Developing novel supplementation strategies for dairy cows to improve nutrient efficiency and animal health and welfare.

End of Project Report to AgriSearch

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STRUCTURE OF REPORT

This report begins with an Executive Summary which provides the background to the research, details of the work undertaken, key findings, and practical implications.

The main body of the report describes three studies. From the first study there are three scientific papers (Chapters 1-3), from the second there are two (Chapters 4 and 5) and from the third there is one (Chapter 6). Two of these papers have already been published in scientific journals, while the remaining four are nearing submission. Papers contained within Chapters 1-4 are as follows:

Chapter 1: Law, R. A., Young, F. J., Patterson, D. C., Kilpatrick, D. J., Wylie, A. R. G., M. A. McCoy, Ferris, C. P. and Mayne, C. S. (2011) Effect of pre- and post calving dietary energy content on animal production and blood metabolites of dairy cows during lactation. *Journal of Dairy Science,* 94(2):808-823.

Chapter 2: Law, R. A., Young, F. J., Patterson, D. C., Kilpatrick, and Mayne, C. S. (2011) Effect of pre- and post-calving dietary energy content on behavioural activities of dairy cows in early and mid lactation. (for submission)

Chapter 3: Law, R. A., Young, F. J., Patterson, D. C., Kilpatrick, Wylie, A. R. G. and Mayne, C. S. (2011) Effect of pre- and post-calving dietary energy content on the fertility of dairy cows in early and mid lactation. (for submission)

These chapters examine the effects of dietary energy content, pre- and post-calving, on milk production, energy status, blood metabolite concentrations, behaviour and fertility in high yielding dairy cows.

Chapter 4: Young, F.J., C. S. Mayne, R. A. Law, D.C. Patterson, A.W. Gordon, H. Hartley and A. R. G. Wylie. (2011) The effect of nutritional strategy and individual animal management on the performance and fertility of Holstein Friesian dairy cows in early lactation. (for submission)

Chapter 5: Gilmore, H., Young, F.J., Patterson, D.C., Wylie, A., Law, R.A., Elliot, C. and Mayne, C.S. (2011) An evaluation of the effect of altering nutrition and nutritional strategies in early lactation on reproductive performance and oestrous behaviour of high yielding Holstein-Friesian dairy cows. *Journal of Dairy Science*, 94(7): 3510-3526.

These chapters examine the effects of various nutritional strategies aimed at improving the energy status and reproductive performance of the high-yielding dairy cow. An earlier version of Chapter 5 was included within the PhD thesis submitted by Hazel Gilmore (copy provided to AgriSearch). However, the version included within this report has been modified following peer review after being submitted to the Journal of Dairy Science.

Chapter 6: The effect of offering additional concentrates from either week-2, 6 or 10 of lactation on the performance of Holstein Friesian dairy cows

This chapter examines the effects of concentrate allocation strategy on production performance, energy status and the reproductive performance of the high yielding dairy cow.

The report finishes with a summary of key presentations and publications which have arisen from this work.

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EXECUTIVE SUMMARY

Intensive genetic selection has resulted in a dramatic increase in the milk production potential of the modern Holstein-Friesian dairy cow. However, this increase in milk production potential has been accompanied by a decline in functional traits including health, fertility and longevity. Furthermore, severe and prolonged periods of NEB can predispose the dairy cow to metabolic disorders, immunosuppression, reproductive failure, and behavioural abnormalities, all of which contribute to a decline in the cow's general well-being (Nielsen, 1999). Thus, minimising the extent and duration of NEB experienced by dairy cows would appear to be an important objective within dairy systems.

Chapter 1

- The objective of this study was to evaluate the effects of precalving nutrition on body condition score (BCS) at calving and on subsequent BCS loss during lactation. The study was also designed to evaluate the effect of pre- and post-calving nutrition, and their interactions, on feed intake, animal performance, and energy status during lactation.

- 80 high-yielding Holstein-Friesian dairy animals (40 primiparous and 40 multiparous) were allocated to one of 4 treatments based on a 2 x 2 factorial arrangement involving two levels of energy intake (high and low) precalving and two levels of energy intake (high and low) postcalving. Precalving treatments began -80 and -100 days precalving for primiparous and multiparous animals respectively.

- Multiparous animals offered a high energy diet precalving had a significantly higher body condition score at calving than those offered the low energy diet precalving; however, the numerical difference was relatively small (0.27 units). Body condition score was not affected by precalving treatment in primiparous animals.

- Precalving diet had no significant effect on plasma non-esterified fatty acids (NEFA) concentrations during the last 3 weeks precalving in primiparous or multiparous animals.

Primiparous animals offered a high energy diet precalving had significantly higher postcalving plasma NEFA concentrations, suggesting greater mobilisation of body reserves (Figure 1.1). Non-esterified fatty acids were higher in weeks 2, 3, & 4 postcalving in multiparous animals offered a high energy precalving diet compared to those offered a low energy precalving diet.

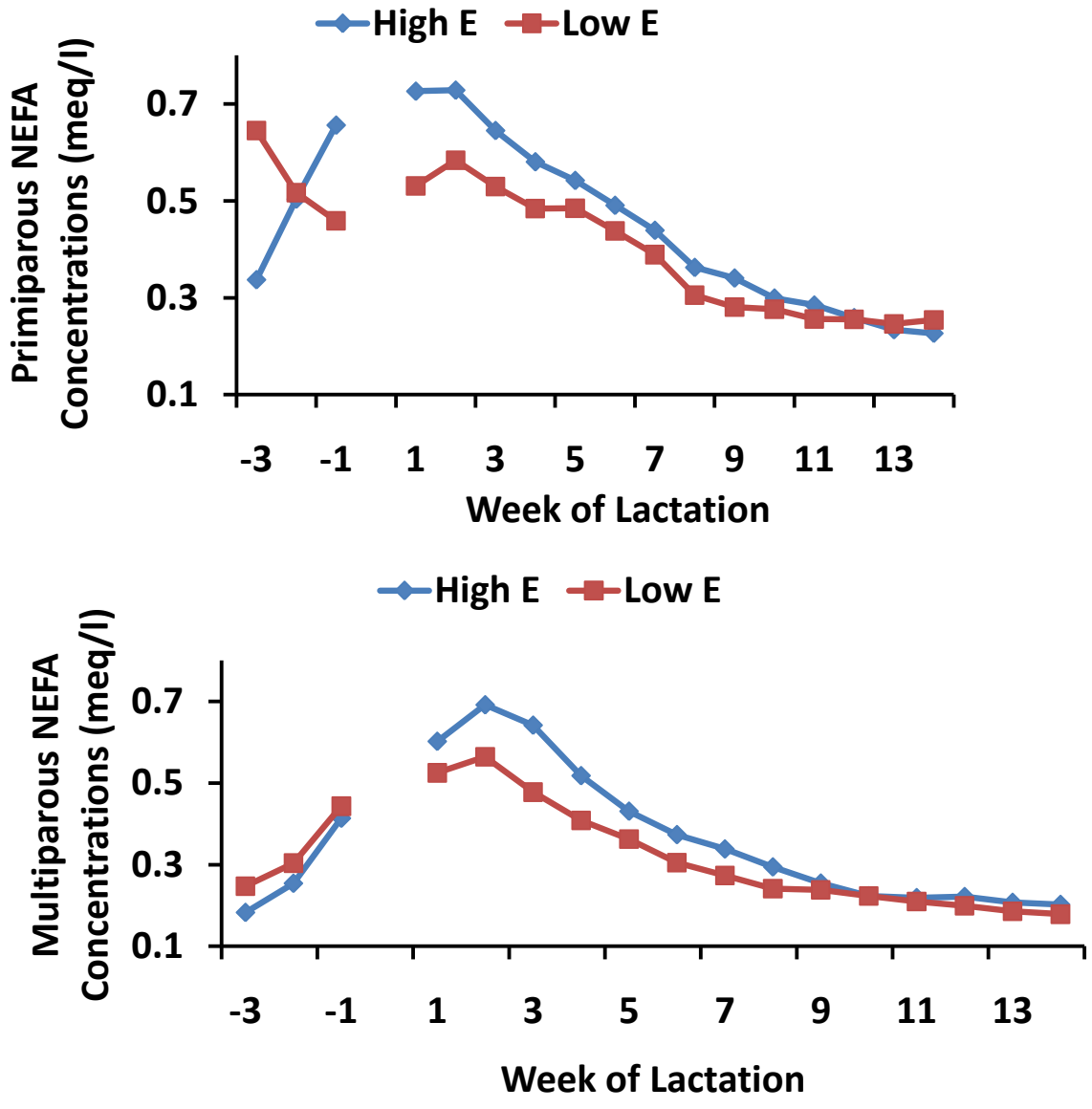


Figure 1.1: NEFA concentrations of primiparous and multiparous animals offered two levels of energy intake (high and low), precalving.

- Primiparous and multiparous animals offered a high energy diet postcalving had a significantly higher dry matter intake (DMI), milk yield, and energy status postcalving compared to animals offered a low energy diet postcalving.

- Precalving treatment had no significant effect on daily or cumulative energy balance. However, precalving treatment did effect the energetic response to postcalving treatment (pre-*post-calving interaction) indicating that precalving nutrition has an influence on the postcalving energy trajectory (Figure 1.2).

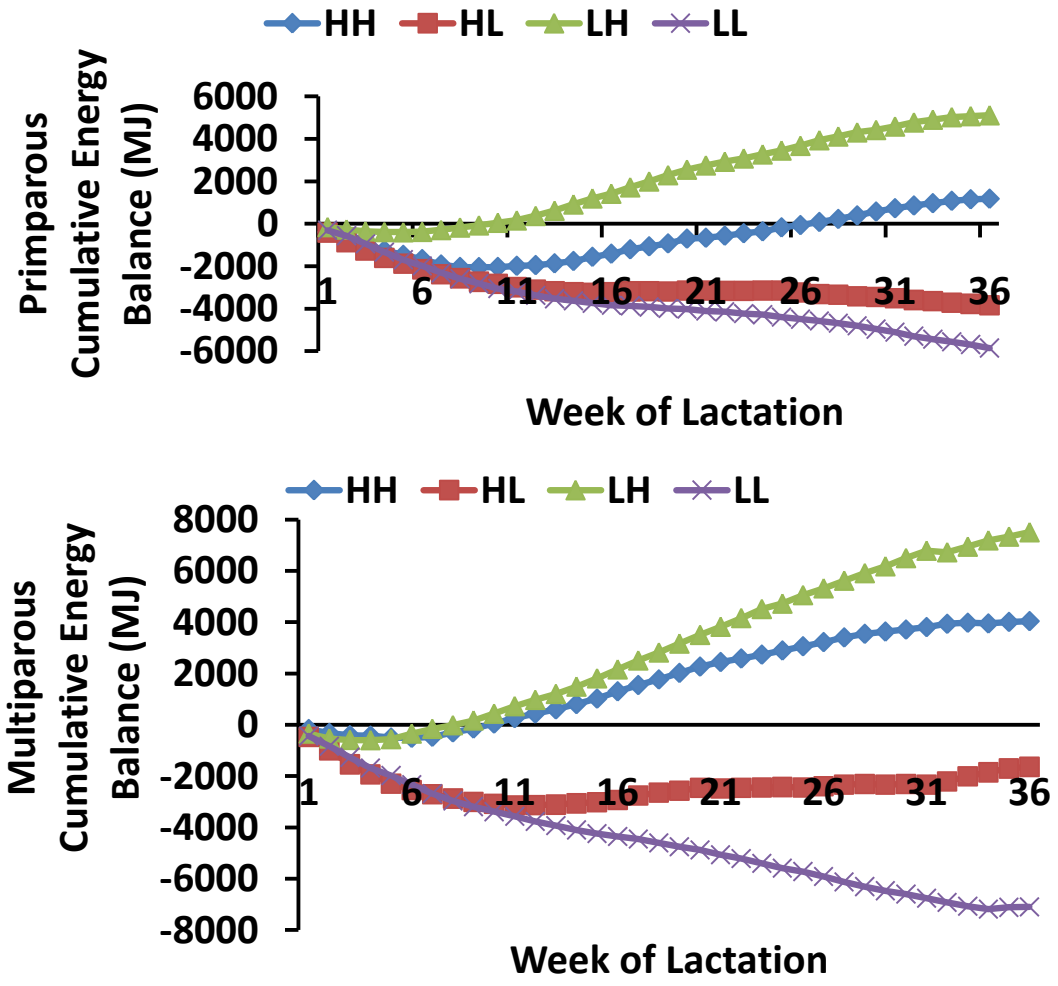


Figure 1.2: Cumulative energy balance of primi- and multi-parous animals offered two levels of energy intake (high and low), pre- and post-calving.

- In the current study, it is concluded that:
    a. Attempting to substantially alter BCS during the dry period is a difficult task and should be addressed well in advance of drying off.
    b. Feeding a low energy precalving diet reduced BCS loss postcalving and supported a more rapid return to positive energy status in animals offered a high plane of nutrition postcalving. This would suggest that initiating BCS loss during the dry period reduces mobilisation postpartum and conditions the liver to better deal with higher NEFA concentrations in early lactation.
    c. Postcalving, a low plane of nutrition is detrimental to both milk production and energy balance, and is not suitable for the high-yielding dairy cow.
    d. Energetic responses to high and low energy diets, pre- and post-calving, in the current study highlight the importance of addressing a dairy cow's energy status precalving, as well as during early lactation.

Chapter 2

- Changes in the expression of behavioural activities have been characterised as a mechanism by which an animal adapts to the environment in which it is placed (O'Connell et al., 1989). It is generally accepted that 'normal' behaviour patterns are associated with good animal welfare, while deviations from normal behaviour patterns are indicative of stress. This study was designed to examine relationships between energy balance and behavioural patterns in an attempt to identify possible behavioural indicators of metabolic stress and compromised welfare.

- It was hypothesized that animals experiencing more severe NEB will display different behavioural patterns to animals experiencing less severe NEB. Furthermore, it is hypothesised that cows in severe negative energy balance will show a greater motivation to feed than to lie down after a period of lying deprivation, such as during milking.

- 40 high-yielding Holstein-Friesian dairy animals (multiparous) were allocated to one of 4 treatments based on a 2 x 2 factorial arrangement involving two levels of energy intake

(high and low) precalving and two levels of energy intake (high and low) postcalving, as described in Chapter 1.

- Maintenance and feeding behaviours were recorded. Maintenance behaviour included lying idle, lying ruminating, standing idle, standing ruminating, walking, feeding and drinking. All animals were observed for one 24-hr period every 14 d, from calving until all animals had reached day-140 postpartum.

- Precalving treatment had no effect ($P > 0.05$) on any of the 24-hr behaviours measured postcalving.

- Animals offered the high energy postcalving diet had a significantly lower total ruminating time, ruminating time per unit dry matter intake, time spent lying ruminating, and average length of lying bouts compared to those offered the low energy diet. The reverse was true for dry matter intake, individual meal intakes, eating rate, time spent lying idle and the number of lying bouts per day.

- Daily energy balance was significantly correlated with the majority of recorded behavioural activities including time spent standing ruminating in the two hr period post evening milking (r^2, 0.748). A number of additional potential explanatory factors were examined in a multiple regression analysis which showed that daily energy balance, milk yield and locomotion score were significantly correlated with time spent standing ruminating in the two hr period, post evening milking (r^2, 0.841; Figure 1.3).

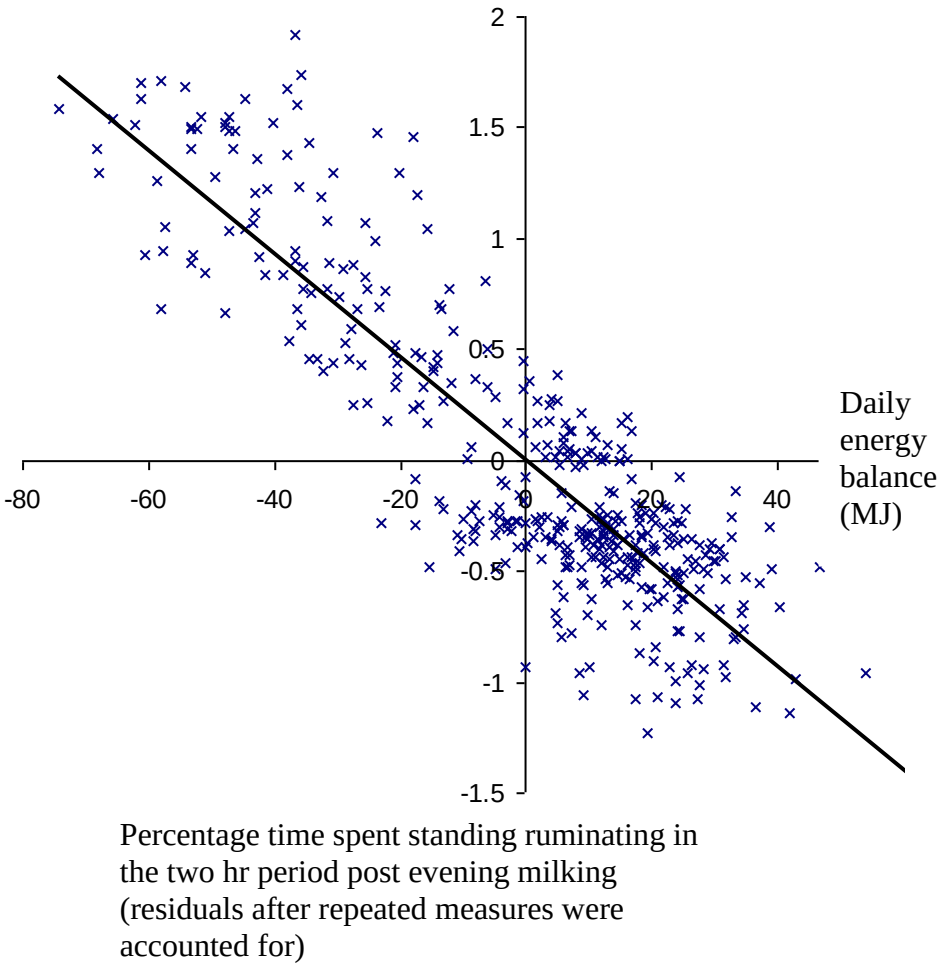


Figure 1.3: Regression of daily energy balance against the percentage time spent standing ruminating in the 2 hr period post evening milking, $r^2 = 0.748$

- Significant associations between average DEB and behavioural activities highlight potential to develop indicators of energy imbalance. However, these results are specific to this study and further work is needed before solid conclusions can be made on the link between energy balance and dairy cow behavioural patterns.

