat (kgs)	256	250	10	0.7
Cumulative milk protein (kgs)	217	211	9.8	0.6
Milk yield decline from 1st peak (kg/week)	4.48	4.67	0.21	0.4
Protein to fat ratio	0.855	0.853	0.010	0.8
Number of days in milk	266	261	7.3	0.6
Start Dec `04 to dry off				
Cumulative milk yield (kg)	4686	5177	173	0.05
Cumulative milk fat (kgs)	200	230	8.6	0.02
Cumulative milk protein (kgs)	181	201	6.1	0.03
Milk yield decline from 2nd peak (kg/week)	2.47	2.92	0.12	0.009
Protein to fat ratio	0.940	0.913	0.017	0.04
Number of days in milk	327	332	7.3	0.6
Proportion of 1st period produced in 2nd period				
Milk yield	0.74	0.84	0.05	0.18
Milk fat	0.78	0.92	0.06	0.11
Milk protein	0.83	0.95	0.06	0.16

Table 3. The number (and %) of cows achieving lactations of increasing duration.

		Lactation length (days)				
		420	480	540	600	660
Winter feeding treatment						
Low	n = 23	23 (100)	23 (100)	20 (87.0)	11 (47.8)	3 (13.0)
High	n = 23	23 (100)	22 (95.7)	20 (87.0)	10 (43.5)	4 (17.4)
Milk production rank						
R1	n = 15	15 (100)	15 (100)	15 (100)	10 (66.7)	2 (13.3)
R2	n = 15	15 (100)	15 (100)	14 (93.3)	7 (46.7)	4 (26.7)
R3	n = 16	16 (100)	15 (93.8)	11 (68.8)	3 (18.8)	1 (6.3)

Milk production based on Rank

The milk production of the 3 Ranks are summarized in Table 4 and illustrated in Figure 3. The adjustment variable winter feeding level was not significant ($P > 0.5$), but parity tended to be significant ($P = 0.08$). The effect of milk production rank on lactation length is summarized in Table 3. An effect of Rank on mean lactation length was observed ($P < 0.01$), with R3 having a shorter mean lactation length (558 ± 13 days) compared to either R1 (615 ± 10 days) or R2 (607 ± 13 days). The weekly decline in milk yield did not differ between the milk production ranks during the normal or extended lactation periods.

Table 4. Effect of milk production rank on milk production

	R1	R2	R3	SEM	*P*
Calving to end Nov `04					
Milk yield (kg)	7287[a]	6267[b]	5273[c]	308	0.001
Milk fat (kg)	296[a]	253[b]	212[c]	11.1	0.001
Milk protein (kg)	253[a]	213[b]	179[c]	11.2	0.001
Milk yield decline from 1st peak (kg/week)	4.28	4.84	4.80	0.26	0.3
Protein to fat ratio	0.824[a]	0.870[b]	0.863[b]	0.014	0.045
Number of days in milk	266[ab]	276[a]	250[b]	8.7	0.11
Dec `04 to dry off					
Milk yield (kg)	5738[a]	4836[b]	4266[b]	241	0.001
Milk fat (kg)	254[a]	206[b]	186[b]	10.5	0.001
Milk protein (kg)	222[a]	187[b]	164[b]	8.0	0.001
Milk yield decline from 2nd peak (kg/week)	2.57	2.79	2.80	0.16	0.6
Protein to fat ratio	0.829[a]	0.963[b]	0.971[b]	0.023	0.001
Number of days in milk	349[a]	333[a]	308[b]	8.0	0.002
Proportion of 1st period produced in 2nd period					
Milk yield	0.79	0.77	0.81	0.07	0.8
Milk fat	0.86	0.81	0.88	0.07	0.7
Milk protein	0.88	0.88	0.92	0.08	0.9

[abc] Within row means not sharing a common superscript differ at least $P < 0.05$.

Body Condition Score

BCS data up to and including the time of normal dry-off were recorded before the animals commenced the extended lactation study. The BCS results during the 2 year lactation are summarized in Table 5. The winter feeding treatment did not affect BCS. The final BCS recorded at the end of feeding treatment for low and high feeding levels were 3.36 ± 0.08 vs. 3.38 ± 0.08, respectively (P = 0.9). Across all cows, BCS declined during early lactation and during the breeding period of year 1, and remained relatively flat until the time of normal dry off. Thereafter, BCS increased steadily until actual dry-off in Year 2. This resulted in a 0.45 unit increase in BCS between the mating start date (MSD) in year 1 and MSD in year 2 (2.88 ± 0.08 vs. 3.33 ± 0.08; P<0.001). The BCS at mating end date (MED) was 0.8 units greater in year 2 compared to year 1 (2.68 ± 0.08 vs. 3.47 ± 0.08; P<0.001). On average, cows lost 0.2 units of BCS during the breeding period of year 1, but gained 0.15 units of BCS during the breeding period of year 2. There was a significant effect of milk production rank on BCS during year 1, with R3 cows having a higher BCS compared to R1 and R2 cows at MED and time of normal dry-off, respectively. There were no differences in BCS between the 3 milk production ranks during the extended lactation period in year 2.

Table 5. Effect of winter feeding treatment and milk production rank on BCS

	Winter Feeding Treatment		Milk Production Rank			P-values	
	Low	High	Rank1	Rank2	Rank3	Feeding treatment	Rank
Calving	3.20	3.18	3.12	3.22	3.23	0.9	0.70
MSD Year1	2.87	2.89	2.73	2.80	3.09	0.9	0.10
MED Year1	2.71	2.65	2.55[a]	2.65[ab]	2.83[b]	0.9	0.05
Normal dry-off	2.80	2.72	2.70[ab]	2.65[a]	2.92[b]	0.9	0.06
MSD Year2	**3.30**	**3.36**	**3.23**	**3.25**	**3.50**	**0.9**	**0.30**
MED Year2	**3.51**	**3.45**	**3.30**	**3.43**	**3.69**	**0.9**	**0.18**
Actual dry-off	**3.66**	**3.57**	**3.40**	**3.68**	**3.75**	**0.9**	**0.14**

MSD = Mating start data; MED = mating end date; Values in normal type font were recorded prior to the initiation of the extended lactation study. Values in bold type font were recorded during the extended lactation.

Correlation and multiple regression analysis

The correlation between cumulative milk output (volume and solids) and components of the lactation curve and animal factors are summarized in Table 6. The independent variables that were significantly correlated with cumulative milk yield were, with the exception of lactation persistency, also significantly correlated with cumulative milk solids yield. The multiple regression models for predicting cumulative milk yield and cumulative milk solids yield over the combined normal and extended lactations are outlined in Table 7. The independent variables selected for cumulative milk yield were cumulative 305 day milk yield, PTA for milk yield, and mean weekly milk yield at 305 days in milk. The independent variables selected for cumulative milk solids yield were cumulative 305 d milk solids yield, mean weekly milk yield at 305 days in milk, and the categorical variable parity.

Table 6. Correlation between descriptors of the lactation curve and animal factors with cumulative milk yield and cumulative milk solids yield.

	Cum. Milk yield		Cum. MS yield	
	r	P-value	r	P-value
Lactation curve factors				
Milk yield from calving until 305 DIM	0.79	<0.001	0.71	<0.001
Milk yield from 305 DIM until dry off	0.75	<0.001	0.71	<0.001
Milk yield at 305 DIM	0.67	<0.001	0.62	<0.001
MS yield from calving until 305 DIM	0.68	<0.001	0.72	<0.001
MS yield from 305 DIM until dry off	0.65	<0.001	0.73	<0.001
Peak milk yield	0.67	<0.001	0.61	<0.001
Lactation length	0.46	0.001	0.49	<0.001
100-d milk yield/305-d milk yield	-0.31	0.033	-0.26	0.085
Week of peak milk yield	0.16	0.3	0.12	0.4
Animal Factors				
Parity	0.41	0.005	0.36	0.014
PD for milk production	0.62	<0.001	0.41	0.007
EBI for milk solids production	0.33	0.032	0.41	0.008
Proportion of Holstein genetics	-0.12	0.5	-0.20	0.2

Economic analysis

Individual years. Table 8 shows the key herd output parameters from the model for an Efficient Spring (ES) calving herd, R1 and R3 herds in a system with a 12 month calving interval, and a system where R1 and R3 herds had a 24 month calving interval. In the standard 12 month calving interval systems, the highest profit was achieved with the ES system irrespective of milk price (€29,731 compared to €11,875 and €10,385 for Efficient Spring, R1 and R3 at a milk price of 22.3 c/l, respectively; the corresponding figures at a milk price of 30.0 c/l were €71,094, €49,460 and €58,696 respectively). Milk price had a substantial effect on all profitability indicators across the three systems. The increased farm profit for the ES system over both R1 and R3 was associated with lower concentrate supplementation (316, 857 and 537 kg DM/cow for the three groups respectively; higher milk fat and protein concentrations (4.38% and 3.65% for ES; 4.13% and 3.49% for R1; 4.16% and 3.47% for R3); lower

replacement costs (€22,834, €49,797 and €23,692 for the three groups, respectively) resulting in lower total costs (€148,106, €184,627 and €158,992 for the three groups, respectively).

When R1 and R3 were compared in an extended lactation system, there was a substantial difference in the profit in the first 305 day period when compared to the standard ES system. This result arose due to a much reduced replacement cost, with the replacement rate being reduced from 40% to 12% for R1 animals and the replacement rate dropping from 17% to 12% for R3 animals in the first 12 month period. For both R1 and R3 animals, there was a financial loss in the extended lactation period at the low milk price (-€11,880 and -€47,739, respectively), whereas at the higher milk price R3 animals made a loss in the extended lactation period (-€14,829) but R1 animals made a profit of €28,578. The reduction in profitability associated with the extended lactation period for both R1 and R3 animals is due to higher costs of production with reduced milk sales.

Annualised herd affect. Table 9 shows the key herd annualised output parameters from the model for, R1, R3 and an ES system with 12 month calving intervals, R1 animals where 30% of the animals had extended lactations and 24 month calving intervals and a herd with R3 animals that contained 10% of the animals with extended lactations and 24 month calving intervals. At a milk price of 22.3 c/l the ES system had the best financial performance with a total farm profit of €29,731 compared to €11,875, €10,385, €22,870 and -€2,528 for R1 cows with a 12 month calving interval, R3 cows with a 12 month calving interval, R1 in a system with 30% of cows with an extended lactation, and R3 in a system with 10% of cows with an extended lactation, respectively. The corresponding figures for a milk price of 30.0 c/l were €71,094, €58,696, €49,460, €67,782 and €34,697, respectively. The ES system had the lowest costs of milk production and was therefore substantially more profitable than the alternative systems at a low milk price. The farm profit was 30.0% greater than the next most profitable system – R1 with 30% of the cows having extended lactations. At the higher milk price of 30 c/l the difference in profitability was much lower (4.9%). Rank 1 animals became more profitable through extending the lactation of a proportion of the herd when compared to culling and replacing 40% of the herd. The profitability of R3 animals was reduced by having a proportion of the animals with extended lactations.

Table 8. The effect of extended lactation systems on overall profitability for each individual year

	Normal lactation period			Extended lactation period			
	R1	R3	Efficient Spring	R1		R3	
	305 day	305 day	305 day	305 day	Extension	305 day	Extension
Grass kg DM/ Cow	3,921	3,333	3,645	3,921	3,106	3,332	2,551
Grass Silage kg DM/cow	1,358	1,123	1,169	1,358	1,715	1,117	1,459
Concentrate kg DM/cow	857	537	316	857	563	535	567
Cows calving (No.)	80.3	92.7	86.7	80.3	98.3	92.7	118.5
Stocking rate(LU/ha)	2.22	2.61	2.44	2.22	2.31	2.63	2.77
Milk produced (kg)	605,858	510,655	512,112	605,858	460,475	510,655	378,902
Milk sales (kg)	591,090	493,604	496,167	591,090	460,475	493,604	378,902
Fat sales (kg)	24,403	20,558	21,748	24,403	20,496	20,558	16,595
Protein sales (kg)	20,628	17,128	18,129	20,628	18,078	17,128	14,731
Labour costs (€)	33,008	38,517	36,013	33,008	33,968	38,622	40,868
Feed costs /kg milk (c)	7.7	8.3	7.1	7.7	9.5	8.3	12.4
Total costs (€)	184,627	158,992	148,106	149,920	159,506	150,328	168,264
Milk Price at 22.3 c/litre							
Milk returns (€)	153,057	127,760	136,863	153,054	135,197	127,760	109,623
Margin per cow (€)	148	112	343	470	-121	182	-403
Margin per kg milk (c)	1.96	2.03	5.81	6.23	-2.58	3.30	-12.60
Total profit/farm (€)	11,875	10,385	29,731	37,763	-11,880	16,848	-47,739
Milk Price at 30 c/litre							
Milk returns (€)	199,577	166,598	177,963	199,577	175,338	166,598	142,256
Margin per cow (€)	731	534	820	1,053	291	603	-125
Margin per kg milk (c)	9.69	9.69	13.88	13.96	6.21	10.95	-3.91
Total profit/farm (€)	58,696	49,460	71,094	84,584	28,578	55,923	-14,829

Table 9. The annualised herd effect on overall profitability with 30% recycling for R1 and 10% recycling for R3.

	Normal lactation period			Extended lactation period	
	R1	R3	Efficient Spring	R1	R3
	305 day	305 day	305 day	30% Recycling	10% Recycling
Stocking rate(LU/ha)	2.22	2.61	2.44	2.28	2.67
Milk produced (kg)	605,858	510,655	512,112	562,243	471,129
Milk sales (kg)	591,090	493,604	496,167	551,906	459,193
Fat sales (kg)	24,403	20,558	21,748	23,231	19,369
Protein sales (kg)	20,628	17,128	18,129	19,863	16,409
Labour costs (€)	33,008	38,517	36,013	33,589	39,296
Feed costs /kg milk (c)	7.7	8.3	7.7	8.2	9.5
Total costs (€)	184,627	158,992	148,106	152,796	155,709
Milk Price at 22.3 c/litre					
Milk returns (€)	153,057	127,760	136,863	147,697	122,319
Margin per cow (€)	148	112	343	293	-6.5
Margin per kg milk (c)	1.96	2.03	5.81	3.59	-1.47
Total profit/farm (€)	11,875	10,385	29,731	22,870	-2,528
Milk Price at 30 c/litre					
Milk returns (€)	199,577	166,598	177,963	192,305	159,295
Margin per cow (€)	731	534	820	824	385
Margin per kg milk (c)	9.69	9.69	13.88	11.6	6.49
Total profit/farm (€)	58,696	49,460	71,094	67,782	34,697

DISCUSSION

Milk production

Average milk yield during the entire lactation period was approximately similar to previous reports of extended lactations in pasture-based systems (Auldist et al., 2007, Kolver et al., 2007). A strong effect of genetic potential for milk production on milk yield was observed, not just in the normal lactation period, but also in the extended lactation period. Similarly, (Kolver et al., 2007) reported that North American Holsteins had greater total milk production than New Zealand Holsteins, reflecting the greater emphasis placed on milk yield potential in the North American selection indices compared to the New Zealand selection index. Those authors also reported greater responses to additional concentrate supplementation in North American Holsteins compared to New Zealand Holsteins, in agreement with previous reports from lactations of normal length (Horan et al., 2005a).

An interesting feature of extended lactations is the effect on milk solids composition. As can be seen in Figures 1 and 3, milk protein and milk fat concentration both increased, whereas milk lactose concentration declined in tandem with decreasing milk volume. Kolver et al. 2007 reported a trend for increasing protein concentration during extended lactation, but did not observe an effect on milk fat concentration. Auldist et al., 2007 reported an increase in milk protein concentration from month 10 to month 13 of lactation, but observed no further significant increases in milk protein

concentration thereafter. The effects of higher concentrations of protein and fat, and in particular the improved ratio of protein to fat, would have a favourable impact on milk price. Lactose is the primary osmotic regulator of milk volume, and consequently it was expected that lactose concentration would decline as lactation progressed. Milk lactose concentrations are used as a proxy for milk processability in Ireland; a bonus is paid and a penalty is inflicted for milk lactose concentrations above and below certain thresholds (~4.35 and ~4.2%, respectively). In the later stages of lactation, milk lactose concentrations were mostly in the range where no penalty was being inflicted. Nevertheless, low milk lactose concentrations could be a major problem if a large proportion of a herd were in the later stages of an extended lactation.

The milk production data from the current study indicate that only a small proportion of cows are capable of completing 22-month lactations on a low input pasture-based system, but almost 50% of cows are capable of lactating for 20 months. The most profitable pasture-based systems achieve over 6,000 kg milk volume, 500 kg milk solids (fat + protein), with good reproductive performance in a short breeding period that facilitates calving and turnout to pasture in early spring, thus synchronising the supply of and demand for feed (McCarthy et al., 2007). In the current study, Rank1 cows (i.e., the highest yielding cows) were capable of producing the equivalent of two normal lactations, based on milk volume and milk solids yield, in a single two year lactation. This result clearly indicates that modern high genetic merit cows are capable of lactating for considerably longer than the 240 – 300 day lactations normally observed in seasonal-calving pasture based systems. Thus, extended lactations could represent a viable alternative to culling and replacing high-producing cows that fail to become pregnant during a short breeding period. The economic viability of this is discussed below.

Fertility

Declining fertility has resulted in extended lactations in confinement TMR-based year-round calving systems for many years. Year-round calving systems confer greater flexibility than seasonal-based systems when fertility is sub-optimal. The majority of dairy cows in Ireland (>90%) are in spring-calving seasonal systems of production, and the diet is primarily grazed grass with limited use of alternative feeds. At the end of a normal lactation when producers are faced with a large proportion of cows that failed to become pregnant during the preceding breeding period, the choices available are: (i) cull the non-pregnant cows and replace with heifers or purchased cows; or (ii) continue milking the non-pregnant cows for an additional 12 month extended lactation period, and rebreed the cow to calve down after a two year lactation.

Extended lactation has been proposed as a strategy to avoid high culling rates due to infertility in seasonally calving herds and cows in confinement systems (Borman et al., 2004, Knight, 2001). In traditional seasonal-calving systems, cows are inseminated at or near the time of peak milk yield, generally coinciding with nadir BCS. It has been well documented that BCS and BCS loss influence reproductive performance (Berry et al., 2003b, Buckley et al., 2003b). Cows with high genetic potential for milk production are genetically programmed to preferentially partition nutrients to the mammary gland for longer periods into lactation at the expense of body reserves, and hence are typically below target BCS during the breeding period. As a result, they also have reduced conception rates and an overall reduction in fertility performance (Berry et al., 2003a, Evans et al., 2006a). In the current study,

none of the cows successfully established and maintained a pregnancy during the normal lactation period (average 2.8 inseminations per cow). In the extended lactation period, conception rates to first service averaged 52%, and though the number of animals on the study was small, there was no indication that reproductive performance was affected by winter feeding treatment or milk production rank. This indicates that reasonable fertility performance could be achieved with these cows, but that a longer interval between parturition and breeding was required. This is broadly in agreement with the findings of Kolver et al. (2007), who reported improved reproductive performance for both New Zealand and North American strains of Holstein-Friesian cow during the breeding period of an extended lactation compared with the breeding period of a normal lactation. The physiological changes that occurred between the breeding period in the normal lactation and the breeding period during the extended lactation included reduced milk yield and improved BCS. Thus, the signals necessary to establish and support a pregnancy were lacking at breeding in the normal lactation (low BCS, continued preferential partitioning of nutrients to the mammary gland). By allowing a longer interval to insemination, these signals were reversed; in the extended lactation period, BCS was greater and improving whereas milk yield was declining, and thus becoming pregnant was accorded a higher priority during this time.