Chapter 3

- The historical decline in reproductive performance of the high-yielding dairy cow has resulted in huge cost for dairy farmers. This decline has occurred over many years and reproductive failure is now the predominant reason for culling dairy cows (Mayne *et al.*, 2002). Elevated culling rates increases the need for replacements, therefore reducing the average age of the herd and subsequently reducing the herd's overall milk yield potential. There is a common consensus that negative energy balance (NEB) is one of the most important factors contributing to this decline in reproductive performance (Webb *et al.*, 1999).

- As described in Chapter 1, 80 high-yielding Holstein-Friesian dairy animals (40 primiparous and 40 multiparous) were allocated to one of 4 treatments based on a 2 x 2 factorial arrangement involving two levels of energy intake (high and low) precalving and two levels of energy intake (high and low) postcalving. Precalving treatment began -80 and -100 days precalving for primi- and multi-parous animals respectively.

- It was hypothesized that animals experiencing more severe NEB would have poorer reproductive performance than animals with less severe NEB. The effects of cow energy status, as well as nutritional effects, on reproductive performance were examined in this study.

- Pre- and post-calving dietary treatment, and body condition score at calving, had no significant effect on any of the reproductive parameters examined. The average pregnancy rate to first service was low (25%), with the average number of services per conception being 2.8. The average 100-day in-calf rate (100 days from the beginning of the breeding period) was 58% and overall, 86% of cows intended for breeding conceived during the six month breeding period.

- Post-calving dietary treatment had a significant effect on average DEB. The range in DEB for individual animals during the first 21 days of lactation was +33 to -114 MJ/d.

The interval to the commencement of luteal activity was negatively associated with cumulative energy balance and a change in IGF-1 concentrations, and positively associated with weeks to energy nadir.

- On an individual cow basis, body energy status had a significant influence on some important reproductive parameters, with a more severe negative energy balance in early lactation resulting in a greater amount of irregularity in ovarian cyclicity.

- The incidence of a prolonged inter-ovulatory interval, which is indicative of poor fertility, was high in the current study. Furthermore, a high number of animals experiencing prolonged luteal phases in the absence of pregnancy will have reduced reproductive performance.

- The high irregularity of ovarian cyclicity in this experiment is likely to be the main reason for the poor pregnancy rate to first service and the relatively poor 100-day in-calf rates.

- The extent of negative energy balance in early lactation can be reduced by a nutritional strategy involving reduced energy intake pre-calving, followed by high levels of energy intake in early lactation.

- Early lactation energy balance is only one component of a multitude of factors that affect fertility; however, addressing the issue of metabolic stress in early lactation will undoubtedly improve the probability of reproductive success.

Chapter 4

- Chapter 4 marks the commencement of a new study and focuses on further developing feeding strategies to reduce negative energy balance in early lactation and improve the fertility of the high-yielding dairy cow.

- In the literature, various nutritional interventions have been applied experimentally in an effort to minimise the degree of negative energy balance in early lactation and to improve subsequent fertility. One approach, that of reducing dietary crude protein concentrations, was shown by Law et al. (2009) to be an effective technique by which to alleviate NEB in early lactation; however, this also had a negative impact on milk production when adopted throughout lactation. An alternative approach is to design diets to improve reproductive performance. For example, offering either glucogenic or lipogenic diets, or a sequential combination of both, has been suggested as one such approach. The inclusion glucogenic nutrients have been shown to increase circulating insulin concentrations which, in turn, are associated with reducing the interval to the onset of luteal activity. Lipogenic nutrients on the other hand are thought to benefit blastocyst growth.

- The aims of the current study were to assess the performance (including a range of fertility measures) of Holstein-Friesian dairy cows subjected to two contrasting nutritional strategies. These were (i) the manipulation of dietary protein content so as to regulate energy balance and limit the extent of NEB in individual animals in early lactation and (ii) biphasic alteration of dietary starch and dietary fat contents in early-to-mid lactation to encourage, initially, the resumption of ovarian cyclicity and, subsequently, embryo survival.

- In this study, 81 Holstein-Friesian dairy cows (30 primiparous and 51 multiparous of mean parity 2.1) were allocated to one of three experimental treatments: control (C), an individual cow management protocol (ICM) or a sequential glucogenic-lipogenic diet combination (GL) applied from calving until d 210 of lactation. Cows on the control treatment were offered a standard total mixed ration (TMR) containing 176 g of CP / kg DM. The ICM treatment was designed to keep individual cows within a target energy balance range by feeding, as appropriate, one of three diets of contrasting crude protein contents (174, 147 and 200 g of CP / kg DM) reflecting a standard, low and high protein content, respectively. The GL treatment entailed feeding a high-starch diet (177 g rising to 277 g of starch / kg DM) for the first 50 d of lactation and then switching to a high-fat diet (63 g fat / kg DM) from d 51 to d 120.

- There were no significant treatment effects on milk yield or milk composition (fat, protein, lactose and casein nitrogen). Milk urea nitrogen content was reduced by GL compared to C or ICM. Total dry matter intake was increased by ICM when compared to C or GL and this was largely a result of an increase in the dry matter intake of the forage component of the diet. Concentrate dry matter intake was not significantly affected by treatment.

- Both daily and cumulative energy balance were higher for cows on ICM than for cows on C or GL treatments, but live weight and body condition score were unaffected by treatment.

- Plasma BHB and urea concentrations were significantly lower on the GL treatment and blood glucose levels were unaffected by treatment.

- Cow fertility measures were largely unaffected by treatment with only 'days to 1st observed heat' increased by ICM when compared with C or GL cows.

- Overall, the results indicated that production and fertility parameters were unaffected by diet or nutritional strategy. However, results have shown that altering the dietary crude protein concentration at specific points in lactation is an effective approach through which to lessen the severity of the negative energy balance in dairy cows in early lactation without detriment to milk yield in the long term. However, it was also clear that not all animals needed dietary intervention in order to remain within a daily energy balance target. Further investigation of this approach with larger numbers of animals may prove useful in developing future nutritional strategies to minimise NEB in the critical early lactation period.

- Ultimately, fertility problems in the high-yielding Holstein Friesian are multi-factorial in origin, with nutrition being only one contributor. Further work is required with larger numbers of animals before a definitive nutritional impact on Holstein Friesian dairy cow fertility can be identified.

Chapter 5

- The aim of this study was to compare the effectiveness of four nutritional strategies in improving the reproductive performance of high-yielding dairy cows. It was hypothesized that offering cows a high starch ration in early lactation would enhance the onset of luteal activity, and that reducing the severity of negative energy balance in the early postcalving period would improve reproductive parameters.

- This study involved 96 high-yielding dairy cows. Cows were allocated to either a control diet (C), an individual cow management protocol (ICM), a sequential glucogenic-lipogenic diet combination (GL), or a low protein diet supplemented with methionine (LP). Diets were offered from calving until d 210 of lactation. Cows on the control treatment were offered a standard total mixed ration (TMR) containing 176 g of CP / kg DM. The ICM treatment was designed to keep individual cows within a target energy balance (EB) range by feeding, as appropriate, one of three diets of contrasting crude protein contents (174, 147 and 200 g of CP / kg DM) reflecting a standard, low and high protein content, respectively. The GL treatment entailed feeding a high-starch diet (177 g rising to 277 g of starch / kg DM) for the first 50 d of lactation and then switching to a high-fat diet (63 g fat / kg DM) from d 51 to d 120. The LP diet contained 140g CP/kg DM and was supplemented with protected methionine at an inclusion level of 40g per animal per d.

- The nutritional strategies implemented in this study had no significant effect on cow fertility measures, including the onset of luteal activity, conception rate, 100d in-calf rate and the incidence of atypical cycles. The individual cow feeding strategy improved energy balance in early lactation but had no benefit on conception rate (CR) to first insemination. However, CR to second insemination, 100 d pregnancy rate (from the commencement of breeding), and overall pregnancy rate tended to be higher ($P > 0.05$) in this group. The GL treatment tended to decrease the proportion of delayed ovulations and increase the proportion of animals cycling by day-50 postcalving. Although the concept of offering high starch diets in early lactation (to reduce the interval from calving to commencement of cyclicity), followed by a high fat diet (to improve embryo quality)

appears to be based on sound scientific principles, the results of the current experiment provide no evidence of an increase in overall conception rates with the 'fertility improver' ration. It is possible that this lack of response was related to the relatively low numbers of cows on each of the diets, and that a different result may have been obtained if much larger group sizes had been used.

- With regards to oestrous behaviour, results indicate that as the size of the sexually active group increased, the intensity of oestrus and the expression of mounting or attempting to mount another cow also increased. Furthermore, cows that became pregnant displayed more intense oestrous behaviour than cows that failed to become pregnant.

Chapter 6

- While many studies have examined the response of dairy cows to concentrate supplementation at a fixed point in time during the lactation, few studies have compared the response to concentrates offered at different stages of the lactation.

- This experiment was conducted to examine the effect of introducing additional concentrates into the diet of lactating dairy cows at three time points in early/mid lactation, namely at week-2 (pre peak-yield but at the time of maximum lipid mobilisation and milk yield acceleration), week-6 (peak-yield) and week-10 (after peak milk yield and peak lipid mobilisation) of lactation. The study was designed to measure the food intake, milk production, tissue change and fertility responses to additional concentrate supplementation at these times.

- The study involved 80 winter calving Holstein cows, 40 primiparous and 40 multiparous. All animals were offered a basal diet containing proportionally 0.5 concentrate and 0.5 forage on a dry matter basis. The forage component of the diet comprised proportionally 0.5 grass silage and 0.5 maize silage (DM basis). An additional 4.0 kg of concentrate was introduced into the diets of cows at weeks 2, 6 and 10 post calving. Cows remained on their treatment diets until week-28 of lactation.

- Within the current experiment total DM intake increased with the inclusion of additional concentrates in the diet (although not always significantly so), with the magnitude of the intake response similar irrespective of stage of lactation when the additional concentrates were offered. The intake responses achieved were normally observed within 2 – 4 weeks of the additional concentrates being offered.

- When examined over the entire experimental period, the inclusion of additional concentrates in the diet at 2, 6 or 10 weeks post calving had no effect on milk yield, milk fat + protein yield or milk composition with cows of either parity. However, the absence of a significant response may reflect the high concentrate inclusion level in the Control diet (proportionally 0.53, DM basis) and the relatively high quality silage offered within the study.

- Nevertheless, examining milk yield responses over the entire period tended to mask the immediate milk yield responses observed during the periods immediately after the additional concentrates were offered. With both primiparous and multiparous cows, while most of the milk yield and fat plus protein yield responses tended to be non-significant, there was evidence that the response to additional concentrates declined with stage of lactation, being greatest at week-2 post calving, and lowest at week-10 post calving. This supports the findings of a study undertaken almost 40 years ago involving cows of a much lower yield potential.

- Within the current study the expectation might have been that these higher yielding cows would have had the genetic potential to respond to additional concentrates in mid lactation. However the absence of a response may reflect the high quality basal diet offered. Indeed the results of the current study suggest that when high yielding cows are offered a quality diet in mid lactation, their potential to exhibit a milk yield response to additional concentrates is limited.

- Treatment had remarkably few effects on body tissue reserves within the current study. The exception to this was live weight, which was higher with multiparous cows on

treatments S6 and S10 at the end of the study, compared to the Control treatment. A similar although non-significant trend was observed with body condition score.

- In contrast, both primiparous and multiparous cows on the Control treatment remained in negative energy balance for considerably longer than cows on any other treatment. This more prolonged period of negative energy balance with the Control treatment reflects the fact that intakes were significantly lower with this treatment, and yet milk yield was not significantly affected by treatment (over the entire lactation).

- The increased levels of tissue deposition observed with the multiparous cows offered concentrates at 6 and 10-weeks post calving reflects the numerically higher intakes of these cows, the higher ME content of the diets offered, and the absence of a significant milk yield response to additional concentrates as lactation proceeded.

- That no such increase in tissue deposition was observed with the primiparous cows is perhaps surprising given the more dramatic lift in food intake following concentrate supplementation, and the much reduced trend for a milk yield response. The need for primiparous cows to partition energy toward growth, especially during their first lactation, may provide an explanation for how part of the additional energy was utilised.

- The almost immediate change in trajectory of the energy balance curves after additional concentrates were offered demonstrate that relatively small increases in additional concentrate supplementation during early lactation can be used to manipulate energy balance. Indeed, given the clear relationship that has been established between dairy cow fertility and the extent of negative energy balance, enhanced fertility with treatments S2, S6 and S10 might have been expected in the current study. This is especially true in view of the fact that cows on the Control treatment remained in negative energy balance for considerably longer than cows on any other treatment. Nevertheless, in common with other studies involving relatively small numbers of cows, there was no evidence that any aspect of dairy cow fertility performance was affected by treatment.

CHAPTER 1: Effect of Precalving and Postcalving Dietary Energy Level on Performance and Blood Metabolite Concentrations of Dairy Cows throughout Lactation

ABSTRACT

The effects of the level of energy intake (high E and low E) offered before and after calving, on body condition score at calving, production performance and energy status in the first 250 d of lactation were evaluated in a 2x2 factorial design experiment involving 80 Holstein-Friesian dairy animals (40 primiparous and 40 multiparous). From day 80 until day 21 precalving, primiparous animals were offered either high or low pasture allowances. Thereafter, these animals were housed and had *ad libitum* access to a high energy density diet (high E) or restricted access (6 kg dry matter (DM) /d) to a low energy density diet (low E), respectively, until calving. From day 100 until day 42 precalving, multiparous animals were offered either *ad libitum* or restricted (10 kg DM / d) access to a late lactation diet, and thereafter, had *ad libitum* access to a high E diet or restricted access (7 kg DM complete diet / d) to a low E diet, respectively, until calving. The forage to concentrate (F:C) ratios (DM basis) of these high E and low E diets [d 42 (d 21 in primiparous animals) until calving] were 64:36 and 83:17, respectively. Cows offered high E and low E precalving diets were allocated to either a high E or low E postcalving diet [F:C ratio (DM basis) of 30:70 and 70:30 respectively] and remained on these diets until d 250 of lactation. Multiparous animals offered a high E diet precalving had a significantly higher body condition score at calving than those offered the low E diet precalving. This effect was not evident in primiparous animals. Precalving diet had no significant effect on plasma NEFA concentrations during the last 3 weeks precalving in primi- or multi-parous animals. Primiparous animals offered a high E diet precalving had significantly higher postcalving plasma NEFA concentrations, suggesting a greater mobilisation of body reserves. Primi- and multi-parous animals offered a high E diet postcalving had a significantly higher dry matter intake (DMI), milk yield, and energy status postcalving compared to animals offered a low E diet postcalving. Milk yields of primi- and multi-parous animals offered high E and low E diets postcalving were 29.7 and 24.8 kg / d, and 33.5 and 28.2 kg / d respectively. It is concluded

that altering body condition score during the dry period is difficult but that specific dietary regimes applied precalving can have a significant influence on postcalving production and energy-related parameters.
Keywords: Precalving nutrition, milk yield, energy balance, plasma NEFA

INTRODUCTION

Intensive genetic selection for productivity has resulted in a dramatic increase in the milk production potential of the modern high-yielding dairy cow. However, in general the observed increases in milk energy output in early lactation have not been matched by a proportionate increase in energy intake (Veerkamp et al., 1995; Ingvarsten et al., 1999). The resulting energy deficit [negative energy balance (NEB)] leads to a loss of body condition. A positive correlation between milk production potential and reductions in BCS during early lactation was previously demonstrated by Ruegg and Milton (1995). Severe and prolonged NEB may also predispose the animal to metabolic disorders, immunosuppression, and behavioural abnormalities, all of which can affect production, fertility and the cow's general well-being (Nielsen, 1999). Reducing NEB and the accompanying BCS loss is likely to help minimize some of these problems.

While the relationship between milk yield and DMI postcalving will influence the extent and the duration of NEB, both of these parameters will also be affected by BCS at calving. For example, BCS at calving has been positively correlated with the rate of BCS loss postcalving (Kokkonen et al., 2005), with fat animals having a greater energy deficit (lower intake relative to milk yield) in early lactation than thin animals. Friggens et al. (2004a) argued that the nutritional manipulation of cows to be either fatter or leaner than normal (BCS of 2.5 - 3.25) at calving provokes a change in the subsequent rate of BCS loss such that normal levels of body fatness are re-established approximately 3 to 4 months after calving. This suggests that there is a genetic component to postcalving BCS loss (Friggens et al., 2004a) and that the extent to which this is expressed is influenced by the difference between actual BCS at calving and that of the genetically desirable state. It may be that only when BCS loss exceeds this genetically pre-determined rate, do dietary effects influence the energetic trajectory of these high-yielding

animals. Garnsworthy and Topps (1982) demonstrated that a higher BCS at calving suppressed DMI after parturition, resulting in more severe NEB. This suggests that energy intake may be a response dictated by BCS loss. Thus, minimising genetically driven BCS loss in early lactation, by avoiding over-fat cows, may alleviate the suppression of DMI and maximise the potential impact of postcalving nutrition in reducing BCS loss. Quantifying the effects of precalving nutrition on BCS at calving and subsequent effects on milk production, DMI, and BCS loss during lactation may allow a fuller understanding of factors affecting energetic responses to postcalving feeding strategies and more successful exploitation of such strategies.