Financial

Milk production systems are composed of complex interactions between a series of individual biological and mechanical components. One system may be optimal in one environment but not in another, for a range of economic, policy, environmental or biological reasons. This results in huge variation in the optimum systems of milk production throughout the world. In most countries the optimum systems are developed around maximising output per cow, but optimum systems in New Zealand and Ireland revolve around maximising the utilisation of grass in the diet and minimising costs of production (Dillon et al., 1995). The additional costs or increased profit associated with systems of milk production that contain extended lactations will be dependent on a number of factors, including the system of milk production on the farm, the milk price and milk pricing regime operated, additional labour costs and the overall fertility of the herd.

Within grass based systems of milk production the effect of extending lactations beyond 305 days distorts the synchrony between supply of feed in the form of pasture and the requirement for feed to produce milk, which will ultimately lead to increased costs of production (Borman et al., 2004). In systems where the relative costs of grazed grass, conserved feed and concentrate are not substantially different, the increased costs will not be substantially increased with extended lactations. This study has shown that high-producing R1-type cows are most suited to extended lactations systems, in agreement with previous work from New Zealand (Kolver et al., 2007). The results of the current study indicate that cows suited to extended lactation systems are capable of achieving peak milk yields of ~40 kg/day, cumulative milk yields during the normal lactation period of ~7,200 kg, maintaining milk yields of >20 kg/day at 305 DIM, and have a PTA for milk yield of +225 kg. Cows with lower production potential will not be profitable in extended lactation systems, and should instead be culled.

Methods of milk payment that are aimed at distorting the seasonal nature of milk production will alter the competitiveness of one system over another. Specific schemes that are aimed to increase the production of milk out of season in seasonal

systems of milk production are largely developed on a contract basis to produce milk for specific fresh products that require a supply of milk all year round. This study has shown that at higher milk prices the competitiveness of extending the lactation increases. Substantial price volatility is expected in Irish milk production systems as a result of the relaxation of the market management regimes within CAP; as a consequence, optimum systems will have to be able sustain low milk prices for considerable periods of time.

Infertility and the associated costs substantially affect the profitability of milk production systems right across the world. An economic analysis by (Evans et al., 2006b) across 14 commercial dairy herds in Ireland over the period 1990 to 2003 showed that their overall profitability per litre of milk produced did not improve over the period due to substantial declines in reproductive performance, even though milk yield per cow increased substantially. The current study indicates that in seasonal calving systems where herd fertility is poor, extended lactations for cows not going in calf could be a good short term method of reducing the costs associated with infertility. However, the results also indicate that extended lactation systems will be substantially less profitable than systems with a 12 month calving interval, and that this is the optimum system to strive to achieve.

3.4 Experiment 4. An examination of the effects of trans-10, cis-12 conjugated linoleic acid on reproductive performance in lactating dairy cows.

3.4.1 Introduction

Conjugated linoleic acids (CLA) are geometric and positional isomers of linoleic acid, and are normally found in the rumen as intermediates in the biohydrogenation of linoleic acid to stearic acid. Trans-10, cis-12 CLA was identified as a specific CLA isomer that inhibits milk fat synthesis (Baumgard *et al.*, 2000), and has been demonstrated to reduce mammary milk fat synthesis in a dose-dependent manner (de Veth *et al.*, 2004). This effect has been confirmed in cows consuming both pasture (Kay et al., 2006, Mackle et al., 2003) and TMR diets (Baumgard et al., 2005, Odens et al., 2007), and at varying stages of lactation. However, the dose needed to evoke milk fat depression immediately postpartum is considerably greater than necessary in established lactation (Moore et al., 2004).

As fat is the most energetically expensive component of milk, the milk fat depressing effects of trans-10, cis-12 CLA could therefore be used as a management tool to temporarily reduce milk energy output. Importantly, milk volume and milk protein concentration are not decreased by CLA supplementation, and milk fat depression caused by Trans-10, cis 12 CLA is reversible, with milk fat content returning to similar levels as control groups at the termination of supplementation (Castaneda-Gutierrez et al., 2005b).

This reduction in milk energy output has previously been reported in some studies (Kay et al., 2006), but in other reports the energy spared by reducing milk fat output was partitioned to increased milk volume (Bernal-Santos et al., 2003b). Beneficial effects of CLA supplementation on fertility indices have been reported, including trends towards decreased interval to first ovulation, increased plasma IGF-I, elevated plasma progesterone during the early luteal phase and greater numbers of cows pregnant (Bernal-Santos et al., 2003a, Castaneda-Gutierrez et al., 2007a, Castaneda-Gutierrez et al., 2005a) This study was carried out to examine the effects of CLA supplementation during early lactation on milk production and reproductive indices.

3.4.2 MATERIALS AND METHODS

Animals and treatments

Forty multiparous Holstein-Friesian cows were blocked on the basis of expected calving date, previous lactation milk yield, and body condition score (BCS), and 32 primiparous Holstein-Friesian cows were blocked on the basis of expected calving date, BCS and bodyweight. Cows were then randomly assigned to receive 60 g per day of lipid encapsulated CLA (LE-CLA; Lutrell pure; BASF AG, Ludwigshaven, Germany) or 60 g per day of calcium salts of palm fatty acids (CSFA; Megalac; Church and Dwight Co. Inc., Princeton, NJ) from parturition until 60 days in milk. The LE-CLA contained a 50:50 mix of cis-9, trans-11 CLA and trans-10, cis-12 CLA, resulting in a daily intake of 6 g per day of each isomer. Cows were assigned to their treatments and treatments were initiated during a 4 month period. Cows were housed in a free stall cubicle shed and managed as a single group of animals throughout the study period. To facilitate administration of the treatments, the fatty acid supplements were incorporated into concentrate pellets such that 2 kg of concentrate contained 60 g of LE-CLA or CSFA supplements. The concentrate pellets were fed using automatic feeders. Individual dry matter intake (DMI) was measured daily from 3 weeks prior to parturition to 13 weeks postpartum using the Griffith-Elder

Mealmaster feeding system (Griffith Elder & Co Ltd, Suffolk, UK). Cows were housed on a straw bed for a variable period of 1 to 4 days around the time of parturition.

Weekly samples of the feeds offered were dried and ground, and composited on a monthly basis for nutrient analysis. The ingredient composition of the basal diet and the concentrate supplements are outlined in Table 28.

Table 28. Ingredient and nutrient composition of the feeds offered.

Ingredient (% of DM)	Prepartum	Postpartum
TMR diet		
Grass silage	100	50
Soya hulls		25
Coarse ground barley		15
Soyabean meal		9
Dicalcium phosphate		0.4
Calcined magnesite		0.33
Limestone		0.15
Salt		0.13
	CSFA	LE-CLA
Concentrate supplement (% as fed)		
Barley	20.0	20.0
Citrus pulp	26.1	26.1
Maize gluten feed	26.5	26.5
Soyabean meal	19.0	19.0
CSFA	4.4	-
LE-CLA	-	4.4
Dicalcium phosphate	1.6	1.6
Calcined magnesite	1.3	1.3
Limestone	0.6	0.6
Salt	0.3	0.3

Cows were fed daily at 0900 h, and were allowed access to their fat supplements from 0900 to 1700. Cows consumed their allocated fat supplements in a single meal, and the allotted time allowed ample access time for all cows. Following parturition, cows were milked twice daily at 0700 and 1600 h, and milk samples for composition analysis (fat, protein, and lactose) were collected weekly. Milk fatty acid composition was examined at 30 days in milk using Gas-Liquid chromatography. Milk lipids were extracted and methylated, and the fatty acid methyl esters were analyzed using a Hewlett Packard Model 5890 Series II GLC. Individual fatty acids were reported as a percentage of total fatty acids.

During the period from day 21 before calving to day 28 postpartum blood samples were collected on three days per week (Monday, Wednesday, and Friday). Thereafter, blood samples were collected every 2 weeks until 10 weeks postpartum. In addition, blood samples were collected from each cow 3 times per week for 3 weeks after the first postpartum insemination. All blood samples were collected from the coccygeal vessels into vacutainers containing lithium heparin (Becton Dickinson, Plymouth, United Kingdom) between 0700 and 0800 h after the morning milking but before feeding. The samples were immediately centrifuged at 2000 × g for 15

minutes. The plasma was decanted into 1.5 mL tubes, sealed with an airtight cap, and stored at -20 °C until analysis.

Hormone and metabolite analysis
Blood samples were analysed for circulating concentrations of glucose, non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHBA), insulin, and insulin-like growth factor-I (IGF-I) on days -14, -7, 0, 7, 14, 21, 28, 42, 56 and 70 relative to parturition. Progesterone was measured in the blood samples collected after the first postpartum insemination. The assays were carried out as previously described.

Ovarian ultrasonography.
Ovarian structures were examined by linear array ultrasonography using a 7.5-MHz transrectal transducer (Aloka SSD-900; Aloka Ltd., Tokyo, Japan). Ultrasound examinations commenced on day 8 to 10 postpartum, and were carried out on 3 days per week (Monday, Wednesday and Friday) until first ovulation. Pregnancy diagnoses were carried out at 30 to 36 and 60 to 66 days post-insemination. Visualization of a fluid-filled horn and a viable embryo were used for positive identification of pregnancy.

Statistical analysis
All statistical analyses were carried out using SAS (SAS System Inc., Cary, NC). Daily measurements of milk yield and dry matter intake were collapsed into weekly means. Milk production and milk composition data were analysed using the MIXED procedure of SAS with repeated measures, using the satterthwaite adjustment to calculate denominator degrees of freedom and an unstructured (UN) covariance structure. A first order autoregressive covariance structure (AR1) was used for the DMI data. The appropriate covariance structure for each repeated measures analysis was identified based on Akaike's Information Criterion (AIC) model fit statistic. Measurements made during the final 3 weeks prepartum were used as covariates for DMI, EB, and plasma analytes. Parity and calving day of year were included as adjustment variables in all repeated measures models; if non-significant, these variables were removed and the models were rerun. All values reported are least square means and SEM.

3.4.3 RESULTS
Milk production, energy balance and BCS.
The milk production results are summarized in Table 29 and Figure 12. Milk fat concentration and yield were significantly reduced by the CLA supplement, although not immediately postpartum. Milk fat concentration did not differ between treatments for the first 5 weeks postpartum, but from week 6 to week 10 postpartum was significantly lower ($P < 0.001$) in LE-CLA cows, with a maximal reduction of 15.7% at week 8. Milk fat yield was also reduced by up to 15.2% during this period ($P = 0.008$). Supplementation with CLA ceased at 60 DIM, and thereafter milk fat concentration in CLA cows returned toward concentrations similar to the control group after week 10 postpartum. Supplementation with CLA had no effect on milk yield, the concentration or yield of milk protein, or the concentration or yield of milk lactose (all $P > 0.1$). Mean daily milk energy output tended ($P = 0.06$) to be lower for cows on the CLA treatment.

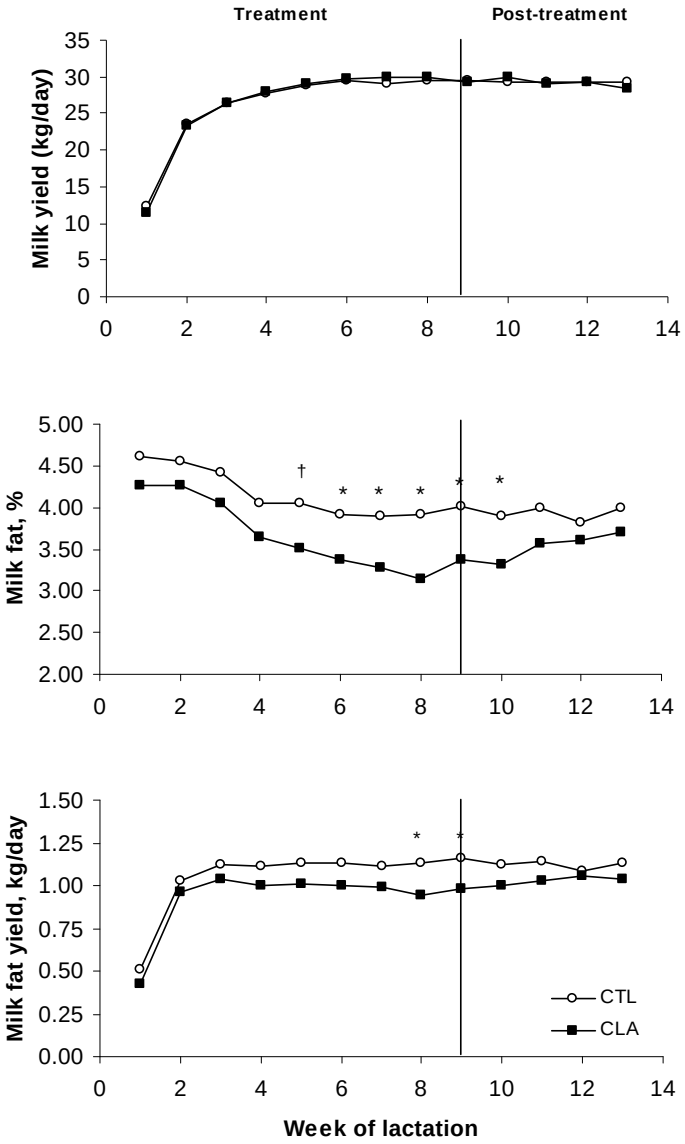

Figure 12. Temporal changes in milk yield, milk fat concentration and milk fat yield during the treatment and post-treatment periods. The P-values for the effect of treatment on milk yield, milk fat concentration, and milk fat yield were 0.9, <0.001, and 0.008, respectively. The interaction between treatment and time was not significant for any of the variables (all P > 0.4). The pooled SEM for milk yield, milk fat concentration, and milk fat yield were 0.9 kg/day, 0.11%, and 0.04 kg/day, respectively. † Treatment means are different P < 0.1; * Treatment means are different P < 0.05.

Table 29. Least square means for milk yield and milk composition during the treatment period

				P-value	
	Control	LE-CLA	SEM	Trt	Trt × Time
Milk yield (kg/day)	26.5	26.5	0.9	0.9	0.8
Milk fat (%)	4.26	3.78	0.11	<0.001	0.4
Milk fat (kg/day)	1.08	0.98	0.04	0.008	0.5
Milk protein (%)	3.12	3.07	0.05	0.4	0.14
Milk protein (kg/day)	0.81	0.78	0.03	0.15	0.7

Milk lactose (%)	4.63	4.62	0.03	0.7	0.6
Milk lactose (kg/day)	1.23	1.22	0.04	0.8	0.8
Milk energy (UFL/day)	11.4	10.7	0.4	0.06	0.7
Milk energy (Mcal/day)	19.4	18.3	0.7	0.06	0.7

Dry matter intake and energy intake were not affected by LE-CLA supplementation (P = 0.25; Figure 2), and there was no treatment by time interaction (P = 0.5). Energy balance was significantly greater for the cows on the CLA treatment (P < 0.001), and consequently mean postpartum BCS tended to be greater for these cows (P = 0.09) (Figure 13). Body condition score declined in both treatments for the first 4 weeks of lactation. Thereafter CLA cows did not loose any further BCS whereas control cows continued to mobilise body reserves (week 5 to 8 BCS: 2.96 vs. 2.83; treatment by time, P < 0.05).

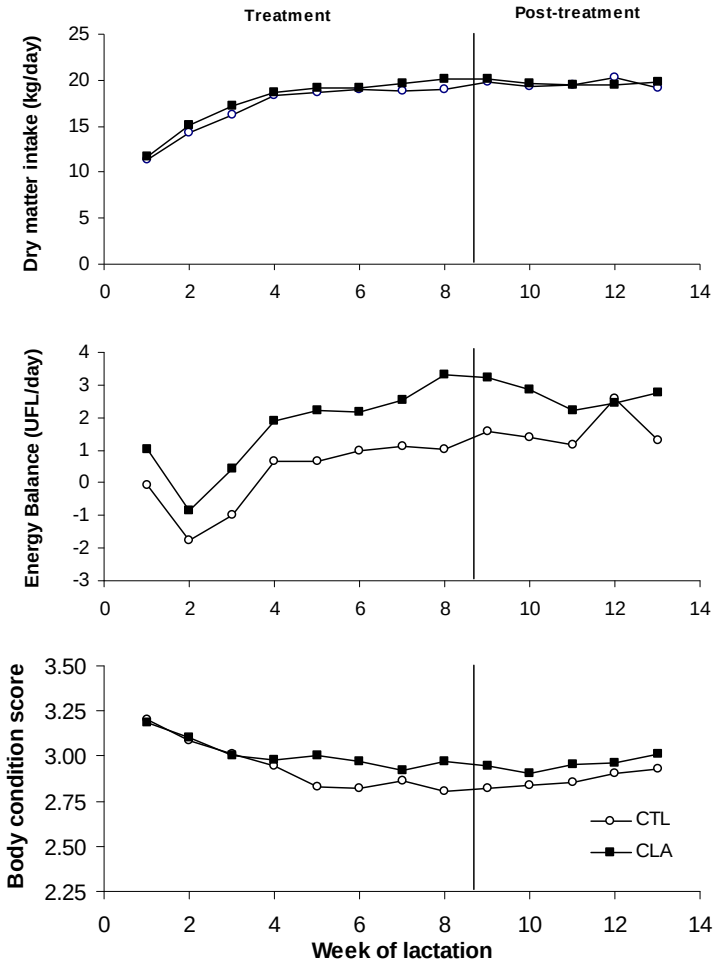

Figure 13. Effect of treatment on dry matter intake, energy balance and body condition loss. Upper panel: The effect of treatment and the interaction between treatment and time were not significant for DMI (P = 0.25 and 0.5, respectively; pooled SEM = 0.4 kg DMI). Middle panel: Energy balance was improved by CLA supplementation (treatment effect, P < 0.001; treatment by time interaction, P = 0.8; pooled SEM 0.35 UFL/day). Lower panel: BCS tended to be greater for cows on the CLA treatment (treatment, P = 0.09), and a significant interaction between treatment and time was observed (P = 0.05). The pooled SEM was 0.036 BCS units.

Blood metabolite data

Plasma metabolite data are illustrated in Figure 14. Plasma glucose concentrations were significantly greater in cows assigned to the CLA treatment than

control cows (P = 0.02). No differences in circulating NEFA or BHBA were observed.