Previous data on the effects of precalving nutrition on BCS at calving and subsequent postcalving performance are conflicting. Douglas et al. (2006) demonstrated that restricting DMI to 80% of requirements during the precalving period resulted in a lower BCS precalving and improved DMI postcalving. In support of this, Dann et al. (2006) identified significant effects of restricting DMI to 80% of requirements during the precalving period on postcalving energy balance, with a high plane of nutrition in the 'far-off' period (between drying-off and 25 d before calving) resulting in more severe NEB in the first 10 d postcalving. However, the latter group noted no significant effect of precalving treatment on DMI or milk yield during the postcalving period. Furthermore, there was no significant effect of nutrition in the close-up period (from 24 d before calving until calving) on energy balance during this period. Agenas et al. (2003) found significant effects of precalving dietary energy intake (71, 106, and 177 MJ / d) on BCS at calving but not on postcalving DMI, milk yield, or energy balance, whilst Winkelman et al. (2008) found no direct effects of precalving nutrition (feeding to requirement vs. ad libitum) on BCS pre- and post-calving or on energy balance postcalving.

The objective of the current study was to evaluate the effects of precalving nutrition on BCS at calving, and on subsequent BCS loss during lactation. The study was also designed to evaluate the effect of pre- and post-calving nutrition, and their interactions, on feed intake, animal performance, and energy status during lactation.

MATERIALS AND METHODS

Animals and Housing

This experiment involved 80 Holstein-Friesian dairy animals [40 primiparous and 40 multiparous (mean parity, 3.2; SEM, 0.06)], calving between 27th August and 21st December 2004 (mean calving date 16th October; SEM, 3.4). Primiparous refers to animals calving for the first time and multiparous refers to animals calving for the second time or greater. Primiparous animals were housed from 21 d before calving while multiparous animals were housed for the entire duration of the precalving period. Primiparous animals were penned separately from multiparous animals in the same free stall house with concrete flooring. Following calving, all animals were housed as a single group in a free stall house with concrete flooring. The cubicle to cow ratio was ≥ 1:1 at all times, thus meeting the recommendations of FAWC (1997). Cubicles (2.20m x 1.25m) were fitted with rubber mats and bedded with sawdust thrice weekly. Concrete passageway floors were scraped a minimum of four times daily by an automated system. Lights were left on in the cow house at all times.

Experimental Design, Diets and Feeding

In the current experiment, treatments were in a 2 x 2 factorial arrangement involving two levels of energy intake (high E and low E) precalving and two levels of energy intake (high E and low E) postcalving. Primiparous animals were assigned to precalving treatments in a balanced manner based on heifer rearing regime, calving date, BCS and BW. Multiparous animals were assigned to precalving treatments in a balanced manner according to parity, milk yield, calving date, BCS and BW. From d 80 until d 21 precalving, primiparous animals were offered either high or low pasture allowances (25 or 10 kg DM / animal / d, respectively, assessed above 5cm cutting height). Thereafter, animals offered the high and low pasture allowances were housed and had *ad libitum* access to a high energy density diet (high E) and restricted access (6 kg DM / d) to a low energy density diet (low E), respectively, until calving. Animals were fed individually and dietary restriction was achieved using electronic feeding gates (American Calan Inc., Northwood, New Hampshire, USA) which were programmed to prevent individual animals gaining access to food after they had consumed their daily allocation of diet.

From d 100 to d 42 precalving (late lactation period), multiparous animals were offered either *ad libitum* or restricted (10 kg DM / d) access to a late lactation diet. This was a complete diet with a forage to concentrate (F:C) ratio of 36:64. The forage component consisted of grass silage and maize silage (1:1 ratio; DM basis). Concentrate composition and analysis is presented in Table 1.1. From d 42 precalving (drying off) until calving, animals offered the *ad libitum* and restricted late lactation diets had *ad libitum* access to a high energy density diet (high E) and restricted access (7 kg DM complete diet / d) to a low energy density diet (low E), respectively. The F:C ratios (DM basis) of high E and low E diets [d 42 (d 21 in primiparous animals) until calving] were 64:36 and 83:17, respectively (Table 1.3). Dietary restriction was achieved using electronic feeding gates as described previously. The forage component of both high E and low E diets was grass silage.

Postcalving, animals managed on the high E and low E precalving treatments were offered *ad libitum* access to either a high (high E) or low (low E) energy lactation diet and remained on these diets until d 250 of lactation. The F:C ratios of the high E and low E postcalving diets were 30:70 and 70:30 respectively (DM basis), providing 12.5 and 11.7 MJ of metabolisable energy (ME) / kg DM respectively (Table 1.3). The forage component of the postcalving diet consisted of grass silage and maize silage (1:1 ratio, DM basis). Concentrate composition and analysis is presented in Table 1.1. The energy and protein concentrations of individual ingredients were based on published values (AFRC, 1993).

Primiparous animals were assigned to postcalving treatment according to precalving treatment, calving date, and BCS and BW at d 21 precalving. Multiparous animals were assigned to postcalving treatment according to precalving treatment, parity, previous 305-d milk yield, calving date, and BCS and BW at d 21 precalving. Consequently, there were four treatments groups: high E precalving, high E postcalving (HH); high E precalving, low E postcalving (HL); low E precalving, high E postcalving (LH); low E precalving, low E postcalving (LL). The CP contents of pre- and post-calving diets were maintained at 170 and 190 g/kg DM respectively. Animals on the low E postcalving diet received an additional 50 g / d of a mineral preparation to ensure that total mineral intakes were similar on both treatments. Diets were prepared daily in a

mixer wagon and offered as a total mixed ration (TMR) between 1000 and 1100h via feed boxes placed on a series of computer-linked load-cells. Access to feed boxes was controlled by an electronic identification system (described previously), enabling fresh intakes of individual animals to be recorded continuously. Dry matter intakes were then calculated using daily oven DM values for grass and maize silage and fortnightly oven DM values for concentrates, as detailed below. Daily intakes were used to calculate an average daily intake for each week of lactation. Postcalving, diet allocation included a target excess of 7 percent daily and uneaten material was removed thrice weekly.

Table 1.1: Ingredient composition and chemical composition of the concentrate component of the diet offered in late lactation, during the dry period and postcalving.

Constituent	Inclusion rate (g/kg, DM)		
	Late lactation	Dry cow[3]	Lactation
Barley (milled)	207	162	162
Wheat (milled)	207	162	162
Unmolassed sugar beet pulp	180	132	132
Citrus pulp	178	162	162
Soya bean meal (Hi-Pro)	63	260	260
Rape meal	56	41	41
Rumen-inert fat[2]	29	22	22
Dairy cow minerals	46	34	34
Molasses[1]	34	25	25
ME (MJ/kg DM)	12.8	13.1	13.1
CP (g/kg DM)	136	220	220
ADF (g/kg DM)	110	109	109
NDF (g/kg DM)	211	217	217
Ash (g/kg DM)	84.8	83.9	83.9
Starch (g/kg DM)	280	218	218

[1] Molaferm, United Molasses, Belfast, NI, UK

[2] Megalac, Volac Ltd. Orwell, Hertfordshire, UK

[3] Dry cow mineral was added to dry cow diet at a level of 120 grams / head / day; Trouw Nutrition, Belfast, NI, UK

Table 1.2: Chemical composition of grass silage and maize silage as offered throughout the experiment.

Analysis	Grass Silage[1]			Maize Silage	
	Late lactation	Dry Period	Postcalving	Late lactation	Postcalving
Oven DM (g/kg)	170	198	228	297	289
VCODM[2] (g/kg)	185	215	248	311	302
pH	3.12	3.59	3.99	3.56	3.68
Composition of DM (g/kg)					
CP	136	141	147	78	86
Ammonia nitrogen (g/kg N)	125	129	101	91	96
Ethanol	3.7	3.7	4.5	-	-
Propanol	0.8	0.9	0.6	-	-
Lactic acid	17	17	21	-	-
Acetic acid	6.4	6.1	5.2	-	-
Propionic acid	0.5	0.4	0.6	-	-
n-butyric acid	1.7	1.2	1.0	-	-
ADF	320	325	315	275	288
NDF	564	565	536	528	552
Ash	72	75	82	37	38
Starch	-	-	-	219	200
Gross energy (MJ/kg DM)	17.1	16.9	18.7	18.1	18.2
Metabolisable energy (MJ/kg DM)[3]	10.3	10.3	11.4	11.1	10.6

[1]During late lactation and the dry period grass silages were produced from primary re-growth herbage. Postcalving, grass silage was produced from predominantly primary growth herbage

[2] VCODM, volatile corrected oven DM

[3] Metabolisable energy determined by near infrared reflectance spectroscopy (Park et al., 1998)

Table 1.3: Composition of TMR as fed, indicating DM, CP and ME contents.

	High precalving[3]		Low precalving[3]			High postcalving			Low postcalving[4]			
	Conc.[1]	Grass silage	Conc.[1]	Soya	Grass silage	Conc.[1]	Grass silage	Maize silage	Conc.[1]	Soya	Grass silage	Maize silage
Proportion of diet	360	640	120	50	830	700	150	150	165	135	350	350
DM content (g/kg)	870	198	870	870	198	870	228	289	870	870	228	289
CP (g/kg DM)[2]	220	141	220	540	141	220	147	86	220	540	147	86
ME (MJ/kg DM)[2]	13.1	10.3	13.1	13.4	10.3	13.1	11.4	10.6	13.1	13.4	11.4	10.6
Total ME (MJ/kg DM)[2]	11.3		10.8			12.5			11.7			
Total CP (g/kg DM)	169		170			189			191			
Starch (g/kg DM)	79		29			183			113			

[1] Conc., concentrate

[2] ME, metabolisable energy

[3] Precalving diet denotes the dry period of multiparous animals and 3 weeks prior to calving in primiparous animals

[4] Animals on a low postcalving diet received an additional 50 grams of dairy cow mineral / head / day

Measurements (diets)

While daily samples were analysed for oven DM (Table 1.2), weekly samples of grass silage and maize silage were analysed by near infrared reflectance spectroscopy to estimate ME content (Park et al., 1998). Twice weekly, fresh samples of maize silage and grass silage were analyzed for gross energy (Porter, 1992) and pH. The same samples were analysed for nitrogen and ammonia-nitrogen (ammonia-N) concentrations as described by Steen (1989), and for lactic acid, VFA's, ethanol, and propanol concentrations as described by Porter and Murray (2001). Four dried silage samples (2 per week) were bulked for each 2-week period and analyzed for concentrations of NDF, ADF, and ash as described by Cushnahan and Gordon (1995). Twice-weekly, samples of maize silage were dried at 60^{o}C and then bulked for each 2-week period and analyzed for starch according to McCleary et al. (1994) using a commercial kit (Megazyme, Megazyme International Ireland Ltd., Bray, Ireland). Each batch of concentrates produced was sampled and bulked 2-wk samples analyzed for oven DM content. The dried samples were analyzed for nitrogen (Steen, 1989), ADF, NDF, and ash concentrations as described by Cushnahan and Gordon (1995). A separate concentrate sample was dried at 60^{o}C and similarly bulked and analyzed for starch as detailed above (Table 1.1). Volatility coefficients (Porter and Murray, 2001) were used with the oven DM contents of the grass silage and maize silage to produce volatile-corrected oven dry matter (VCODM) values.

Measurements (animals)

Cows were milked twice daily, between 0530 and 0700, and 1530 and 1700, through a 50 point rotary parlor. Milk yield was recorded automatically at each milking for each individual animal and a mean daily milk yield was calculated for each animal on a weekly basis. Milk samples were taken for fat, protein, lactose, and SCC analysis weekly from two consecutive collections (a.m. and p.m.) from each animal, with a.m. and p.m. samples analyzed separately using an infrared milk analyzer. Each sample had a preservative tablet added (Lactab Mark III, Thompson and Cooper Ltd, Runcorn, UK) and was stored at 4°C until analyzed. A weighted milk composition was subsequently calculated for each weekly sampling occasion. Body condition

score on a scale from 1 to 5 (Edmonson et al., 1989) and BW were measured weekly from d -42 (-21 in primiparous animals) until the end of the study.

Liver biopsies of approximately 20 mg wet weight (each) were taken from four primiparous and four multiparous animals on each of the four treatments, at 13 d prior to their expected calving dates and at 10, 22, and 56 d postcalving. Samples were obtained through a small incision on the right hand side of the animal between the 10^{th} and 11^{th} ribs, on a line from the hip (tuber coxae) to the upper part of the right front leg (mid-humerus). Before taking the biopsies, a 25 cm^2 area of the skin was shaved and disinfected and 10ml of local anesthetic administered subdermally. Following a minimum wait of 10 minutes, a 0.5cm incision was made in the skin. Liver biopsies were taken from this incision using a PRO-MAG biopsy tool with a 14 gauge x 10cm needle (MDTEH, Florida, USA). The biopsies were immediately frozen in liquid nitrogen and stored at minus 180°C until analysis for triacylglycerol (TAG) content. Prior to analysis, liver samples were homogenized and then centrifuged and supernatants analyzed on an auto analyzer using colorimetric kits (Andersen et al., 2002).

From -21 d precalving until d 100 of lactation, all animals were blood sampled weekly before being offered fresh food. Blood was taken from the coccygeal vein into uncoated, heparin-coated, and fluoride oxalate coated tubes (BD, Oxford, UK). Plasma was recovered by centrifugation from fluoride oxalate tubes for analysis of glucose and non-esterified fatty acids (NEFA), and from heparinised tubes for analysis of total protein, albumin, urea, and β-hydroxybutyrate (BHBA). All analyses were carried out on a clinical analyzer (AU640, Olympus UK Ltd, Middlesex, UK). Plasma concentrations of total protein, albumin, glucose, and urea were determined using Olympus kits (Olympus Life Science Research Euorpa, Munich, Germany). Reagents for total protein, albumin, glucose, and urea were ready for use and placed appropriately into the analyzer. In the analysis of total protein, cupric ions in an alkaline solution react with proteins and polypeptides containing at least two peptide bonds to produce violet colored complex. The absorbance of the complex at 540/660 nm is directly proportional to the concentration of protein in the sample (Young, 2000). For albumin, a coloured complex is formed when bromocresol green (BCG) reacts with albumin. The absorbance of the albumin-BCG complex is measured bichromatically (600/800 nm) and is proportional to the concentration

of albumin in the sample (Young, 2000). Glucose is phosphorylated by hexokinase in the presence of ATP and Mg^{2+} to produce glucose-6-phosphate and ADP. Glucose-6-phosphate dehydrogenase specifically oxidizes glucose-6-phosphate to gluconate-6-phosphate with the concurrent reduction of NAD^+ to NADH. The increase in absorbance at 340 nm is proportional to the glucose concentration in the sample (Young, 2000). Urea is hydrolysed in the presence of water and urease to produce ammonia and carbon dioxide. The ammonia produced in the first reaction combines with 2-oxoglutarate and NADH in the presence of glutamate-byhydrogenase to yield glutamate and NAD^+. The decrease in NADH absorbance per unit time is proportional to the concentration of urea (Young, 2000). NEFA concentrations were determined using a standard Wako reagent kit NEFA-HR(2) (Wako Chemicals GmbH, Neuss, Germany). Reagants were prepared according to Kreb et al. (2000). NEFA is converted to Acyl-CoA, AMP, and pyrophosphoric acid by the action of Acyl-CoA synthetase, under coexistence with coenzyme A and adenosine 5-triphosphate disodium salt (ATP). Obtained Acyl-CoA is oxidized and yields 2,3-trans-Enoyl-CoA and hydrogen peroxide by the action of Acyl-CoA oxidase (ACOD). In the presence of peroxidase, the hydrogen peroxide formed yields a blue purple pigment by quantitative oxidation condensation with 3-Methyl-N-Ethyl-N-(β-Hydroxyethyl)-Aniline (MEHA) and 4-aminoantipyrine (4-AA). Non-esterified fatty acids (NEFA) concentration is obtained by measuring absorbance of the blue purple color. β-hydroxybutyrate concentration of plasma was determined according to McMurray et al. (1984).

Serum was recovered by centrifugation of uncoated tubes for analysis of leptin and IGF-1. Leptin concentrations were determined by radioimmunoassay (RIA) of samples from all animals in each of weeks -3, -2, -1, 2, 5, 8, and 12 postpartum. The leptin RIA was performed as according to Wylie et al. (2008) and used bovine leptin (a gift from Prof A Gertler) as both standard and as iodinated label. Each assay was balanced for dietary treatment and for parity and control samples were included in each assay. Serum IGF-1 concentrations were also determined by RIA of week 2, 5, 8, 16, and 20 samples from the same 7 multiparous and 5 primiparous animals randomly selected from each of the 4 dietary treatments. IGF-1 RIAs were balanced for dietary treatment and parity, with control samples included in each assay. IGF-1 binding proteins were removed by acid-ethanol cryo-precipitation (Wylie et al., 1997) prior to analysis. The

primary antibody (AFP 4892898) was rabbit anti-human somatomedin C (a gift from Dr A Parlow; NIDDK, Torrance, CA., USA) and was used at a final dilution of 1:400,000.

Calculation of Energy Balance

The average daily energy balance (DEB) for each animal was calculated for each week of lactation using the equations described by Thomas (2004) {DEB = ME intake – ME requirement [-10 + (ME_{preg} + $ME_{maintmilk}$ * $BW^{0.75}$)] + [(0.0013*BW)/K_m)]; K_m, efficiency of energy use for maintenance (0.35 x ME/GE + 0.503)}. Milk yields, DMI, milk compositions, BW, and feed composition data were all used in the calculations. Missing values (less than 2% of all values) were estimated from data for the week prior to, and the week following, the week of which observations were missing.