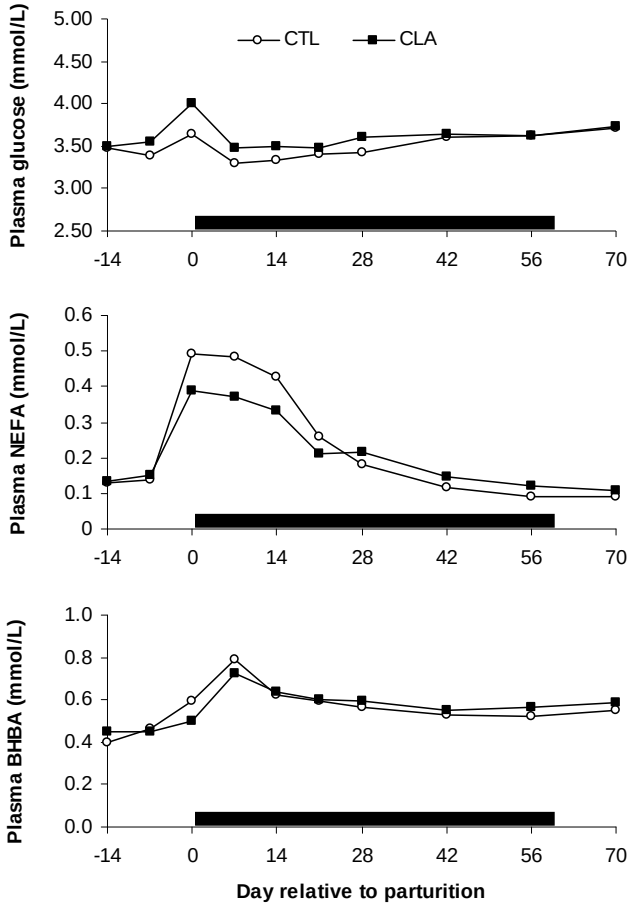

Figure 14. Temporal changes in circulating glucose, NEFA and BHBA in cows supplemented with control and CLA supplements. Upper panel: Circulating glucose concentrations were increased in cows supplemented with CLA (treatment, P = 0.02; treatment by time, P = 0.26; pooled SEM = 0.07 mmol/L). Middle panel: Treatment and treatment by time effects were not significant for circulating NEFA concentrations (treatment, P = 0.4; treatment by time, P = 0.3; pooled SEM = 0.03 mmol/L). Lower panel: Treatment and treatment by time effects were not significant for circulating BHBA concentrations (treatment, P = 0.9; treatment by time, P = 0.7; pooled SEM = 0.04 mmol/L).

Milk Fatty Acid Analysis

CLA supplementation reduced the proportion of the majority of short and medium chain fatty acids in milk fat compared to the control animals (Table 30), with the exception of C4:0 which was increased (P = 0.03) and C12:1 and C13:0 which were not affected (both P > 0.2). The proportion of C16:0 in milk fat was also decreased by CLA supplementation (P = 0.01). The proportion of *trans*-10, *cis*-12 CLA in milk fat was increased by CLA supplementation (P = 0.01), as were proportions of other long chain fatty acids C18:0, *cis*-9 18:1, C18:3 n-3 and C20:0 (all P < 0.03). The proportion of *cis*-9, *trans*-11 CLA tended to be reduced (P = 0.07).

Table 30: Milk fatty acid composition (g/100g total fatty acids) of cows on the control and CLA supplements at week 4 of lactation.

	Control	LE-CLA	S.E.M	P value
4:0	3.36	3.58	0.067	0.03

6:0	1.93	1.80	0.035	0.03
8:0	1.11	0.98	0.029	<0.01
10:0	2.40	2.04	0.087	0.01
10:1	0.28	0.22	0.013	<0.01
11:0	0.06	0.04	0.005	0.02
12:0	2.75	2.28	0.103	<0.01
12:1	0.07	0.06	0.008	0.69
13:0	0.10	0.09	0.005	0.23
14:0	9.95	8.98	0.235	0.01
14:1	1.09	0.90	0.037	<0.01
15:0	1.15	1.08	0.027	0.07
All <C:16	23.09	20.94	0.475	<0.01
16:0	30.90	28.62	0.650	0.01
cis-9 16:1	1.42	1.43	0.062	0.98
All C:16	32.08	29.55	0.652	<0.01
17:0	0.71	0.77	0.015	0.02
17:1	0.35	0.39	0.012	0.01
18:0	8.44	9.68	0.352	0.02
trans-11 18:1	1.40	1.49	0.072	0.37
cis-9 18:1	17.59	20.22	0.697	0.01
cis-11 18:1	0.43	0.47	0.030	0.44
18:2n-6	1.64	1.69	0.051	0.5
18:3n-3	0.51	0.56	0.017	0.02
20:0	0.12	0.15	0.006	<0.01
cis-9, *trans*-11 CLA	0.51	0.58	0.026	0.06
trans-10, *cis*-12 CLA	0.01	0.02	0.003	0.01
20:1	0.09	0.10	0.005	0.25
20:4n-6	0.10	0.11	0.004	0.25
All >C16	33.37	37.67	1.04	<0.01

Postpartum onset of cyclicity and reproductive performance

There was no difference between treatments in the postpartum interval to onset of cyclicity (21.0 ± 2.5 vs. 23.3 ± 2.6 days postpartum, CTL and CLA, respectively). Reproductive performance data is summarized in Table 31. Conception rate to first service was numerically higher for cows on the CLA treatment as was conception rate to second service, and the number of services per conception also tended to be reduced (P = 0.07). Calving to service interval and calving to conception interval did not differ between the treatments (Table 3).

Table 31. Reproductive performance of cows on the control and CLA supplements

	CTL	CLA	P-value
Conception rate to first service (%)	38.9 (14/36)	51.5 (17/33)	0.3
Conception rate to second service (%)	66.7 (14/21)	76.9 (10/13)	0.7

Conceived to first and second services (%)	77.8 (28/36)	81.8 (27/33)	0.7
Services per conception for pregnant cows (n)	1.63 (± 0.15)	1.48 (± 0.14)	0.3
Services per conception for all cows (n)	2.00 (± 0.15)	1.72 (± 0.16)	0.07
Calving to service interval (days)	81.1 (± 6.1)	83.2 (± 6.5)	0.7
Calving to conception interval (days)	111.0 (± 8.8)	109.1 (± 8.6)	0.8

Circulating progesterone after insemination.
Circulating concentrations of progesterone during the first 21 days after insemination were not affected by CLA supplementation in all cows, regardless of a successful/unsuccessful establishment of pregnancy (both $P > 0.5$).

Discussion
Following parturition, the energy required for milk production and maintenance often exceeds energy ingested, and dairy cows typically enter a state of NEB (Reist et al., 2003). The duration and severity of NEB are associated with prolonged interval to first ovulation, and subsequently reduced likelihood of conception (Beam and Butler, 1999, Buckley et al., 2003a). In the present study, CLA supplementation did not affect milk fat concentration or yield until 5-6 weeks after the initiation of supplementation. From then on, milk fat concentration declined until supplementation ceased at 60 days in milk when milk fat depression was greatest at 15.7%.
In previous studies, a dietary supplement of CLA during established lactation resulted in an immediate reduction in milk fat content in cows either consuming a TMR (Giesy et al., 2002, Perfield et al., 2002) or a pasture diet (Kay et al., 2007, Mackle et al., 2003). Conversely, studies conducted in cows during the transition period indicated that CLA supplementation does not cause a depression in milk fat until approximately five weeks after the initiation of supplementation (Bernal-Santos et al., 2003b, Castaneda-Gutierrez et al., 2005b). Supplementation with very high doses of CLA (~3X) during the early lactation period can induce milk fat depression (Kay et al., 2006). (Moore et al., 2004) suggested that the mammary gland is less sensitive to CLA in early lactation, and that the milk fat synthesising genes are resistant to manipulation at this time for evolutionary purposes. In the current study, the supplement provided an intake of 6g/day of trans-10, cis-12 CLA, similar to the work of (Castaneda-Gutierrez et al., 2007b), though much less than other transition period studies (Kay et al., 2006, Odens et al., 2007). However, the magnitude of milk fat depression (up to 50%) caused by high supplementation levels would not be viable for commercial dairy producers, as fat is an economically important component of milk. Therefore the level of supplementation utilised in the present study is designed to induce sufficient milk fat depression to improve energy status and fertility indices, while not significantly decreasing income from milk.
CLA supplementation improved calculated Energy Balance compared to the cows on the control diet, which was reflected in modest improvements in the BCS of the CLA supplemented cows compared to control animals. This improvement in energy balance can be directly attributed to milk fat depression, as DMI and milk yield did not differ between control and CLA treatment groups, and is consistent with previous research that has identified beneficial effects of feeding CLA on EBAL indices (Kay et al., 2007, Odens et al., 2007).
Theoretically, the improvement in EBAL should reduce the demand to mobilise adipose tissue reserves and decrease plasma NEFA, however in the present study

there was no effect of CLA supplementation on circulating NEFA, consistent with results from (Kay et al., 2007), who also improved EBAL by feeding a CLA supplement. It is not clear why NEFA levels are not affected by CLA supplementation, although it has been hypothesised that the signal to mobilise adipose tissue in early lactation is independent of EBAL, and instead reflects the oxidation needs of extra-mammary tissues in a homeorhetic effort to spare glucose for mammary lactose synthesis (Moore et al., 2004).
The increase in plasma glucose concentrations with CLA supplementation in the present study is not consistent with the majority of other similar studies (Bernal-Santos et al., 2003b, Kay et al., 2006). Only (Odens et al., 2007) established comparable effects, which they attributed to a possible decrease in insulin sensitivity.

The general trend of changes in milk fat composition evident in the present study, of decreasing proportions of short and medium chain fatty acids and increasing proportions of long chain fatty acids with CLA supplementation, are consistent with other reports (Castaneda-Gutierrez et al., 2005b, Mackle et al., 2003, Perfield et al., 2002), suggesting that although all milk fatty acids are decreased, there is greater reduction in the short chain de novo synthesised fatty acids than in the long chain fatty acids derived from the circulation. This is consistent with the work of (Baumgard et al., 2002), who demonstrated that the mechanism by which *trans*-10, *cis*-12 CLA decreases milk fat production involves reduction in mRNA expression for key enzymes involved in fat synthesis in the mammary gland, as well as enzymes involved in the uptake and transport of circulating fatty acids.
Although there are only a limited number of studies that have considered the effect supplemental CLA has on dairy cow reproductive performance, beneficial effects of CLA on fertility indices have been reported (Bernal-Santos et al., 2003b, Castaneda-Gutierrez et al., 2007b, Castaneda-Gutierrez et al., 2005b, Chagas et al., 2007). The results obtained from the current work would appear to support these reports. (Bernal-Santos et al., 2003b) and (Castaneda-Gutierrez et al., 2005b) both identified a tendency for reduced number of days to ovulation in CLA supplemented cows, although no such effect was established here. (Castaneda-Gutierrez et al., 2005b) also noted a numerical improvement in the number of services per conception with CLA supplementation, consistent with the current study where services per conception tended to be reduced. We also found a numerical improvement in conception rate to first service and conception rate to second service in CLA supplemented cows.
Other variables associated with reproductive performance such as circulating concentrations of progesterone and IGF-1 were not affected by CLA supplementation in the present study, however (Castaneda-Gutierrez et al., 2007b) established significant increases in plasma concentrations of IGF-1, and a trend towards greater concentrations of plasma progesterone during the early luteal phase in cows receiving the CLA supplement.
A great deal of interest has focussed on the potential benefits feeding fat supplements may have on reproductive performance, not only through improvements in energy status but also effects related to the specific fatty acid composition of the supplement. Fatty acids have been implicated in a number of reproductive processes, including prostaglandin synthesis, follicle development and oocyte competence, ovarian steroidogenesis and embryo quality (Santos et al., 2008), which raises the possibility that supplemental CLA may enhance reproductive performance through interactions with the pituitary, ovaries and uterus in addition to the energy sparing effects confirmed in the present study.

Conclusions

Supplementation with *trans*-10, *cis*-12 CLA induced milk fat depression in early lactation dairy cows, with no effect on milk yield, or the yield of milk protein or lactose. The reduction in milk fat content resulted in an improvement in calculated energy balance, reflected in reduced loss of BCS in CLA supplemented cows. There were numerical improvements in conception rates, and a tendency towards fewer services per conception in CLA treated cows. The data presented here together with results from previous studies indicate the potential beneficial effects of feeding CLA on reproductive performance, and additional work should be carried out on a larger scale to fully determine these effects.

Overall Summary

Strain comparison Study

- There were no differences observed between NA and NZ strains for the timing and magnitude of the energy balance (EB) nadir, interval to neutral EB, or mean daily EB for week 1-20 of lactation.
- Plasma concentrations of glucose and insulin were greater for NA cows during the transition period (d 14 *pre partum* to d 28 *post partum*). Plasma IGF-I concentrations were similar for the strains at this time, but NZ cows had greater plasma IGF-I concentration from d 29 to d 100 of lactation.
- Abundance of IGF-I mRNA was greater in the NZ strain concomitant with a tendency for increased expression of ALS mRNA.
- The NZ strain had a greater clearance rate of glucose and tended to have a shorter glucose half-life in mid-lactation when infused with glucose.
- The NA cows had a greater glucose response to epinephrine infusion in early and mid-lactation.
- Pre-ovulatory oestradiol concentrations, follicle diameter, post-ovulatory progesterone concentrations, and CL diameter did not differ between the two strains.
- The proportion of transferable embryos recovered following superovulation was greater in the NZ cows compared with the NA cows. A greater proportion of the recovered structures were at the blastocyst stage in the NZ cows.
- Results of this study do not support the premise that the NZ strain has a more favourable energy balance or metabolic status during the transition period. The results indicate that NZ cows begin to partition nutrients towards body reserves during mid-lactation whereas NA cows continue to partition nutrients to milk production. This occurs due to greater hepatic expression of IGF-I and greater circulating IGF-I concentrations, in addition to greater glucose clearance rates in mid-lactation. Superior embryo quality on day 7 post-AI is consistent with previous reports showing superior fertility performance. Strain differences in nutrient partitioning from the time of peak SCM yield through late lactation may provide the key signals responsible for superior embryo quality in NZ cows.

Dry Period Duration and Feeding Level Study

- Cows were assigned to one of two dry period treatments (standard 8 week dry period (**SDP**) or no planned dry period (**NDP**)) and one of two dietary energy density treatments (standard TMR (**STMR**) or high quality TMR (**HTMR**)).
- Milk yield during weeks 1 to 12 postpartum was reduced in cows assigned to the NDP treatment. Energy balance and body condition score during weeks 1 to 4 postpartum were increased in cows assigned to the NDP treatment compared to the cows assigned to the SDP, and BCS increased from weeks 5 to 12 postpartum in the NDP cows compared to the SDP cows.
- During the first 12 weeks postpartum, cows assigned to the HTMR had greater milk yields and reduced milk fat concentration compared to the cows assigned the STMR diet. BCS was greater from weeks 5 to 12 postpartum in HTMR cows compared to STMR cows.
- Circulating concentrations of insulin, glucose and IGF-I were greater in cows on the NDP treatment compared to cows on the SDP treatment.
- Cows assigned to the HTMR had greater circulating insulin and glucose.

- The first postpartum ovulation occurred earlier for cows on the NDP treatment compared to cows on the SDP treatment, but dietary treatment had no effect. Energy balance and metabolic status can be improved by either eliminating the dry period or by feeding a higher energy diet, but effects on the reproductive axis appear to be different.
- Reducing the duration of the dry period could be employed as a strategy to improve postpartum BCS in cows with high PTA for milk kg, as these cows typically partition nutrients to the mammary gland at the expense of body reserves.

Extended Lactation Study

- High winter feeding resulted in greater milk production over the winter indoor period (20.0 ± 0.3 vs. 17.8 ± 0.3 kg/day), and had a carryover effect of increased milk production during the remainder of the extended period (5177 vs. 4686 kg during the extended period, $P<0.05$).
- At the end of the study period, cows were ranked on the basis of cumulative milk solids, and separated into 3 groups (R1, R2, and R3). R1 produced 7287 (549) and 5738 (476) kg, R2 produced 6267 (466) and 4836 (393) kg, and R3 produced 5273 (391) and 4266 (350) kg milk in the normal and extended periods, respectively (milk solids in parentheses).
- 85% of cows became pregnant during the breeding season of year 2, with a conception rate to first service of 52%, which was not affected by either feeding treatment or Rank.
- An economic analysis indicated that within a spring system where 30% of R1 animals are subjected to extended lactations, the profitability of the system was greater than culling and replacing, but still less profitable than an efficient spring system with a compact calving pattern. The results indicate that extended lactations may be a viable alternative to culling non-pregnant cows, and economically is more suited to higher producing cows.

CLA supplementation study

- CLA supplementation resulted in decreased milk fat content from week 5 postpartum, with consequent improvements in energy balance and BCS.
- Plasma glucose concentrations were greater in cows assigned to the CLA treatment. Circulating concentrations of progesterone, NEFA, BHBA, insulin and IGF-1 were not affected by CLA supplementation.
- There was no effect of CLA supplementation on the postpartum interval to first ovulation. Services per conception tended to be reduced and there was a numerical improvement in conception rate to first service in CLA supplemented cows.

4. References

Annen, E. L., R. J. Collier, M. A. McGuire, and J. L. Vicini. 2004a. Effects of dry period length on milk yield and mammary epithelial cells. Journal of Dairy Science 87(E. Suppl.):E66-E76.

Annen, E. L., R. J. Collier, M. A. McGuire, J. L. Vicini, J. M. Ballam, and M. J. Lormore. 2004b. Effect of modified dry period lengths and bovine somatotropin on yield and composition of milk from dairy cows. J Dairy Sci 87(11):3746-3761.

Annen, E. L., A. C. Fitzgerald, P. C. Gentry, M. A. McGuire, A. V. Capuco, L. H. Baumgard, and R. J. Collier. 2007. Effect of continuous milking and bovine somatotropin supplementation on mammary epithelial cell turnover. J Dairy Sci 90(1):165-183.

Auldist, M. J., G. O'Brien, D. Cole, K. L. Macmillan, and C. Grainger. 2007. Effects of varying lactation length on milk production capacity of cows in pasture-based dairying systems. J Dairy Sci 90(7):3234-3241.

Ayalon, N. 1978. A review of embryonic mortality in cattle. J Reprod Fertil 54(2):483-493.

Bauman, D. E. 2000. Regulation of nutrient partitioning during lactation: homeostasis and homeorhesis revisited. Pages 311-327 in Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction . Proceedings of the 9th International Symposium on Ruminant Physiology. Vol. Proceedings of the 9th International Symposium on Ruminant Physiology. P. B. Cronje, ed. CABI Publishing, New York.

Bauman, D. E. and W. B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: a review of mechanisms involving homeostasis and homeorhesis. Journal of Dairy Science 63(9):1514-1529.

Bauman, D. E., S. N. McCutcheon, W. D. Steinhour, P. J. Eppard, and S. J. Sechen. 1985. Sources of variation and prospects for improvement of productive efficiency in the dairy cow: a review. J.Anim Sci. 60(2):583-592.

Baumgard, L. H., B. A. Corl, D. A. Dwyer, A. Saebo, and D. E. Bauman. 2000. Identification of the conjugated linoleic acid isomer that inhibits milk fat synthesis. Am J Physiol Regul Integr Comp Physiol 278(1):R179-184.

Baumgard, L. H., J. K. Kay, C. E. Moore, J. R. Roche, M. J. VanBaale, and R. P. Rhoads. 2005. Regulating milk fat synthesis: potential on-farm applications of basic science. Proceedings of the New Zealand Society of Animal Production 65:33-39.