Statistical Analysis

Data were analyzed by a repeated measures approach using the Residual Maximum Likelihood (REML) procedure in GenStat (Payne et al. 2007). Pre- and post-calving data were analyzed separately. Within each of these data sets, data from primi- and multi-parous animals were also considered separately. For precalving data, the model fitted fixed effects for precalving treatment and weeks prior to calving for each parameter. For postcalving data, the model fitted fixed effects for precalving treatment, postcalving treatment, and week of lactation for each parameter. The model included all 2-way and 3-way interactions among these variables. Linear regression analysis was undertaken for several independent variables relating to energy and liver metabolism. These were: BCS, DEB, cumulative energy balance (CEB) and concentrations of plasma NEFA and BHBA, serum leptin and IGF-1 and liver TAG.

RESULTS

Chemical composition of the silages and concentrates

The chemical composition of concentrates offered during late lactation, the dry period, and lactation are presented in Table 1.1. Concentrates had similar ME, NDF, ADF, and ash contents but differed in CP and starch contents. The chemical composition of silages, as fed, is presented in Table 1.2. The grass silage offered postcalving was considered to be of good quality with a DM content of 228 g / kg DM, a CP concentration of 147 g / kg DM, and an ammonia-N concentration of 101 g / kg total N (consistent with good preservation). The maize silage offered postcalving had a DM content of 289 g / kg, a CP concentration of 86 g / kg DM, and a starch concentration of 200 g / kg DM, typical of average quality maize forage. Table 1.3 details the mean composition of the TMR as fed.

Effect of Precalving Diet on Body Condition Score, DMI, and Energy Indices in the Precalving Period (-21 to 0 d)

During the precalving period, primiparous animals offered the low E diet had lower leptin concentrations ($P = 0.046$) and mean BCS ($P = 0.03$) than those offered the high E diet (Table 1.4). Primiparous animals offered the low E diet also had higher ($P < 0.001$) plasma urea concentrations than animals offered the high E diet (Table 1.4), but there were no precalving dietary effects ($P > 0.05$) on DMI, BCS at calving, BCS change, BW, DEB, or on plasma NEFA, glucose, or BHBA concentrations (Table 1.4).

Multiparous animals offered the high E diet during the precalving period had higher DMI ($P < 0.001$), BCS ($P = 0.04$), BCS at calving ($P = 0.007$), DEB ($P < 0.001$), and plasma glucose concentrations ($P < 0.001$) compared to those offered a low E diet (Table 1.4). Multiparous animals offered a low E precalving diet had higher plasma urea concentrations ($P = 0.044$) and

lost more body condition (P = 0.015) than animals offered a high E precalving diet (Table 1.4). There were no (P > 0.05) precalving dietary effects on plasma NEFA or BHBA concentrations, or serum leptin concentrations (Table 1.4) in multiparous animals.

Regression analysis of data from multiparous animals revealed a strong positive association between BCS at drying off and BCS at calving (P < 0.001; r^2, 0.63). Precalving treatment had a significant effect on the intercept (P = 0.01; r^2, 0.054) and trajectory (P = 0.022; r^2, 0.042) of this relationship. The fitted equation for this relationship was "Y = 1.295 – 1.119 (Precalving treatment, low E) + 0.477 (X) + 0.356 (X; Precalving treatment, low E)".

Effect of Precalving and Postcalving Diet on Energy and Production Parameters (1-250 d)

Primiparous Animals

During the postcalving period (1-250 d), primiparous animals offered a low E diet precalving were significantly heavier (519 vs. 478 kg / d; SED, 12.5; P = 0.001) than primiparous animals offered a high E diet precalving (Table 1.5). Diet identity precalving had no direct effect (P > 0.05) on BCS, ME requirement, ME intake (Table 1.5; Figure 1.1), DMI, DEB, CEB, milk energy output, yields of milk, milk fat, protein, fat plus protein or lactose, nor on concentrations of milk fat, protein, or lactose (Table 1.5 and 1.6).

Primiparous animals receiving a high E postcalving diet had higher ME requirement (207 vs. 187 MJ / d; SED, 5.9; P = 0.01), ME intakes (219 vs. 167 MJ / d; SED, 5.1; P < 0.001), DMI (17.8 vs. 14.5 kg / d; SED, 0.43; P < 0.001), milk yields (29.7 vs. 24.8 kg / d; SED, 1.27; P < 0.001) milk protein yields (0.99 vs. 0.81 kg / d; SED, 0.037; P < 0.001), milk fat plus protein yields (2.07 vs. 1.84 kg / d; SED, 0.079; P = 0.005), milk energy outputs (89.5 vs. 79.3 MJ / d; SED, 3.42; P = 0.03), DEB (12.5 vs. -19.2 MJ / d; SED, 5.2; P < 0.001), and CEB (727 vs. -3296 MJ; SED, 678; P < 0.001) than animals receiving the low E postcalving diet (Tables 5 and 6).

Animals offered a low E postcalving diet had higher milk fat concentration (41.7 vs. 37.0 g / kg; SED, 1.47; $P = 0.003$) than animals offered the high E postcalving diet (Table 1.6). Postcalving diet did not affect ($P > 0.05$) BW, BCS, milk fat yield, or concentrations of milk protein and lactose.

Table 1.4: Effect of precalving diet on mean DMI, BCS, BW, energy status, and blood metabolite concentrations during the precalving period (-21 to 0 d)

	Primiparous animals						Multiparous animals					
	Precalving regimen			P-value			Precalving regimen			P-value		
Variate	High E	Low E	SED	Diet	Time[2]	Diet * Time	High E	Low E	SED	Diet	Time[2]	Diet*time
DMI (kg / d)	6.0	5.9	0.19	0.74	0.03	0.50	10.2	6.6	0.32	<0.001	0.006	0.21
Mean BCS	2.80	2.67	0.047	0.03	0.24	0.02	2.71	2.44	0.126	0.04	0.02	0.50
BCS at calving[1]	2.79	2.63	0.089	0.11	-	-	2.68	2.34	0.091	0.007	-	-
BCS change[3]	0.02	-0.03	0.061	0.10	-	-	-0.10	-0.26	0.049	0.015	-	-
BCS												
Mean BW (kg)	549	560	10.1	0.26	<0.001	0.44	665	638	22.4	0.25	0.03	0.02
DEB (MJ / d)	-39.7	-40.1	4.39	0.74	<0.001	0.96	0.2	-40.9	4.18	<0.001	<0.001	0.13
Serum Leptin (ng / ml)	1.95	1.35	0.288	0.046	<0.001	0.18	2.11	2.05	0.277	0.78	0.03	0.27

Plasma NEFA (meq / l)	0.50	0.52	0.063	0.21	0.15	<0.001	0.26	0.31	0.027	0.10	<0.001	0.40
Plasma Glucose (mmol / l)	3.45	3.36	0.063	0.26	0.50	0.22	3.45	3.08	0.066	<0.001	0.08	0.008
Plasma Urea (mmol / l)	5.1	6.4	0.23	<0.001	0.02	0.88	6.0	6.8	0.28	0.044	0.45	0.60
Plasma BHBA (mmol / l)	0.52	0.51	0.05	0.58	0.46	0.009	0.50	0.52	0.031	0.43	0.44	0.09

[1] BCS at calving was calculated as an average of BCS in wk -1 and +1 postcalving.

[2] Time, week precalving

[3] BCS change (total change) for multiparous animals -42 to 0 d precalving and primiparous animals -21 to 0 d precalving

In primiparous animals, there was a precalving * postcalving diet interaction for DEB ($P = 0.03$), CEB ($P = 0.01$), and milk fat yield ($P = 0.02$). For both DEB and CEB, the magnitude, but not direction, of the energy-related response to postcalving treatment was influenced by precalving treatment (Table 1.5) such that differences in DEB and CEB between the postcalving treatments were greater in animals offered a low E diet precalving than in those offered a high E diet precalving. With regards to milk fat yield, animals offered a high E diet precalving had a higher milk fat yield when offered a high E diet postcalving, compared to those offered a low E diet postcalving. However, when offered a low E diet precalving, milk fat yield was higher in animals offered the low E, compared to a high E diet, postcalving (Table 1.6).

There were also significant 3-way interactions (week of lactation * precalving treatment * postcalving treatment) for milk yield ($P < 0.001$; SED, 1.59; Figure 1.2), milk lactose yield ($P < 0.001$), BCS ($P < 0.001$), BW ($P = 0.012$), total energy requirement ($P = 0.005$), and CEB ($P < 0.001$; SED, 979; Figure 1.3). For all of these parameters, the precalving treatment significantly affected the postcalving response trajectory.

Table 1.5: Effect of pre- and post-calving diets on mean BW, BCS, and body energy status of multiparous animals from d 1 to 250 of lactation

		Dietary regimes (Precalving / Postcalving)[1]					P-value					
							Treatment			Interaction[3]		
		HH	HL	LH	LL	SED	Pre	Post	Time[2]	1	2	3
	BW (kg)	480	476	536	502	17.7	0.001	0.15	<0.001	0.24	<0.001	<0.001
	BCS	2.49	2.49	2.60	2.53	0.074	0.12	0.56	<0.001	0.58	<0.001	<0.001
Primi-	ME Requirement (MJ / d)	210	180	203	193	8.3	0.48	0.001	<0.001	0.11	0.56	<0.001
parous	ME Intake (MJ / d)	214	165	224	170	7.2	0.06	<0.001	<0.001	0.67	0.02	<0.001
	Daily Energy Status (MJ / d)	4.7	-15.3	20.3	-23.2	7.37	0.17	<0.001	<0.001	0.03	<0.001	0.22
	Cumulative Energy Status (MJ)	-665	-2915	2119	-3676	958	0.06	<0.001	<0.001	0.01	0.02	<0.001
	BW (kg)	607	575	603	580	24.4	0.95	0.12	<0.001	0.80	<0.001	0.009
	BCS	2.78	2.29	2.65	2.38	0.145	0.54	0.002	<0.001	0.14	0.005	<0.001
Multi-	ME Requirement (MJ / d)	236	210	230	217	10.5	0.87	0.01	<0.001	0.36	0.02	0.005
parous	ME Intake (MJ / d)	252	203	262	189	10.6	0.99	<0.001	<0.001	0.114	0.65	<0.001
	Daily Energy Status (MJ / d)	15.8	-6.2	32.2	-28.2	10.5	0.87	<0.001	<0.001	0.02	0.73	<0.001
	Cumulative Energy Status (MJ)	1772	-2388	3113	-4555	1377	0.76	<0.001	<0.001	0.09	0.99	<0.001

[1] HH, high E precalving, high E postcalving; HL, high E precalving, low E postcalving; LH, low E precalving, high E postcalving; LL, low E precalving, low E postcalving.

[2] Time, week of lactation.

[3] Interaction 1, 2, and 3 are: pre- * post-calving treatment, precalving treatment * week of lactation, and postcalving treatment * week of lactation respectively.

Table 1.6: Effect of pre- and post-calving diets on mean DMI, milk yield, milk constituent variables, and milk energy from d 1 to 250 d of lactation

		Dietary regimes (Precalving / Postcalving)[1]					P-value					
							Treatment			Interaction[3]		
		HH	HL	LH	LL	SED	Pre	Post	Time[2]	1	2	3
Primi-parous	DMI (kg / d)	17.4	14.2	18.1	14.7	0.60	0.06	<0.001	<0.001	0.75	0.11	<0.001
	Milk Yield (kg / d)	29.9	23.9	29.5	25.8	1.80	0.42	<0.001	<0.001	0.38	<0.001	<0.001
	Milk Fat (g / kg)	39.1	41.8	34.8	41.5	2.08	0.06	0.003	<0.001	0.20	0.27	0.49
	Milk Protein (g / kg)	33.2	32.7	34.4	32.7	0.92	0.23	0.11	<0.001	0.36	0.88	0.22
	Milk Lactose (g / kg)	49.6	49.2	49.8	49.4	0.47	0.48	0.29	<0.001	0.97	0.03	0.007
	Milk Fat Yield (kg / d)	1.15	0.99	0.99	1.07	0.067	0.29	0.32	0.02	0.02	0.90	0.67
	Milk Protein Yield (kg / d)	0.99	0.77	1.00	0.84	0.052	0.16	<0.001	<0.001	0.53	0.03	<0.001
	Milk F + P Yield (kg / d)	2.14	1.77	1.99	1.90	0.111	0.97	0.005	<0.001	0.08	0.80	<0.001
	Milk Energy Output (MJ / d)	92.6	76.2	86.3	82.3	4.83	0.22	0.03	<0.001	0.35	0.50	0.91
Multi-	DMI (kg / d)	20.5	17.6	21.2	16.4	0.87	0.87	<0.001	<0.001	0.12	0.33	<0.001
	Milk Yield (kg / d)	34.3	27.7	32.6	28.7	2.11	0.88	<0.001	<0.001	0.38	0.92	<0.001

parous	Milk Fat (g / kg)	35.6	41.6	37.9	42.3	2.74	0.50	0.009	<0.001	0.69	0.02	0.38
	Milk Protein (g / kg)	32.6	33.6	33.4	34.4	1.04	0.29	0.19	<0.001	0.98	0.61	<0.001
	Milk Lactose (g / kg)	48.8	47.7	48.4	48.7	0.49	0.46	0.21	<0.001	0.051	0.048	0.92
	Milk Fat Yield (kg / d)	1.21	1.14	1.20	1.19	0.090	0.79	0.59	<0.001	0.65	<0.001	0.079
	Milk Protein Yield (kg / d)	1.12	0.92	1.07	0.97	0.057	0.85	<0.001	<0.001	0.28	0.31	<0.001
	Milk F + P Yield (kg / d)	2.32	2.06	2.27	2.16	0.130	0.80	0.057	<0.001	0.45	0.002	0.50
	Milk Energy Output (MJ / d)	101.2	87.9	97.8	92.1	5.85	0.42	0.02	<0.001	0.86	0.02	0.45

[1] HH, high E precalving, high E postcalving; HL, high E precalving, low E postcalving; LH, low E precalving, high E postcalving; LL, low E precalving, low E postcalving.

[2] Time, week of lactation.

[3] Interaction 1, 2, and 3 are: pre- * post-calving treatment, precalving treatment * week of lactation, and postcalving treatment * week of lactation respectively.

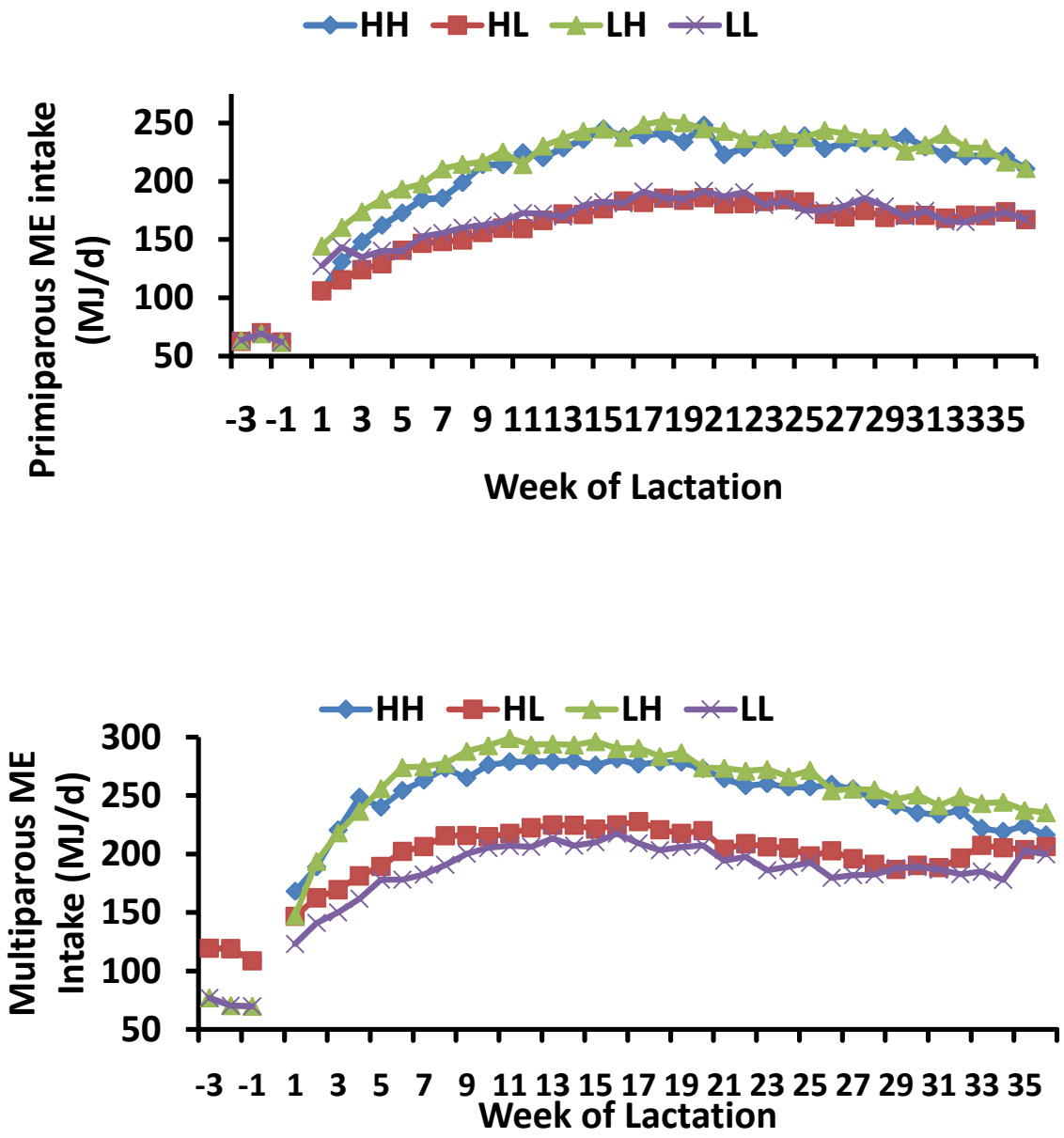


Figure 1.1: ME intake of animals offered two levels of energy intake (high and low), pre- and post-calving. Thus, HH refers to animals that were offered a high precalving and high postcalving diet. Postcalving effects in primiparous animals: precalving treatment, *P* = 0.06 (SED, 5.1); postcalving treatment, *P* < 0.001 (SED, 5.1); week of lactation * precalving treatment * postcalving treatment, *P* = 0.93 (SED, 10.4). Postcalving effects in multiparous animals: precalving treatment, *P* = 0.99 (SED, 7.5); postcalving

treatment, $P < 0.001$ (SED, 7.5); week of lactation * precalving treatment * postcalving treatment, $P = 0.98$ (SED, 13.6).