Baumgard, L. H., E. Matitashvili, B. A. Corl, D. A. Dwyer, and D. E. Bauman. 2002. trans-10, cis-12 Conjugated Linoleic Acid Decreases Lipogenic Rates and Expression of Genes Involved in Milk Lipid Synthesis in Dairy Cows. J. Dairy Sci. 85(9):2155-2163.

Beam, S. W. and W. R. Butler. 1997. Energy balance and ovarian follicle development prior to the first ovulation postpartum in dairy cows receiving three levels of dietary fat. Biol Reprod 56:133-142.

Beam, S. W. and W. R. Butler. 1999. Effects of energy balance on follicular development and first ovulation in postpartum dairy cows. Journal of Reproduction and Fertility:411-424.

Bell, A. W., W. S. Burhans, and T. R. Overton. 2000. Protein nutrition in late pregnancy, maternal protein reserves and lactation performance in dairy cows. Proceedings of the Nutrition Society 59(1):119-126.

Bernal-Santos, G., J. W. Perfield, 2nd, D. M. Barbano, D. E. Bauman, and T. R. Overton. 2003a. Production responses of dairy cows to dietary supplementation with conjugated linoleic acid (CLA) during the transition period and early lactation. J Dairy Sci 86(10):3218-3228.

Bernal-Santos, G., J. W. Perfield, II, D. M. Barbano, D. E. Bauman, and T. R. Overton. 2003b. Production Responses of Dairy Cows to Dietary Supplementation with Conjugated Linoleic Acid (CLA) During the Transition Period and Early Lactation. J. Dairy Sci. 86(10):3218-3228.

Berry, D. P., F. Buckley, P. Dillon, R. D. Evans, M. Rath, and R. F. Veerkamp. 2003a. Genetic parameters for body condition score, body weight, milk yield, and fertility estimated using random regression models. J Dairy Sci 86(11):3704-3717.

Berry, D. P., F. Buckley, P. Dillon, R. D. Evans, M. Rath, and R. F. Veerkamp. 2003b. Genetic relationships among body condition score, body weight, milk yield, and fertility in dairy cows. J Dairy Sci 86(6):2193-2204.

Borman, J. M., K. L. Macmillan, and J. Fahey. 2004. The potential for extended lactations in Victorian dairying: a review. Australian Journal of Experimental Agriculture 44:507-519.

Buckley, F., K. O'Sullivan, J. F. Mee, R. D. Evans, and P. Dillon. 2003a. Relationships Among Milk Yield, Body Condition, Cow Weight, and Reproduction in Spring-Calved Holstein-Friesians. J. Dairy Sci. 86(7):2308-2319.

Buckley, F., K. O'Sullivan, J. F. Mee, R. D. Evans, and P. Dillon. 2003b. Relationships among milk yield, body condition, cow weight, and reproduction in spring-calved Holstein-Friesians. J Dairy Sci 86(7):2308-2319.

Butler, S. T., S. H. Pelton, and W. R. Butler. 2006. Energy balance, metabolic status, and the first postpartum ovarian follicle wave in cows administered propylene glycol. J Dairy Sci 89(8):2938-2951.

Butler, W. R. 2003. Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows. Livestock Production Science 83(2-3):211-218.

Butler, W. R. and R. D. Smith. 1989. Interrelationships between energy balance and postpartum reproductive function in dairy cattle. Journal of Dairy Science 72:767-783.

Castaneda-Gutierrez, E., B. C. Benefield, M. J. de Veth, N. R. Santos, R. O. Gilbert, W. R. Butler, and D. E. Bauman. 2007a. Evaluation of the mechanism of action of conjugated linoleic acid isomers on reproduction in dairy cows. J Dairy Sci 90(9):4253-4264.

Castaneda-Gutierrez, E., B. C. Benefield, M. J. de Veth, N. R. Santos, R. O. Gilbert, W. R. Butler, and D. E. Bauman. 2007b. Evaluation of the Mechanism of Action of Conjugated Linoleic Acid Isomers on Reproduction in Dairy Cows. J. Dairy Sci. 90(9):4253-4264.

Castaneda-Gutierrez, E., T. R. Overton, W. R. Butler, and D. E. Bauman. 2005a. Dietary supplements of two doses of calcium salts of conjugated linoleic acid during the transition period and early lactation. J Dairy Sci 88(3):1078-1089.

Castaneda-Gutierrez, E., T. R. Overton, W. R. Butler, and D. E. Bauman. 2005b. Dietary Supplements of Two Doses of Calcium Salts of Conjugated Linoleic Acid During the Transition Period and Early Lactation. J. Dairy Sci. 88(3):1078-1089.

Chagas, L. M., J. J. Bass, D. Blache, C. R. Burke, J. K. Kay, D. R. Lindsay, M. C. Lucy, G. B. Martin, S. Meier, F. M. Rhodes, J. R. Roche, W. W. Thatcher, and R. Webb. 2007. New perspectives on the roles of nutrition and metabolic priorities in the subfertility of high-producing dairy cows. Journal of Dairy Science 90(9):4022-4032.

de Feu, M. A., J. Patton, A. C. O. Evans, P. Lonergan, and S. T. Butler. 2008. The effect of strain of Holstein-Friesian cow on size of ovarian structures, periovulatory circulating steroid concentrations, and embryo quality following superovulation. Theriogenology 70(7):1101-1110.

de Veth, M. J., J. M. Griinari, A. M. Pfeiffer, and D. E. Bauman. 2004. Effect of CLA on milk fat synthesis in dairy cows: comparison of inhibition by methyl esters and free fatty acids, and relationships among studies. Lipids 39(4):365-372.

Dillon, P., S. Crosse, G. K. Stakelum, and F. Flynn. 1995. The effect of calving date and stocking rate on the performance of spring-calving dairy cows. Grass and Forage Science 50:286-299.

Evans, R. D., P. Dillon, D. P. Berry, M. Wallace, V. Ducrocq, and D. J. Garrick. 2006a. Trends in milk production, calving rate and survival of cows in 14 Irish dairy herds as a result of the introgression of Holstein-Friesian genes. Animal Science 82:423-433.

Evans, R. D., M. Wallace, L. Shalloo, D. J. Garrick, and P. Dillon. 2006b. Financial implications of recent declines in reproduction and survival of Holstein-Friesian cows in spring-calving Irish dairy herds. Agricultural Systems 89(1):165-183.

Giesy, J. G., M. A. McGuire, B. Shafii, and T. W. Hanson. 2002. Effect of Dose of Calcium Salts of Conjugated Linoleic Acid (CLA) on Percentage and Fatty Acid Content of Milk Fat in Midlactation Holstein Cows. J. Dairy Sci. 85(8):2023-2029.

Gumen, A., R. R. Rastani, R. R. Grummer, and M. C. Wiltbank. 2005. Reduced dry periods and varying prepartum diets alter postpartum ovulation and reproductive measures. Journal of Dairy Science 88(7):2401-2411.

Hansen, L. B. 2000. Consequences of selection for milk yield from a geneticist's viewpoint. Journal of Dairy Science 83(5):1145-1150.

Harris, B. L. and E. S. Kolver. 2001. Review of holsteinization on intensive pastoral dairy farming in New Zealand. J. Dairy Sci. 84(E-Suppl):E56-61.

Horan, B., P. Dillon, P. Faverdin, L. Delaby, F. Buckley, and M. Rath. 2005a. The interaction of strain of Holstein-Friesian cows and pasture-based feed systems on milk yield, body weight, and body condition score. Journal of Dairy Science 88(3):1231-1243.

Horan, B., J. F. Mee, P. O'Connor, M. Rath, and P. Dillon. 2005b. The effect of strain of Holstein-Friesian cow and feeding system on postpartum ovarian function, animal production and conception rate to first service. Theriogenology 63(3):950-971.

Horan, B., J. F. Mee, M. Rath, P. O'Connor, and P. Dillon. 2004. The effect of strain of Holstein-Friesian cow and feeding system on reproductive performance in seasonal-calving milk production systems. Animal Science 79:453-467.

Jarrige, J. 1989. INRAtion, 1989. V2.7. Micro soft computer program of ration formulation for ruminant livestock. 1. Dairy cows (J. B. Coulon and P. Faverdin). 2. Sheep (F. Bocquier and A. Brelurut). 3. Growing and finishing cattle (D. Micol, J. Robelin and J. Agabriel). 4. Suckler cows (J. Agabriel). 5. Goats (D. Sauvant). CNERTA, 26 Boulevard du Docteur Petit Jean 21000, Dijon, France.

Kay, J. K., T. R. Mackle, D. E. Bauman, N. A. Thomson, and L. H. Baumgard. 2007. Effects of a supplement containing trans-10, cis-12 conjugated linoleic acid on bioenergetic and milk production parameters in grazing dairy cows offered ad libitum or restricted pasture. Journal of Dairy Science 90(2):721-730.

Kay, J. K., J. R. Roche, C. E. Moore, and L. H. Baumgard. 2006. Effects of dietary conjugated linoleic acid on production and metabolic parameters in transition dairy cows grazing fresh pasture. Journal of Dairy Research 73(3):367-377.

Knight, C. H. 2001. Lactation and gestation in dairy cows: flexibility avoids nutritional extremes. Proceedings of the Nutrition Society 60(4):527-537.

Kolver, E. S., J. R. Roche, C. R. Burke, J. K. Kay, and P. W. Aspin. 2007. Extending lactation in pasture-based dairy cows: I. Genotype and diet effect on milk and reproduction. J Dairy Sci 90(12):5518-5530.

Mackle, T. R., J. K. Kay, M. J. Auldist, A. K. H. McGibbon, B. A. Philpott, L. H. Baumgard, and D. E. Bauman. 2003. Effects of Abomasal Infusion of Conjugated Linoleic Acid on Milk Fat Concentration and Yield from Pasture-Fed Dairy Cows. J. Dairy Sci. 86(2):644-652.

McCarthy, S., D. P. Berry, P. Dillon, M. Rath, and B. Horan. 2007a. Effect of strain of Holstein–Friesian and feed system on calving performance, blood parameters and overall survival. Livestock Science 111(3):218-229.

McCarthy, S., D. P. Berry, P. Dillon, M. Rath, and B. Horan. 2007b. Influence of Holstein-Friesian strain and feed system on body weight and body condition score lactation profiles. J Dairy Sci 90(4):1859-1869.

McCarthy, S., B. Horan, P. Dillon, P. O'Connor, M. Rath, and L. Shalloo. 2007c. Economic comparison of divergent strains of Holstein-Friesian cows in various pasture-based production systems. J Dairy Sci 90(3):1493-1505.

McCarthy, S. D., S. T. Butler, J. Patton, M. Daly, D. G. Morris, D. A. Kenny, and S. M. Waters. 2009. Differences in the expression of genes involved in the somatotropic axis in divergent strains of Holstein-Friesian dairy cows during early and mid lactation. J. Dairy Sci. 92(10):5229-5238.

Mertz, W. 1993. Chromium in human nutrition: a review. J Nutr 123(4):626-633.

Moore, C. E., H. C. Hafliger, O. B. Mendivil, S. R. Sanders, D. E. Bauman, and L. H. Baumgard. 2004. Increasing amounts of conjugated linoleic acid (CLA) progressively reduces milk fat synthesis immediately postpartum. Journal of Dairy Science 87(6):1886-1895.

Odens, L. J., R. Burgos, M. Innocenti, M. J. VanBaale, and L. H. Baumgard. 2007. Effects of varying doses of supplemental conjugated linoleic acid on production and energetic variables during the transition period. Journal of Dairy Science 90(1):293-305.

Patton, J., J. J. Murphy, F. P. O'Mara, and S. T. Butler. 2008. A comparison of energy balance and metabolic profiles of the New Zealand and North American strains of Holstein Friesian dairy cow. Animal 2(6):969-978.

Patton, J., J. J. Murphy, F. P. O'Mara, and S. T. Butler. 2009. Responses of North American and New Zealand strains of Holstein-Friesian dairy cattle to homeostatic challenges during early and mid-lactation. Animal 3(2):251-260.

Perfield, J. W., II, G. Bernal-Santos, T. R. Overton, and D. E. Bauman. 2002. Effects of Dietary Supplementation of Rumen-Protected Conjugated Linoleic Acid in Dairy Cows during Established Lactation. J. Dairy Sci. 85(10):2609-2617.

Pryce, J. E., M. P. Coffey, and G. Simm. 2001. The relationship between body condition score and reproductive performance. J Dairy Sci 84(6):1508-1515.

Rastani, R. R., R. R. Grummer, S. J. Bertics, A. Gumen, M. C. Wiltbank, D. G. Mashek, and M. C. Schwab. 2005. Reducing dry period length to simplify feeding transition cows: milk production, energy balance, and metabolic profiles. Journal of Dairy Science 88(3):1004-1014.

Reist, M., D. K. Erdin, D. von Euw, K. M. Tschumperlin, H. Leuenberger, H. M. Hammon, C. Morel, C. Philipona, Y. Zbinden, N. Kunzi, and E. W. Blum. 2003. Postpartum reproductive function: association with energy, metabolic and endocrine status in high yielding dairy cows. Theriogenology 59(8):1707-1723.

Santos, J. E. P., T. R. Bilby, W. W. Thatcher, C. R. Staples, and F. T. Silvestre. 2008. Long chain fatty acids of diet as factors influencing reproduction in cattle. Reproduction in Domestic Animals 43:23-30.

Shalloo, L., P. Dillon, M. Rath, and M. Wallace. 2004. Description and validation of the Moorepark Dairy System Model. Journal of Dairy Science 87(6):1945-1959.

Tyrrell, H. F. and J. T. Reid. 1965. Prediction of the energy value of cow's milk. J Dairy Sci 48(9):1215-1223.

Veerkamp, R. F. 1998. Selection for economic efficiency of dairy cattle using information on live weight and feed intake: a review. J Dairy Sci 81(4):1109-1119.

Veerkamp, R. F., P. Dillon, E. Kelly, A. R. Cromie, and A. F. Groen. 2002. Dairy cattle breeding objectives combining yield, survival and calving interval for pasture-based systems in Ireland under different milk quota scenarios. Livestock Production Science 76:137-151.

Villa-Godoy, A., T. L. Hughes, R. S. Emery, L. T. Chapin, and R. L. Fogwell. 1988. Association between energy balance and luteal function in lactating dairy cows. Journal of Dairy Science 71:1063-1072.

Wiebold, J. L. 1988. Embryonic mortality and the uterine environment in first-service lactating dairy cows. J Reprod Fertil 84(2):393-399.

5. Publications from this project

Peer review publications

Butler, S. T., L. Shalloo, J. J. Murphy. Extended lactations in a seasonal-calving pastoral system of production to modulate the effects of reproductive failure. *Journal of Dairy Science, Submitted May 2009.*

de Feu, M.A., A. C. O. Evans, P. Lonergan and S. T. Butler. The effect of dry period duration and dietary energy density on milk production, bioenergetic status and postpartum ovarian function in Holstein-Friesian dairy cows. Journal of Dairy Science, Fully accepted, August 2009.

de Feu, M. A., J. Patton, A. C. O. Evans, P. Lonergan, and S. T. Butler. 2008. The effect of strain of Holstein-Friesian cow on size of ovarian structures, periovulatory circulating steroid concentrations, and embryo quality following superovulation. Theriogenology 70:1101-1110.

Hutchinson, I. A., C. Stanton, M. J. de Veth, and S. T. Butler. Effects of CLA supplementation on milk production and reproductive performance of dairy cows. In preparation, Journal of Dairy Science.

McCarthy, S. D., S. T. Butler, J. Patton, M. Daly, D. G. Morris, D. A. Kenny, and S. M. Waters. 2009. Differences in the expression of genes involved in the somatotropic axis in divergent strains of Holstein-Friesian dairy cows during early and mid lactation. Journal of Dairy Science 92:5229-5238.

Patton, J., J. J. Murphy, F. P. O'Mara, and S. T. Butler. 2008. A comparison of energy balance and metabolic profiles of the New Zealand and North American strains of Holstein Friesian dairy cow. Animal 2:969-978.

Patton, J., J. J. Murphy, F. P. O'Mara, and S. T. Butler. 2009. Responses of North American and New Zealand strains of Holstein-Friesian dairy cattle to homeostatic challenges during early and mid-lactation. Animal 3:251-260.

Conference abstracts

Butler, S.T., L. Shalloo, and J. J. Murphy (2006). An evaluation of extended lactation as a strategy to alleviate reproductive loss in a seasonal pasture-based system. *Journal of Dairy Science;* 89 (Suppl. 1): 194. Presented at the ADSA/ASAS meeting in Minneapolis, MN July 9 – 13th, 2006.

Butler, S. T., M. A. de Feu, B. O' Brien, and J. J. Murphy (2007). The effect of dry period duration and dietary energy density in early lactation on the rennet gelation properties of milk. *Journal of Dairy Science;* 90 (Suppl. 1): 734. Presented at the ADSA/ASAS meeting in San Antonio, TX, July 8 – 12th, 2007.

Patton, J., S. T. Butler and J.J. Murphy. (2008). Responses of North American and New Zealand strains of Holstein Friesian to homeostatic challenges during early and mid lactation. Book of abstracts of the *59th Annual Meeting of the European Association for Animal Production*, page 156. Presented at the European Association of Animal Production (EAAP) annual meeting, Vilnius, Lithuania, August 24-27 2008.

Conference proceedings

Butler, S.T., L. Shalloo and J. J. Murphy (2006). The role of extended lactation to reduce the cost of high empty rates in seasonal spring calving dairy herds. In: *Moorepark Dairy Levy Research Update: Alternative options for dairying*, pages

26 – 32. Presented at the Dairy Levy Update Conference, Moorepark, January 11 and 12 2006.

Butler, S.T., L. Shalloo and J. J. Murphy (2006). An evaluation of extended lactation with a two year calving interval on a grass based system of production. Proceedings of the 2006 Agricultural Research Forum, page 70. Tullamore.

Butler, S. T. (2007). Conjugated linoleic acids and reproductive performance of dairy cows. In: Proceedings of the British Society of Animal Science international conference "Fertility in Dairy Cows – bridging the gaps", page 22. 30-31 August 2007, Liverpool, UK.

Butler, S.T. and M. J. de Veth (2007). Effects of CLA supplementation on milk production and reproductive performance of dairy cows. In: Proceedings of the British Society of Animal Science international conference "Fertility in Dairy Cows – bridging the gaps", page 20. 30-31 August 2007, Liverpool, UK.

de Feu, M. A., J. Patton, A. C. O. Evans, P. Lonergan, and S. T., Butler **(**2006). Effects of genotype of Holstein-Friesian cow on embryo quality following superovulation. Proceedings of the 2006 Agricultural Research Forum, page 28. Tullamore.

de Feu, M., A.C.O. Evans, P. Lonergan and S. T. Butler (2007). Effects of dry period duration and dietary energy density on transition period metabolic status and postpartum ovarian function in dairy cows. In: Proceedings of the British Society of Animal Science international conference "Fertility in Dairy Cows – bridging the gaps", page 41. 30-31 August 2007, Liverpool, UK.

Patton, J., O'Mara, F.P., and Butler, S.T., 2006. Effect of strain of Holstein-Friesian cow on response to metabolic challenges. Proceedings of the 2006 Agricultural Research Forum, page 85. Tullamore.