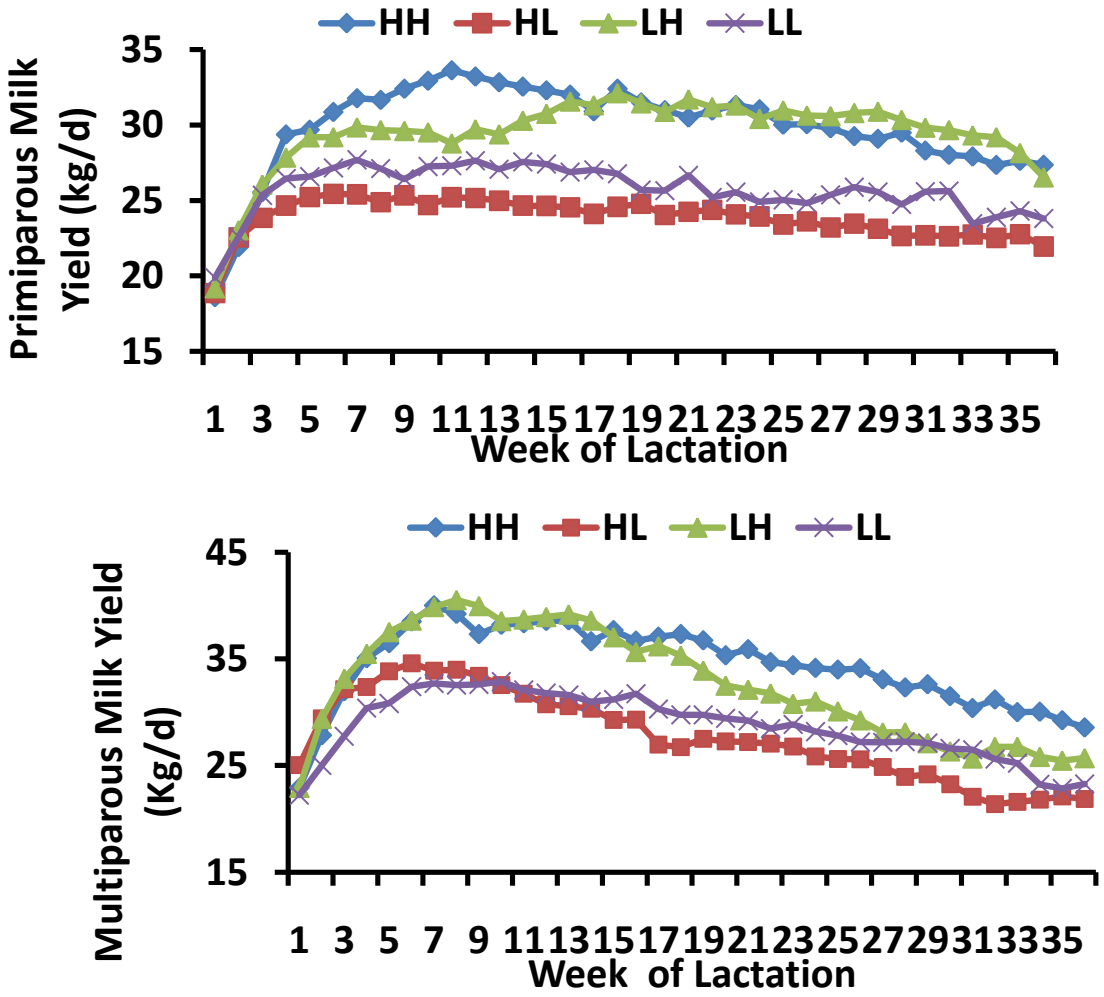


Figure 1.2: Milk yields of animals offered two levels of energy intake (high and low), pre- and post-calving. Thus, HH refers to animals that were offered a high precalving and high postcalving diet. Postcalving effects in primiparous animals: precalving treatment, $P = 0.42$ (SED, 1.27); postcalving treatment, $P < 0.001$ (SED, 1.27); week of lactation * precalving treatment * postcalving treatment, $P < 0.001$ (SED, 1.80). Postcalving effects in multiparous animals: precalving treatment, $P = 0.88$ (SED, 1.49); postcalving treatment, $P < 0.001$ (SED, 1.49); week of lactation * precalving treatment * postcalving treatment, $P < 0.001$ (SED, 2.32).

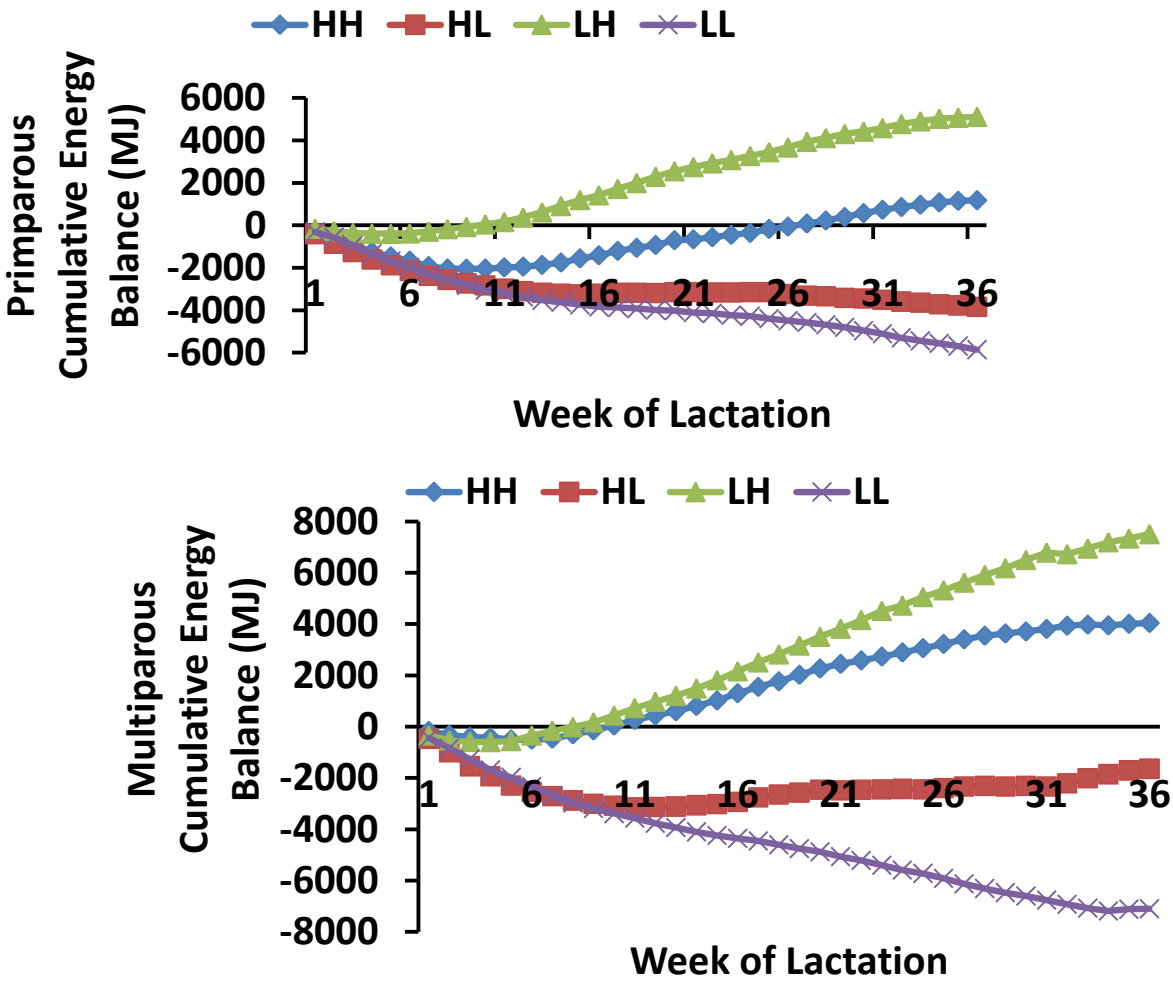


Figure 1.3: Cumulative energy balance of animals offered two levels of energy intake (high and low), pre- and post-calving. Thus, HH refers to animals that were offered a high precalving and high postcalving diet. Postcalving effects in primiparous animals: precalving treatment, $P = 0.06$ (SED, 678); postcalving treatment, $P < 0.001$ (SED, 678); week of lactation * precalving treatment * postcalving treatment, $P < 0.001$ (SED, 979). Postcalving effects in multiparous animals: precalving treatment, $P = 0.76$ (SED, 974); postcalving treatment, $P < 0.001$ (SED, 974); week of lactation * precalving treatment * postcalving treatment, $P < 0.001$ (SED, 1433).

Multiparous animals

For multiparous animals, there were no significant ($P > 0.05$) effects of precalving treatment on any of the parameters listed in Tables 5 and 6.

During the first 250 d of lactation, multiparous animals offered a high E diet postcalving had a higher BCS (2.67 vs. 2.33; SED, 0.10; $P = 0.002$), ME requirement (233 vs. 214 MJ / d; SED, 7.5; $P = 0.01$), ME intake (257 vs. 196 MJ / d; SED, 7.5; $P < 0.001$), DMI (20.9 vs. 17.0 kg / d; SED, 0.62; $P < 0.001$), milk yield (33.5 vs. 28.2 kg / d; SED, 1.49; $P < 0.001$), milk protein yield (1.09 vs. 0.95 kg / d; SED, 0.041; $P < 0.001$), milk energy output (99.5 vs. 90.0 MJ / d; SED, 4.14; $P = 0.02$), DEB (24.0 vs. -17.2 MJ / d; SED, 7.40; $P < 0.001$), and CEB (2443 vs. -3471 MJ; SED, 974; $P < 0.001$) than those offered the low E postcalving diet (Tables 5 and 6). Animals offered the low E postcalving diet had higher milk fat concentrations (36.8 vs. 42.0; SED, 1.94; $P = 0.009$) than animals offered the high E postcalving diet (Table 1.6).

In multiparous animals, there was a precalving * postcalving diet interaction for DEB ($P = 0.02$) with the magnitude, but not the direction, of the energy-related response to postcalving treatment influenced by precalving treatment (Table 1.5). The difference between high E and low E postcalving treatments was greater in animals receiving the low E diet precalving than in those receiving the high E diet precalving.

There were also 3-way interactions ($P < 0.001$; week of lactation * precalving treatment * postcalving treatment) for milk yield (Figure 1.2; SED, 1.59), milk fat yield, milk protein yield, milk fat plus protein yield, total energy requirement, DEB (Figure 1.4; SED, 14.1), and CEB (Figure 1.3; SED, 979). For all of these parameters, precalving treatment significantly affected on the postcalving response trajectory.

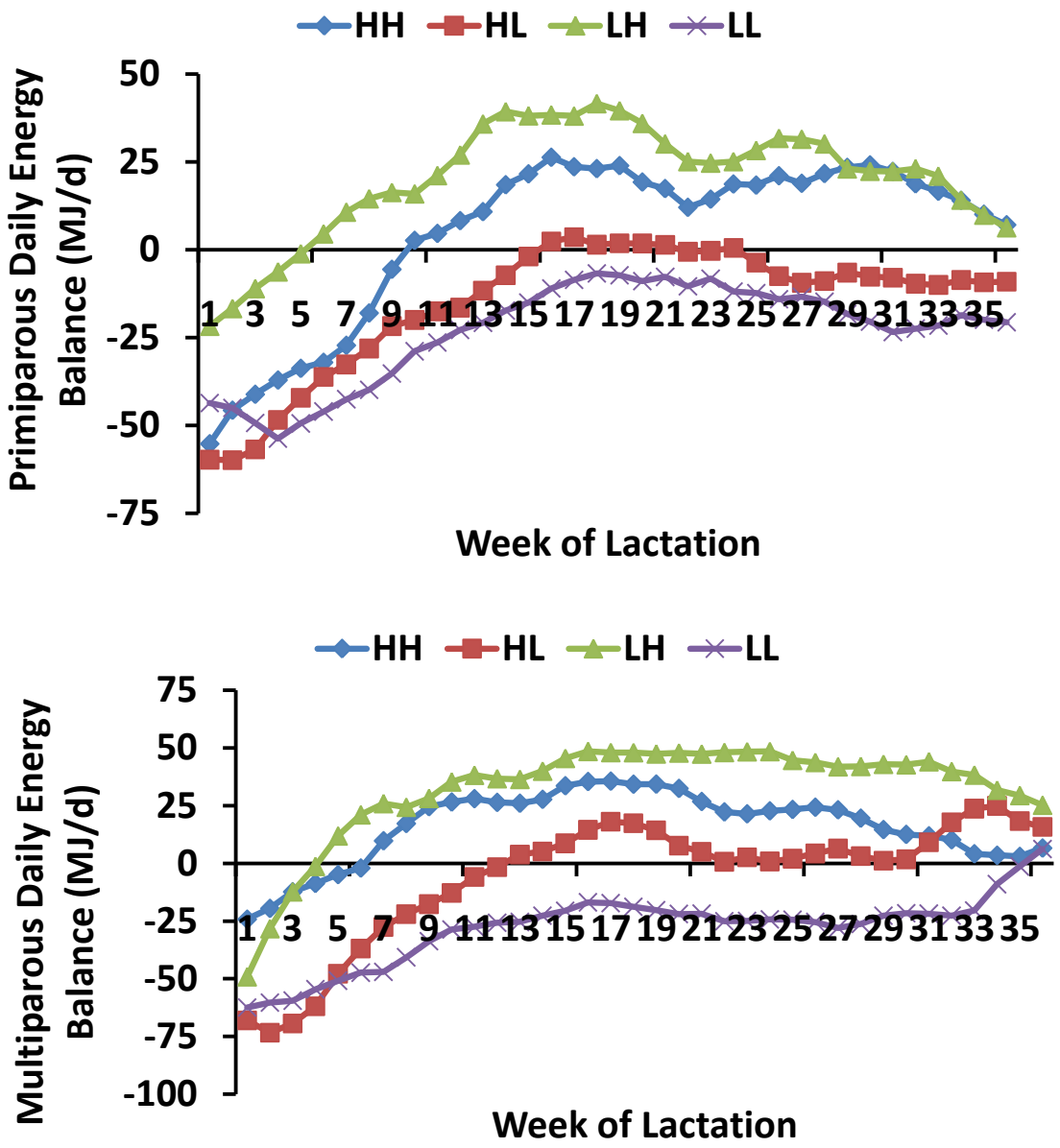


Figure 1.4: Daily energy balance of animals offered two levels of energy intake (high and low), pre- and post-calving. Thus, HH refers to animals that were offered a high precalving and high postcalving diet. Postcalving effects in primiparous animals: precalving treatment, $P = 0.17$ (SED, 5.22); postcalving treatment, $P < 0.001$ (SED, 5.22); week of lactation * precalving treatment * postcalving treatment, $P = 0.41$ (SED, 11.0). Postcalving effects in multiparous animals: precalving treatment, $P = 0.87$ (SED, 7.4); postcalving treatment, $P < 0.001$ (SED, 7.4); week of lactation * precalving treatment * postcalving treatment, $P < 0.001$ (SED, 14.1).

Effect of Precalving and Postcalving Diets on Blood and Liver Parameters (1-100 d)

Primiparous Animals

Precalving diet had a significant effect on postcalving plasma NEFA (Table 1.7; Figure 1.5) and BHBA concentrations (Table 1.7), with those managed on high E and low E precalving diets having mean NEFA concentrations of 0.44 and 0.39 meq / l (SED, 0.023; $P = 0.006$) and BHBA concentrations of 0.55 and 0.49 mmol / l (SED, 0.025; $P = 0.003$) respectively, during the postcalving period. There were no significant effects ($P > 0.05$) of precalving treatment on plasma glucose or urea concentrations, on serum IGF-1 or leptin concentrations, or on liver TAG concentrations.

Primiparous animals offered a high E postcalving diet had higher serum IGF-1 concentrations ($P < 0.001$; 115 vs. 99 ng / ml, SED; 7.3) than those offered a low E diet postcalving. Animals offered a high E diet postcalving had lower concentrations of plasma urea (5.1 vs. 6.5 mmol / l; SED, 0.16; $P < 0.001$) and BHBA (0.44 vs. 0.60 mmol / l; SED, 0.025; $P < 0.001$) compared to those offered a low E diet postcalving. There was no significant ($P > 0.05$) effect of postcalving dietary regimen on plasma NEFA or glucose concentrations, on serum leptin concentrations, or on liver TAG concentrations during lactation (Table 1.7).

Multiparous animals

Amongst multiparous animals, precalving diet had no effect ($P > 0.05$) on blood or liver parameters during the postcalving period (Table 1.7).

Multiparous animals offered a high E postcalving diet had higher plasma glucose concentrations (3.5 vs. 3.3 mmol / l, SED; 0.04; $P < 0.001$) than those offered the low E diet postcalving. Animals offered a high E postcalving diet had lower plasma concentrations of urea (5.5 vs. 7.0

mmol / l; SED, 0.14; $P < 0.001$), BHBA (0.46 vs. 0.57 mmol / l; SED, 0.030; $P = 0.002$), and NEFA (0.31 vs. 0.39 meq / l; SED, 0.032; $P = 0.02$) compared to those offered the low E diet postcalving. In these multiparous animals, there was no effect ($P > 0.05$) of postcalving dietary regimen on serum leptin or IGF-1 concentrations or on liver TAG concentrations during lactation (Table 1.7).

Regression analysis of all data (primiparous and multiparous animals) showed that a more negative DEB was associated with increased plasma NEFA (r^2, 0.284; $P < 0.001$) and liver TAG (r^2, 0.325; $P < 0.001$) concentrations. Postcalving treatment had a significant effect on the intercept in both regressions (NEFA, $r^2 = 0.07$, $P = 0.005$; TAG, $r^2 = 0.05$, $P < 0.02$). The fitted equation for NEFA was "Y = 0.451 – 0.201 (Postcalving treatment, low E) – 0.00477 (X)", while that for TAG was "Y = 4.523 – 3.07 (Postcalving treatment, low E) – 0.0866 (X)". Additionally, plasma NEFA concentrations were positively associated with liver TAG concentrations (r^2, 0.319; $P < 0.001$) and postcalving treatment did not affect this regression ($P = 0.19$). Thus, the fitted equation for TAG was "Y = 1.02 + 9.83 (X)".

Additional regression analysis indicated that a more negative DEB was associated with increased plasma BHB (r^2, 0.097; $P < 0.001$) while a more negative CEB was associated with lower serum IGF-1 concentrations (r^2, 0.079; $P = 0.008$). However, in both these regressions, the r^2 value was low indicating a weak relationship.

Table 1.7: Effect of pre- and postcalving diets on blood †and liver ± parameters from d 1 to 100 of lactation

		Dietary regimes (Precalving / Postcalving)[1]					P-value					
							Treatment			Interaction[3]		
		HH	HL	LH	LL	SED	Pre	Post	Time[2]	1	2	3
	Plasma NEFA (meq / l)	0.44	0.45	0.37	0.41	0.032	0.006	0.26	<0.001	0.57	0.71	0.74
	Plasma Glucose (mmol / l)	3.54	3.51	3.66	3.54	0.060	0.06	0.10	<0.001	0.34	0.76	0.84
Primi-	Plasma Urea (mmol / l)	4.95	6.33	5.29	6.78	0.224	0.14	<0.001	<0.001	0.74	0.11	0.23
parous	Plasma BHBA (mmol / l)	0.46	0.64	0.42	0.57	0.035	0.003	<0.001	<0.001	0.72	0.35	0.24
	Serum IGF-1 (ng / ml)	109	100	121	98	8.6	0.29	<0.001	0.20	0.46	0.64	0.29
	Serum Leptin (ng / ml)	1.34	1.58	1.18	0.96	0.190	0.22	0.76	0.78	0.08	0.81	0.99
	Liver TAG (mg / g)	5.27	8.32	5.92	4.64	4.000	0.56	0.70	<0.001	0.37	0.99	0.46
	Plasma NEFA (meq / l)	0.34	0.42	0.29	0.35	0.046	0.09	0.02	<0.001	0.94	0.58	0.72
	Plasma Glucose (mmol / l)	3.48	3.29	3.55	3.37	0.063	0.14	<0.001	<0.001	0.96	0.15	0.54
Multi-	Plasma Urea (mmol / l)	5.82	7.22	5.67	6.83	0.202	0.06	<0.001	0.001	0.50	0.31	0.78
parous	Plasma BHBA (mmol / l)	0.49	0.56	0.44	0.57	0.042	0.43	0.002	0.04	0.24	0.59	0.08
	Serum IGF-1 (ng / ml)	108	104	114	106	6.6	0.81	0.06	0.005	0.23	0.25	0.75
	Serum Leptin (ng / ml)	1.34	1.41	1.47	1.76	0.204	0.38	0.41	0.007	0.64	0.14	0.52
	Liver TAG (mg / g)	3.16	5.08	4.58	6.02	2.371	0.36	0.12	0.001	0.66	0.72	0.61

[1] HH, high E precalving, high E postcalving; HL, high E precalving, low E postcalving; LH, low E precalving, high E postcalving; LL, low E precalving, low E postcalving.

[2] Time, week of lactation.

[3] Interaction 1, 2, and 3 are: pre- * post-calving treatment, precalving treatment * week of lactation, and postcalving treatment * week of lactation respectively.

† Based on weekly sampling

± Based on mean of four samples from 4 animals on each treatment

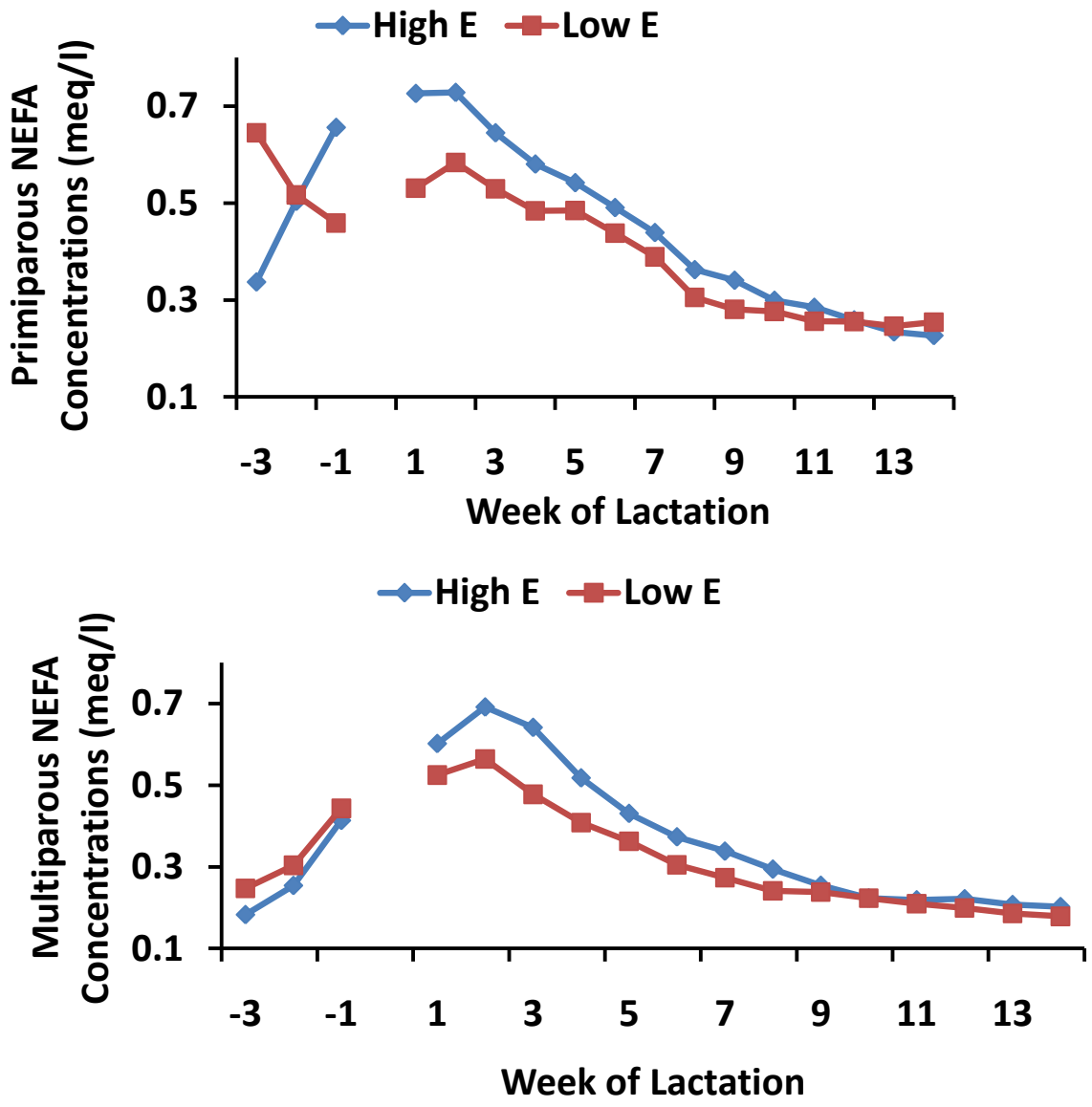


Figure 1.5: NEFA concentrations of animals offered two levels of energy intake (high E and low E), precalving. Precalving effects in primiparous animals: precalving treatment, *P* = 0.21 (SED, 0.06); precalving treatment * week precalving, *P* < 0.001 (SED, 0.072). Postcalving effects in primiparous animals: precalving treatment, *P* = 0.006 (SED, 0.023), precalving treatment * week of lactation, NS (SED, 0.071). Precalving effects in multiparous animals: precalving treatment, *P* = 0.10 (SED, 0.032); precalving treatment * week precalving, *P* = 0.71 (SED, 0.045). Postcalving effects in multiparous animals: precalving treatment, *P* = 0.09 (SED, 0.032); precalving treatment * week of lactation, *P* = 0.85 (SED, 0.077).

DISCUSSION

In the current study, the period of energy restriction commenced at d 80 and d 100 precalving for primi- and multi-parous animals respectively. During this period, primi- and multi-parous animals were offered different dietary regimes. The treatments were designed to represent commercial practices in grassland regions of the UK and Ireland. After drying-off, high yielding dairy cows are usually housed during the dry period to allow BCS to be monitored and improved prior to parturition. However, primiparous animals do not normally need to gain body condition during the precalving period and producers often keep such animals at grass for as long as possible, housing them only about 3 wk prior to their expected calving date. As first-calving animals are immature and inherently different in many ways to multiparous animals, this confounding was deemed reasonable.

Dietary Effects on Body Energy Status Precalving

This current study was undertaken to compare the effects of two contrasting levels of precalving nutrition (a restricted low energy diet vs. an *ad libitum* high energy diet) on the BCS at calving and postcalving performance of high-yielding dairy cows. It was further designed to examine how, and to what degree, pre- and post-calving nutrition influence the trajectory of changes in energy-related parameters in high-yielding dairy cows during lactation.

While the high E and low E diets adopted succeeded in establishing a 0.34 unit difference in BCS at calving in multiparous animals, no significant difference in BCS at calving was found in primiparous animals. This may have been because primiparous animals, when offered a low E diet precalving, suffered less of a restriction in DMI during the precalving period prior to housing, which reflected in a difference in BCS at calving of only 0.16 units in the current study. In support of this explanation, precalving treatment had no effect on the DMI of primiparous animals during the last 21 days of gestation. Overall, the relatively small differences incurred in BCS at calving between precalving treatments, in primi- and multi-parous animals, highlights the

difficulty in achieving large changes in BCS of high-yielding dairy cows during their precalving period. This concurs with the view of Friggens et al. (2004b), who presented a strong correlation between BCS at drying-off and BCS at calving and argued that the strategy of using dry period nutrition as a mechanism to markedly change BCS is unlikely to succeed. A highly significant positive relationship between BCS at drying-off and BCS at calving was also identified in the current experiment.

In the current experiment, multiparous animals offered high E and low E diets precalving consumed, on average, 116.3 and 72.6 MJ / d respectively from d -21 until calving. Similarly, the difference in DEB of these animals (-21 to 0 d postcalving) was 41.1 MJ / d. Over 21 d, this equates to theoretical difference in BCS of 0.48 units (1770 MJ/ unit BCS loss; AFRC, 1993) compared to an actual difference of 0.34 units achieved from d -100 to 0 precalving. Interestingly, Garnsworthy (2006) stated that Holstein cows have more internal fat relative to external (subcutaneous) fat when compared with cows of other dairy and beef breeds, suggesting that at equal BCS, a modern Holstein cow will have a greater variance in total body fat content compared to cows from other dairy breeds. Accordingly, a relatively small difference in BCS at calving could reflect a larger difference in total body fat reserves.

Dietary Effects on Production Parameters

The positive effects of feeding a low E precalving diet on postcalving BW in primiparous animals can be partially attributed to the degree of BW gain in late gestation. In the last 3 weeks of gestation, the BW gain in animals offered high E and low E precalving diets was 16.4 and 24.2 kg, resulting in mean precalving BWs of 554 and 572 kg, respectively. In contrast, Grummer et al. (1995) reported that primiparous animals on a high plane of nutrition precalving had higher precalving BW than animals on a control diet. In addition to a lower BW precalving, primiparous animals offered a high E diet precalving had a higher degree of BW loss in early lactation. In the first 12 weeks of lactation the mean daily BW losses for animals on high E and low E precalving diets were 0.17 and 0.03 kg / d respectively. In support of this, the mean DEBs during this period for animals offered high E and low E precalving diets were -30.1 and -18.2 MJ

/ d respectively (Figure 1.4). In contrast, Grummer et al. (1995) found no effect of precalving treatment on postcalving BW in primiparous animals. However, Keady et al. (2001) found that offering 5 kg concentrates daily to primi- and multi-parous animals during the last 28 days of gestation, increased BW loss in the first 12 weeks of lactation above that sustained by animals offered no concentrates (0.33 vs. 0.04 kg / d BW loss for 5 vs. 0 kg / d concentrate respectively). These are similar to the findings from the current study in primiparous animals; however, Keady et al. (2001) saw no effect of parity on postcalving BW loss. Precalving treatment had no significant effect on the postcalving BW of multiparous animals in the current study.

In the current study, there was no significant effect of precalving treatment on ME intake or DMI during the postcalving period. This is in agreement with the observations of Holcomb et al. (2001), Keady et al. (2001), and Agenäs et al. (2003). However, Garnsworthy and Topps (1982) stated that increased BCS at calving will suppress DMI following parturition. In the current study, a more pronounced effect may have been observed had the differences in BCS at calving been greater. The trajectory of ME intake in Figure 1.1 would suggest only minimal effects of precalving nutrition on postcalving ME intake. However, in primiparous animals there was a significant precalving treatment * week of lactation interaction (Table 1.5), whereby animals allotted to the low E precalving treatment had 30 MJ higher ME intake at the beginning of lactation. A significant precalving treatment * week of lactation interaction for postcalving BCS was also apparent in both primi- and multi-parous animals. Both primi- and multi-parous animals offered the low E precalving diet had lower BCS losses postcalving than did animals offered the high E precalving diet. Primiparous animals offered a low E precalving diet sustained a much flatter BCS trajectory than those offered the high E precalving diet.

McNamara et al. (2003) observed a higher postcalving milk yield (wks 1 to 8) in multiparous animals offered a high plane of nutrition precalving but there was no significant difference in milk yield between multiparous animals offered high E or low E diets precalving in the current study. However, precalving treatment did affect the milk yield trajectory as there was a significant 3-way interaction (week of lactation * precalving treatment * postcalving treatment) for milk yield in both primi- and multi-parous animals, with the effect of precalving treatment on the milk yield trajectory becoming more pronounced as lactation advanced (Figure 1.2). In both

primi- and multi-parous animals, a high E precalving diet negatively impacted milk yield in animals offered a low E diet postcalving. This treatment group (HL) experienced excessive NEB in early lactation (Figure 1.4) which could potentially impair liver function (Top et al., 1995) and, therefore, milk production, if there was an accumulation of TAG. The data presented in Table 1.7 would suggest that liver TAG concentrations were indeed high in animals within this treatment group, especially in primiparous animals.

Postcalving, a high E diet increased DMI and milk yield, in agreement with the findings of Agnew et al. (1996) and Kuoppala et al. (2004). In the current study, concentrate was offered as part of a TMR and animals offered high E and low E consumed, on average, 12.4 and 4.4 kg DM concentrates / d (1 – 250 d) respectively. In the current study, this equates to a milk yield response of 0.61 kg for each kg DM of concentrate offered above 4.4 kg DM. In a study by Sutton et al (1996), a milk yield response of 0.25 kg for each kg DM of extra concentrate was noted. However, in that study, the concentrate CP concentrations used were lower than in the current study. In the current study, an increase in the concentrate allocation decreased milk fat concentrations and increased milk protein concentrations, in agreement with research by Friggens et al. (1998) but in contrast to work by Kuoppala et al. (2004). The latter authors reported no significant effects on milk composition of increasing the concentrate level from 9 to 14 kg DM / head / d.

Dietary Effects on Energy Status (d 1 to 250)

Predicting or defining dietary effects on postcalving BCS loss and on subsequent energy status is difficult as there are multiple factors that affect the degree of energy deficit and subsequent BCS loss. The current status (i.e. BCS at calving), and long term objectives of the individual animal may facilitate the expression of a genetic component to BCS loss which aims to achieve a BCS that is optimal for future reproduction [Friggens et al., 2004a; Garnsworthy, 2006]. Garnsworthy (2006) stated that a target BCS that is optimal for reproduction is genetically predetermined and the rate of BCS loss depends on the cow's current BCS status relative to the genetically predetermined BCS optimal for reproduction. In the current study, DEB and CEB represent daily

and total energy deficits which, it is assumed, are buffered by BCS loss. Thus, the difference in DEB between animals on high E and low E precalving treatments, but within the same postcalving regimen, should represent a genetic component to BCS loss (Figure 1.4) i.e. animals with high BCS at calving will be further from the optimal BCS for reproduction, so that a higher proportion of BCS loss may originate from genetic control in comparison to animals with a low BCS at calving. However, in early through to mid-lactation, a period when the dairy cow is under a heavy energy demands, there is, undoubtedly, environmentally driven tissue mobilisation (BCS loss) which exceeds the genetically determined rate. Environmentally driven mobilisation can be influenced by diet and subsequently has the potential to alter the energy trajectory of these animals. Despite the small difference in BCS at calving in the current study, the existence of a 3-way interaction for CEB (week of lactation * precalving treatment * postcalving treatment; Figure 1.3), in both primi- and multi-parous animals, suggests that precalving dietary regimen has a significant influence on the energy profile of high-yielding dairy animals. This interaction may be a result of the un-sustainability of the high rate of BCS loss observed in animals offered the HL diet. An extremely high rate of mobilization in this early period can cause TAG accumulation in the liver, which may impair liver function and be detrimental to other body functions including milk production (Top et al., 1995). A reduction in milk production will reduce the requirement for mobilization, allowing an improvement in energy balance to be achieved. Animals on the LL diet did not respond in a similar manner and remained in severe NEB throughout. It has been suggested that, despite the obvious negative effects of NEB, solely the duration of NEB is not a sufficiently good indicator of unsustainable metabolic load (Pryce and Løvendahl 1999; Thomas et al., 1999). Accordingly, prolonged periods of mild NEB may not negatively impact milk production.

From parturition onwards, DEB values were used to calculate CEB which represents an absolute change in overall energy status. When considering actual CEB values, the mean CEB at wk 36 of lactation for multiparous animals on the LH and HL treatments were 7507 and -7103 MJ respectively. This difference (14610 MJ) equates to a hypothetical difference in BCS of 8.3 units (1770 MJ/ unit BCS loss; AFRC, 1993), or a hypothetical difference in BW of 624 kg (23.4 MJ/kg body BW loss (AFRC, 1990). The actual BW difference between animals in these two treatment groups was 39 kg at wk 36 of lactation with an actual BCS difference of 0.49 units.

The discrepancies in CEB may be partly a result of assumptions used in the energy calculations. These assumptions include:

1. the maintenance requirement of all animals is a constant value which is relative to metabolic body weight, irrespective of the level of production.
2. the net efficiency of energy utilisation for lactation (k_l) does not fall below 0.59 despite being partially scaled to level of intake (Thomas, 2004),.
3. all dietary energy intake is partitioned towards maintenance and milk yield

Energy requirements for maintenance (ME_m) represent a substantial proportion of total energy requirements and have first priority in energy partitioning in order to maintain essential metabolic processes and tissue integrity (Kirkland, 2000). It is likely that ME_m is not a constant, but is influenced by the level of intake (Ketelaars and Tolkamp, 1992; Kirkland, 2000) and by the milk production potential (Ferrell and Jenkins, 1984).

Despite having some degree of flexibility, the limited scaling of net efficiency of energy use for milk production to the level of intake may distort calculations of energy status. As ME intake increases, the net efficiency of energy utilisation for milk production (k_l) will decrease below the base value of 0.59 (Yan, Dr. Tianhai; Agri-Food & Biosciences Institute, Northern Ireland, BT26 6DR, personal communication) due to an increase in the energy requirement of processes related to the digestion of food (Van Es and Van der Honing, 1979).

The assumption that ME intake is partitioned only between maintenance and milk production may also have a significant bearing on the energy trajectory. Garnsworthy (2006) reported that maintenance or gain of BW may occur while body reserves decline, and attributed this to a gut fill. This increase in BW could also be attributed to an increase in gut development (after a precalving suppression) and to body protein accretion.

Precalving and Postcalving Dietary Effects on Plasma NEFA Concentrations

Douglas et al. (2006) noted that reduced feed intake in the dry period increased NEFA concentrations in the blood prepartum and resulted in lower liver lipid content postpartum. In the current study, plasma NEFA concentrations in primi- and multi-parous animals were not significantly affected by precalving treatment during the last 3 wks of gestation. However, Figure 1.5 illustrates a significant difference in plasma NEFA concentrations between precalving treatments in primiparous animals at wk 3 prior to calving with high E and low E precalving diets generating concentrations of 0.34 and 0.79 (SED, 0.105), respectively. Precalving NEFA concentrations in primiparous animals were higher (0.51 meq / l) in the current study than in previous studies (e.g. Meikle et al. (2004), 0.30 m*M*; Douglas et al. (2006), 0.25m*M*. However, Vandehaar et al. (1999) and Meikle et al. (2004) reported higher precalving NEFA concentrations in primiparous animals than in multiparous animals, in agreement with the current study.

In the current study, primiparous animals receiving a high E precalving diet had higher plasma NEFA concentrations during the postcalving period than those receiving a low E precalving diet (0.44 vs. 0.38; SED, 0.022), while there was only a tendency ($P = 0.09$) for lower plasma NEFA concentrations in multiparous animals offered the low E precalving diet (Figure 1.5). Dann et al. (2006) stated that dietary restriction, compared to *ad libitum* access to feed in the close-up precalving period did not affect serum NEFA concentrations postcalving. In agreement with this, primiparous animals offered a low E diet precalving (-21d to parturition) in the current study, had similar DMI during the last 21 d of gestation and yet precalving treatment had a significant effect on postcalving plasma NEFA concentrations. This suggests that energy allocation during the 'far-off' period (-80d to -21 d precalving) had a greater influence on plasma NEFA concentrations postcalving than during the close-up period. Body reserve mobilization during the dry period is hypothesized to prepare the cow for a period of energy deficit in early lactation by conditioning the liver to cope more efficiently and effectively with the increase in blood NEFA concentrations that occurs during this period (Friggens et al., 2004b).

CONCLUSIONS

Allocating high-genetic merit Holstein-Friesian animals to either a high E or low E precalving dietary regime significantly affected mean BCS during the precalving period. However, between-treatment differences in BCS in both primiparous and multiparous animals were small. Attempting to substantially alter BCS during the dry period is a difficult task and should be addressed well in advance of drying off. Feeding a low E precalving diet significantly reduced BCS loss postcalving (reflected by changes in plasma NEFA concentrations) and supported a more rapid return to positive energy status in animals offered a high plane of nutrition postcalving. This suggests that initiating BCS loss during the dry period reduces mobilisation postpartum and conditions the liver to better deal with higher NEFA concentrations in early lactation. Postcalving, a low plane of nutrition was detrimental to both milk production and energy balance, and is not suitable for the high-yielding dairy cow. With regards to the assessment of energy status, it is likely that both a cow's maintenance requirements and the net efficiency of energy utilisation for milk production vary with the level of ME intake and production. However, energetic responses to high E and low E diets, pre- and post-calving, in the current study highlight the importance of addressing a dairy cow's energy status precalving, as well as during early lactation.

CHAPTER 2: Effect of Pre- and Post-calving Dietary Energy Content on Behaviour of Dairy Cows in Early and Mid Lactation

ABSTRACT

The experiment examined the effects of energy intake (high E and low E), both pre- and post-calving, on behavioural parameters of 40 multiparous Holstein Friesian dairy cows. From day -100 until day -42 precalving, animals were offered either *ad libitum* or restricted (10.0 kg DM / cow / d) access to a late lactation diet. Thereafter, these animals had either *ad libitum* access to a high energy density diet (high E) or restricted access (7.0 kg DM complete diet / d) to a low energy density diet (low E), until calving. The forage to concentrate (F:C) ratio (DM basis) of these high E and low E precalving diets (d 42 until calving) was 64:36 and 83:17, respectively. Animals offered high E and low E precalving diets were allocated to either a high E or low E postcalving diet (F:C ratio (DM basis) of 30:70 and 70:30, respectively] and remained on these diets until d 250 of lactation. The maintenance behaviour (lying idle, lying ruminating, standing idle, standing ruminating, walking, feeding and drinking) of all animals was observed for one 24 hr period every 14 d, from calving until d 140 postpartum. Precalving treatment had no effect ($P > 0.05$) on any of the 24 hr behaviours measured postcalving. Animals offered the high E postcalving diet had a significantly lower; total ruminating time, ruminating time per unit dry matter intake, time spent lying ruminating, and average length of lying bouts compared to those offered the low E diet, while the reverse was true for dry matter intake, individual meal intakes, eating rate, time spent lying idle and the number of lying bouts per d. Daily energy balance was significantly correlated with the majority of recorded behavioural activities including time spent standing ruminating in the two hr period post evening milking (r^2, 0.748). A number of additional potential explanatory factors were examined in a multiple regression analysis which showed that daily energy balance, milk yield and locomotion score were significantly correlated with time spent standing ruminating in the two hr period, post evening milking (r^2, 0.841).

Keywords: dairy cow, daily energy balance, behavioural activities

INTRODUCTION

Changes in the expression of behavioural activities has been characterised as a mechanism by which an animal adapts to the environment in which it is placed (O'Connell et al., 1989). It is generally accepted that 'normal' behaviour patterns are associated with good animal welfare, while deviations from normal behaviour patterns are indicative of stress. For example, Mench and Mason (1997) attributed the absence or suppression of certain behavioural activities to increased levels of stress. An explicit example of this is play activity in young animals, where its absence is associated with increased stress and poorer welfare (Lawrence, 1987; Lawrence and Appleby, 1996; Jensen et al., 1997). In the case of the high-yielding dairy cow, an array of stressful situations are experienced in early lactation, of which nutritional stress can be most severe. During this period, the cow is unable to consume sufficient food to support high levels of milk production, and this can result in severe and prolonged periods of negative energy balance (NEB) which is manifested in excessive body reserve mobilisation (BRM). When cows experience NEB, the inevitable increase in metabolic load can distort normal physiological function and cause metabolic stress (Thomas *et al.*, 1999; Nielsen, 1999). Animals that experience metabolic stress are predisposed to reduced reproductive efficiency, an increased risk to digestive and metabolic disorders and behavioural abnormalities (Nielsen, 1999). Fregonesi and Leaver (2001) assessed the potential of a range of behaviours to act as indicators of animal welfare in lactating dairy cows and noted that an increase in total lying time and lying synchrony were strong indicators of good welfare. High yielding dairy cows spend about 50% of their time lying. An increase in lying behaviour has been shown to increase rumination time and blood flow to the udder (Haley *et al.*, 2000), the latter being associated with increased milk production (Metcalf *et al.*, 1992). Interestingly, Metz (1985) illustrated that depriving cows from lying and eating for three hours (during milking) greatly increases their motivation to lie down even over eating activity. If increased levels of metabolic stress (NEB) alter the behaviour patterns of high-yielding dairy cows, then it is possible that behavioural changes could have the potential to be used as early indicators of NEB.

This study was designed to examine relationships between energy balance and behavioural patterns in an attempt to identify possible behavioural indicators of metabolic stress and subsequently compromised welfare. It is therefore hypothesized that animals experiencing more severe NEB will display different behavioural patterns to animals with less severe NEB. Furthermore, it is hypothesised that cows in severe negative energy balance will show a greater motivation to feed than to lie down after a period of lying deprivation such as during milking. A full description of cow performance within this study has been presented by Law *et al.* (2011).

MATERIALS AND METHODS

Animals and Housing

This experiment involved 40 multiparous Holstein-Friesian dairy cows (mean parity, 3.2; SEM, 0.06)], which calved between 9th September and 21st December 2004 (mean calving date 19th October; SEM, 3.2). Cows were housed in a free stall house with concrete flooring, with pre- and post-calving cows kept in separate groups. The cubicle to cow ratio was ≥ 1:1 at all times, thus meeting the recommendations of FAWC (1997). Cubicles (2.20m x 1.25m) were fitted with rubber mats and bedded with sawdust thrice weekly. Concrete passageway floors were scraped a minimum of four times daily by an automated system. Lights were left on in the cow house at all times.

Experimental Design, Diets and Feeding

This 2 x 2 factorial design experiment involved two levels of energy intake (high E and low E) precalving and two levels of energy intake (high E and low E) postcalving. Cows were assigned to precalving treatments in a balanced manner, with allocation taking account of parity, milk yield, calving date, BCS and body weight (BW). From d -100 to d -42 precalving (late lactation period), cows were offered a complete diet with a forage to concentrate (F:C) ratio of 36:64 (DM basis), either *ad libitum* or restricted (10.0 kg DM / cow / d). The forage component of this diet

consisted of grass silage and maize silage in equal proportions (1:1 ratio, DM basis). Concentrate composition and analysis, as described by Law *et al.* (2011), is presented in Table 2.1. From drying-off (d -42 precalving) until calving, animals offered the *ad libitum* and restricted diets during late lactation had *ad libitum* access to a high energy density diet (high E) and restricted access (7.0 kg DM / cow / d) to a low energy density diet (low E), respectively. The F:C ratios (DM basis) of these high and low E diets were 64:36 and 83:17, respectively (Table 2.2). Dietary restriction was achieved using electronic feeding gates (American Calan Inc., Northwood, New Hampshire, USA) which were programmed to prevent individual animals gaining access to food after they had consumed their daily allocation of diet.

Postcalving, animals managed on the high E and low E precalving treatments were offered *ad libitum* access to either a high or low E energy lactation diet, remaining on these diets until d 250 of lactation. The F:C ratios of the high E and low E postcalving diets were 30:70 and 70:30, respectively (DM basis), with these diets containing 12.5 and 11.7 MJ of ME / kg DM respectively (Table 2.2). The forage component of the postcalving diet consisted of grass silage and maize silage in equal proportions (1:1 ratio, DM basis). Concentrate ingredient composition and chemical composition is presented in Table 2.1. The metabolisable energy and crude protein concentrations of individual ingredients are based on published values (AFRC, 1993). ME contents of grass and maize silage were determined on a weekly basis using near infrared reflectance spectroscopy (Park et al., 1998). Cows were assigned to postcalving treatment taking account of precalving treatment, parity, previous 305-d milk yield, calving date, and BCS and BW at d 21 precalving.

Consequently, the experiment involved four treatments groups: high E precalving, high E postcalving (HH); high E precalving, low E postcalving (HL); low E precalving, high E postcalving (LH); low E precalving, low E postcalving (LL). The protein contents of pre- and post-calving diets were maintained at 170 and 190 g/kg DM respectively. Animals on the low E postcalving diet received an additional 50 grams / day of a dairy cow mineral preparation to ensure that total mineral intakes were similar on both treatments. Similarly, the concentrate proportion of low E pre- and post-calving diets contained additional soya to ensure all diets (TMR) were iso-proteinic (quantities detailed in Table 2.2). Diets were prepared daily in a mixer

wagon daily and offered as a total mixed ration (TMR) between 1000 and 1100h via feed boxes placed on computer-linked weigh-cells. Access to feed boxes (shared among a maximum of 3 cows) was controlled by an electronic identification system (described previously), enabling fresh intakes of individual animals to be recorded continuously. Dry matter intakes were subsequently calculated based on daily oven DM values for grass and maize silage and fortnightly oven DM values for concentrates, as detailed below. Daily intakes were used to calculate an average daily intake for each week of lactation. Postcalving, diet allocation included a target excess of 7 percent daily and uneaten material was removed thrice weekly.

Table 2.1: Ingredient composition and chemical composition of the concentrate component of the diet offered in late lactation, dry and postcalving periods.

Ingredient composition (g/kg)	Late lactation period	Dry period[3]	Postcalving period
Barley (milled)	207	162	162
Wheat (milled)	207	162	162
Unmolassed sugar beet pulp	180	132	132
Citrus pulp	178	162	162
Soya bean meal (Hi-Pro)	63	260	260
Rape meal	56	41	41
Rumen-inert fat[2]	29	22	22
Dairy cow minerals	46	34	34
Molasses[1]	34	25	25
Chemical composition			
ME (MJ/kg DM)	12.8	13.1	13.1
CP (g/kg DM)	136	220	220
ADF (g/kg DM)	110	109	109
NDF (g/kg DM)	211	217	217
Ash (g/kg DM)	84.8	83.9	83.9
Starch (g/kg DM)	280	218	218

[1] Molaferm, United Molasses, Belfast, NI, UK

[2] Megalac, Volac Ltd. Orwell, Hertfordshire, UK

[3] Dry cow mineral was added to dry cow diet at a level of 120 grams / head / day; Trouw Nutrition, Belfast, NI, UK

Table 2.2: Chemical composition of individual ration components of the high and low energy diets offered pre- and post-calving.

	High precalving[3]		Low precalving[3]			High postcalving			Low postcalving[4]			
	Conc.[1]	Grass silage	Conc.[1]	Soya	Grass silage	Conc.[1]	Grass silage	Maize silage	Conc.[1]	Soya	Grass silage	Maize silage
Proportion of diet	0.36	0.64	0.12	0.05	0.83	0.70	0.15	0.15	0.165	0.135	0.35	0.35
DM content (g/kg)	870	198	870	870	198	870	228	289	870	870	228	289
CP (g/kg DM)[2]	220	141	220	540	141	220	147	86	220	540	147	86
ME (MJ/kg DM)[2]	13.1	10.3	13.1	13.4	10.3	13.1	11.4	10.6	13.1	13.4	11.4	10.6
Starch (g/kg DM)	218	-	218	20	-	218	-	205	218	20	-	205
Total diet												
Total DM (g/kg)	440		312			687			442			
Total ME (MJ/kg DM)[2]	11.3		10.8			12.5			11.7			
Total CP (g/kg DM)	169		170			189			191			
Total Starch (g/kg DM)	79		29			183			110			

[1] Conc., concentrate

[2] ME, metabolisable energy

[3] Precalving diet denotes the dry period of multiparous animals and 3 weeks prior to calving in primiparous animals

[4] Animals on a low postcalving diet received an additional 50 grams of dairy cow mineral / head / day

Measurements

The behaviour of all animals was recorded by direct observation for a 24 hr period once per 14 d, from calving until 140 d post-partum. This 24 hr observational period was divided into three eight-hr periods, over three consecutive days (Botheras et al., 2004). During each assessment the group was scanned at 15 minute intervals and each cow's behaviour recorded. Behaviours recorded were; lying idle, lying ruminating, standing idle, standing ruminating, walking, feeding and drinking, queuing to access the feeders (maintenance behaviours), mounting behaviour, social behaviour (grooming) and aggressive behaviour (butting). Lying behaviour was further categorized into lying position; stretched or normal. In addition, the incidence of abdominal pressing whilst lying was noted (pressing as a result of voluntary blocking of the airway). Lying in the stretched position was defined as one or more limbs being full extended in any direction. When assessing feeding behaviour, meal weight and duration was measured for individual animals. Units of feeding were defined at the level of feeding bouts, and clusters of feeding bouts formed a meal. A new meal was defined when the interval between feeding bouts was greater than or equal to four minutes and 30 seconds. This formed the boundary between within-meal pauses and between meal intervals. Meal interval was determined using the method proposed by Forbes *et al* (1986).

Cows were milked twice daily, between 0530 and 0700, and 1530 and 1700, through a 50 point rotary parlor. Milk yield was recorded automatically at each milking for each individual animal and a mean daily milk yield was calculated for each animal on a weekly basis. Milk samples were taken from each cow during two consecutive milkings (a.m. & p.m.) each week and analyzed for fat, protein, lactose, and somatic cell count, with a.m. and p.m. samples analyzed separately using an infrared milk analyzer. Each sample had a preservative tablet added (Lactab Mark III, Thompson and Cooper Ltd, Runcorn, UK) and was stored at 4°C until analyzed. A weighted milk composition was subsequently calculated for each weekly sampling occasion. Body condition score [scale: 0-5 (Edmonson et al., 1989)] and live weight were measured weekly from d -42 (-21 in primiparous animals) until d 140 postcalving while locomotion score (LS, Manson and Leaver, 1988) was assessed fortnightly during the postcalving period.

Calculation of Energy Balance

The average DEB for each animal was calculated for each wk of lactation using the equations described by Thomas (2004) {DEB = ME intake – ME requirement [-10 + (ME_{preg} + $ME_{maintmilk}$ * live weight$^{0.75}$)] + [(0.0013*live weight)/K_m)]; K_m, efficiency of energy use for maintenance (0.35 x ME/GE + 0.503)}. Milk yields, DMI, milk compositions, live weight and feed composition data were all used in the calculations. Missing values were estimated from data for the wk prior to and the wk following the wk of missing observations. Less than 2% of the data were missing.

Statistical Analysis

A repeated measures approach using the Residual Maximum Likelihood (REML) procedure available in GenStat (Payne et al. 2007) was used to analyze the data set with parity, precalving treatment, postcalving treatment and stage of lactation (ten * fourteen d periods from calving) included as factors. Furthermore, data in the two hr period post milking was also analyzed.

Simple linear and multiple linear regression analysis were performed for several independent variables on all the behavioural activities. In the linear regression analysis DEB was used as the independent variable. Daily energy balance and behavioural data were adjusted to remove cow effects (repeated measures analysis) in order to analyze the effects of DEB on behavioural activities. In the multiple linear regression analysis the independent variables were: dry matter intake (DMI), milk yield, energy corrected milk yield (ECMY), LS and DEB. Further linear and multiple regression analysis was carried out on data collected from the 2 hr period post milking (a.m. and p.m.), after a period of lying deprivation. Behavioural activities with insufficient records were excluded from the analysis. These were walking, mounting, social behaviour, aggressive behaviour, queuing, and abdominal pressing.

RESULTS

Effect of Pre-and Post-calving Diets on Production and Energy Parameters (1-140 d)

Precalving diet had no significant effect ($P > 0.05$) on mean BCS, liveweight, milk yield, ME intake or energy balance during the postcalving period, although cows managed on the high E and low E precalving diets having mean BCS at calving of 2.68 and 2.34, ($P < 0.001$; SED, 0.086) respectively.

Animals offered a high E diet postcalving (d 1-140) had a significantly higher TDMI ($P < 0.001$), milk yield ($P < 0.05$), BCS ($P < 0.05$), DEB ($P < 0.001$), and CEB ($P < 0.001$) than those offered low E diet during the postcalving period (Table 2.3). There was no significant effect of postcalving diet on milk fat plus protein yield ($P > 0.05$)

There were significant ($P < 0.001$) 3-way interactions (wk of lactation * precalving treatment * postcalving treatment) for yield of milk, ME requirement, DEB (Figure 2.1), and CEB (Figure 2.2). For all of these parameters, the precalving treatment significantly affected the postcalving response trajectory. With regards to both DEB and CEB, animals on a high E postcalving diet had an improved energy balance when offered a low E, compared to a high E precalving diet. However, animals on a low E postcalving diet had an improved energy balance when offered a high E, compared to a low E precalving diet.

Table 2.3: Effect of postcalving dietary treatment on production and energy status of dairy cows during the first 140 d of lactation.

	Postcalving treatments		**SED[1]**	**Sig.[1]**
	High E	**Low E**		
Total dry matter intake (kg / d)	20.4	16.7	0.61	***
Milk yield (kg / d)	35.3	31.1	1.68	*
Milk fat + protein yield (kg / d)	2.40	2.33	0.101	NS

Body Condition Score	2.53	2.31	0.096	*
Daily energy balance (MJ / d)	20.7	-27.3	6.48	***
Cumulative energy balance (MJ)	623	-2665	840	**

[1] SED, standard error of the difference; Sig, significance

[2] NS, P > 0.05; *, P < 0.05; **, P < 0.01; ***, P < 0.001

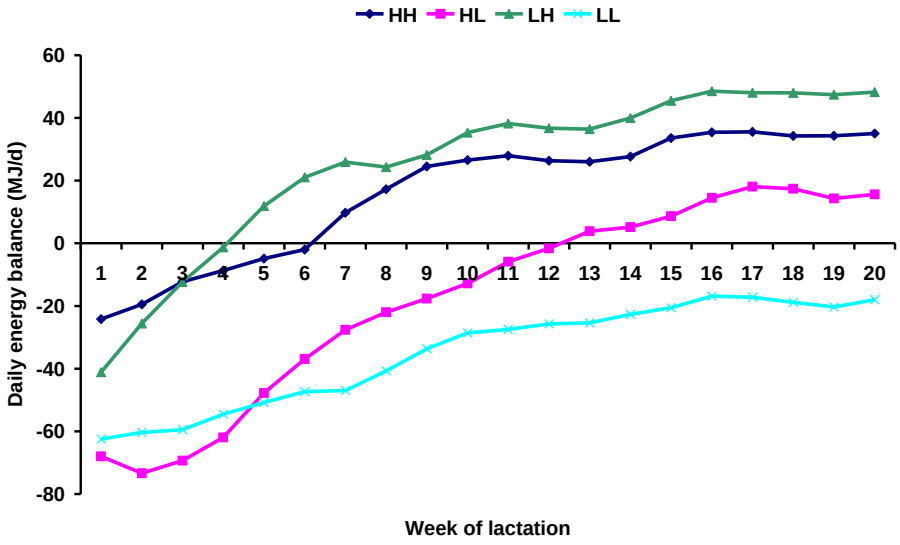


Figure 2.1: The predicted means of daily energy balance of animals (n=41) offered a high (H) or low (L) diet precalving and a high (H) or low (L) diet postcalving with weeks from parturition

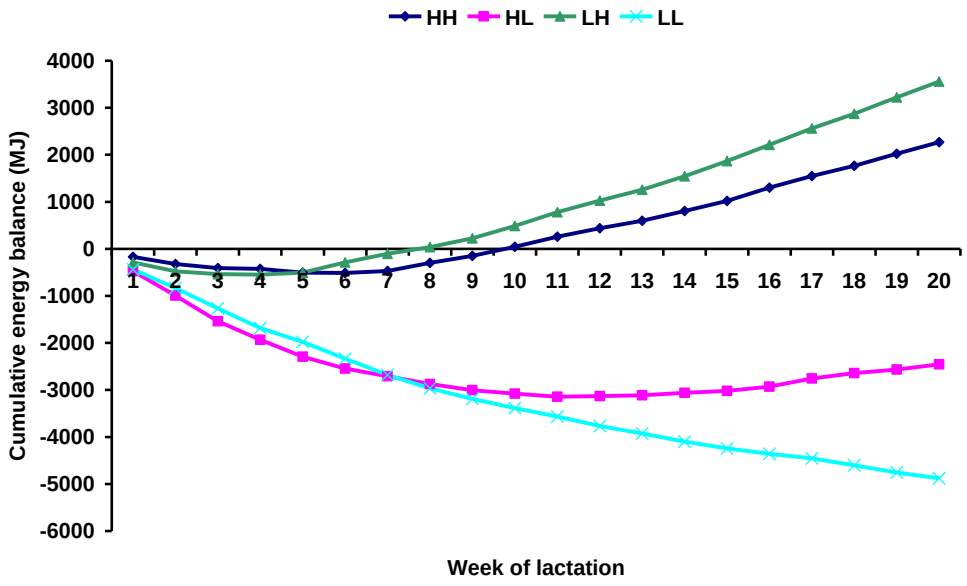


Figure 2.2: The predicted means of cumulative energy balance of animals (n=41) offered a high (H) or low (L) diet precalving and a high (H) or low (L) diet postcalving with weeks from parturition

Effect of Pre- and Post-calving Diet on Behavioural Activities (1-140 d)

Precalving treatment had no significant effect on any of the behaviours recorded.

Animals offered a high E diet postcalving spent less time ruminating ($P < 0.01$), lying ruminating ($P < 0.05$), had a lower ruminating time per unit DMI ($P < 0.001$), and had a shorter duration of the average lying bout ($P < 0.001$), compared to animals offered a low E diet. However, animals offered a high E diet postcalving spent a higher proportion of their time lying idle ($P < 0.05$) and had an increased number of lying bouts per d ($P < 0.001$). Postcalving treatment had no significant effect ($P > 0.05$) on total lying time, the percentage of lying time spent stretched, time standing idle, time standing ruminating, or total standing time. Effects of postcalving treatment on behavioural activities are shown in Table 2.4.

Postcalving treatment had no significant effect on behaviours during the 2-hr period post morning milking (Table 2.5). However, during the 2-hr period post evening milking animals

offered a high E diet spent significantly more time lying ($P < 0.001$) and lying idle ($P < 0.001$), and less time feeding ($P < 0.05$) and standing ($P < 0.001$) than animals offered a low E postcalving diet.

Precalving treatment had no significant ($P > 0.05$) effect on feeding behaviours. Animals offered the high E diet postcalving diet had a significantly ($P < 0.05$) lower number of meals per d compared to animals offered the low E diet postcalving. Animals offered a high E postcalving diet had significantly higher average meal weight ($P < 0.01$), and average eating rate [($P < 0.001$) Table 2.6].

Table 2.4: Effect of postcalving dietary treatment on dairy cow behaviour during the first 140 d of lactation (as a percentage of time spent in the house).

	Postcalving treatments		**SED**[1]	**Sig.**[1]
	High E	**Low E**		
Total ruminating (% of time)	32.3	35.8	1.66	**
Ruminating time / kg DMI (hr/kg)	0.39	0.55	0.008	***
Total lying (% of time)	53.1	51.5	3.62	NS
Lying idle (% of time)	31.2	27.6	2.21	*
Lying ruminating (% of time)	22.0	24.0	2.21	*
Lying stretched (% of lying time)	2.9	2.5	0.76	NS
No. of lying bouts per d	9.5	7.4	0.65	***
Average length of a lying bout (hr)	1.1	1.4	0.09	***
Total standing (% of time)	46.9	48.5	2.63	NS
Standing idle (% of time)	17.7	15.5	1.36	NS
Standing ruminating (% of time)	10.2	11.9	1.80	NS
Feeding (% of time)	15.7	17.2	1.13	NS
Drinking (% of time)	2.3	1.1	0.33	***

[1] SED, standard error of the difference; Sig, significance

[2] NS, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

Effect of Stage of Lactation on Behavioural Activities (1-140 d)

As lactation progressed there was a significant ($P < 0.001$) increase in total lying time, time spent lying idle, and time spent lying ruminating (Figure 2.3). There was also a significant ($P < 0.001$) decrease in total standing time, time spent standing ruminating, time spent standing idle (Figure 2.4), total ruminating time (28 days onwards), and the time spent ruminating per unit DMI. Time spent lying stretched (one or more limbs fully extended) significantly ($P < 0.001$) increased as lactation progressed. Despite time points being significantly different, no clear relationship with stage of lactation could be identified for LS ($P < 0.001$), the number of lying bouts per d ($P < 0.001$), and time spent feeding ($P < 0.05$). The average eating rate significantly ($P < 0.001$) increased with stage of lactation to a maximum at 100 d post partum.

Effect of Daily Energy Status on Behavioural Activities

Animal and time effects were removed from energy and behaviour data to identify underlying effects of DEB on behavioural activities. Average DEB (average calculated over a 7 d period) was significantly correlated with the majority of behavioural activities recorded (Table 2.7). However, the percentage of the residual variation explained by DEB was less than 50 percent for the majority of these behavioural activities. Linear regression analysis of DEB against time spent standing idle, ruminating per kg DMI, and standing ruminating in the two hr period post evening milking produced r^2 values of 0.504, 0.721 and 0.748, respectively. A strong negative relationship existed between standing ruminating in the two hr period post evening milking and DEB (Figure 2.5). Multiple regression analysis, which included the explanatory variables; DMI, milk yield, LS and DEB, was carried out on each of these behavioural activities and the outputs are shown in Table 2.8. Daily energy balance, milk yield and locomotion score significantly ($P < 0.001$) affected the time allocated to standing ruminating in the 2 hr period post evening milking ($r^2 = 0.841$).

Table 2.5: Effect of postcalving dietary treatment on behavioural activities of dairy cows during the two hr period post morning and evening milking (d 1-140)

	2-hr post a.m. milking				2-hr post p.m. milking			
	High E	Low E	SED[1]	Sig.[1]	High E	Low E	SED[1]	Sig.[1]
Lying idle (% of time)	40.6	38.8	3.14	NS	32.0	21.0	2.20	***
Lying ruminating (% of time)	27.8	31.4	2.45	NS	23.8	20.4	2.20	NS
Total lying (% of time)	68.5	70.2	3.86	NS	55.7	41.4	3.12	***
Feeding (% of time)	11.4	10.2	1.98	NS	21.6	27.7	2.86	*
Total standing (% of time)	31.5	29.8	3.86	NS	44.3	58.7	3.12	***
Standing ruminating (% of time)	4.5	5.6	1.64	NS	3.8	6.8	1.96	NS
Standing idle (% of time)	12.3	10.7	1.86	NS	13.4	13.3	1.96	NS

[1] SED, standard error of the difference; Sig, significance

[2] NS, $P > 0.05$; *, $P < 0.05$; ***, $P < 0.001$

Table 2.6: Effect of postcalving dietary treatment on feeding activities of dairy cows during the first 140 d of lactation.

	Postcalving treatments [1]		SED	Sig.[1]
	High E	Low E		
Meals / d	12.5	14.7	0.91	*
Average meal duration (min)	12.5	12.2	0.98	NS
Average dry matter intake (kg DM / d)	20.4	16.7	0.61	***
Average meal weight (kg DM)	1.86	1.38	0.21	**
Average eating rate (kg DM / min)	0.14	0.10	0.003	***

[1] SED, standard error of the difference; Sig, significance

[2] NS, P > 0.05; *, P < 0.05; **,P < 0.01; ***, P < 0.001

Table 2.7: Regression of behaviours against daily energy balance during the first 140 d of lactation

Behavior[1]	**Significance**[2]	**Relationship**	**r^2**
Lying (total)	***	Positive	0.24
Lying idle	***	Positive	0.20
Lying ruminating	***	Positive	0.14
Number of lying bouts per d	***	Positive	0.20
Average length of a lying bout	***	Positive	0.09
Lying stretched	***	Positive	0.45
Total lying (2HPEM)	***	Positive	0.18
Lying idle (2HPEM)	***	Positive	0.13
Lying ruminating (2HPEM)	***	Positive	0.19
Standing (total)	***	Negative	0.24
Standing idle	***	Negative	0.50
Standing ruminating	***	Negative	0.21
Total standing (2HPEM)	**	Negative	0.02
Standing idle (2HPEM)	**NS**		
Standing ruminating (2HPEM)	***	Negative	0.76
Drinking	***	Negative	0.09
Feeding	***	Positive	0.15
Feeding (2HPEM)	***	Positive	0.67
Ruminating	**NS**		
Ruminating per kg DMI	***	Negative	0.67

[1] kg, kilogram; DMI dry matter intake; 2HPEM, 2 hours period post evening milking

[2] NS, $P > 0.05$; **,$P < 0.01$; ***, $P < 0.001$

Table 2.8: Multiple regression analysis for behavioural activities

	Factor [1]	T-value [2]	Significance	r^2
Standing idle	DEB	-14.57	***	0.721
	MY	-13.70		
	LS	-9.70		
Ruminating time per kg DMI	TDMI	-12.32	***	0.761
	DEB	-7.61		
Standing ruminating 2 hours post milking	DEB	-29.29	***	0.841
	MY	-12.59		
	LS	-7.26		

[1] DEB, daily energy balance; MY, milk yield; TDMI, total dry matter intake; DMI, dry matter intake; LS, locomotion score

[2] T-value corresponds to the importance of each factor relative to the least important. The sign (+/-) indicates the nature of the correlation

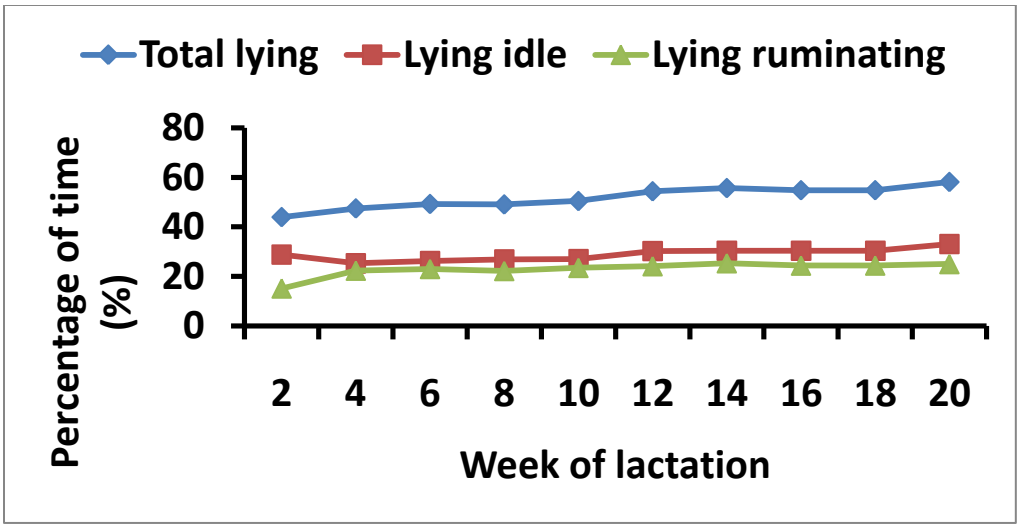


Figure 2.3: The effects of stage of lactation on the predicted means for total lying time, time spent lying idle and time spent lying ruminating

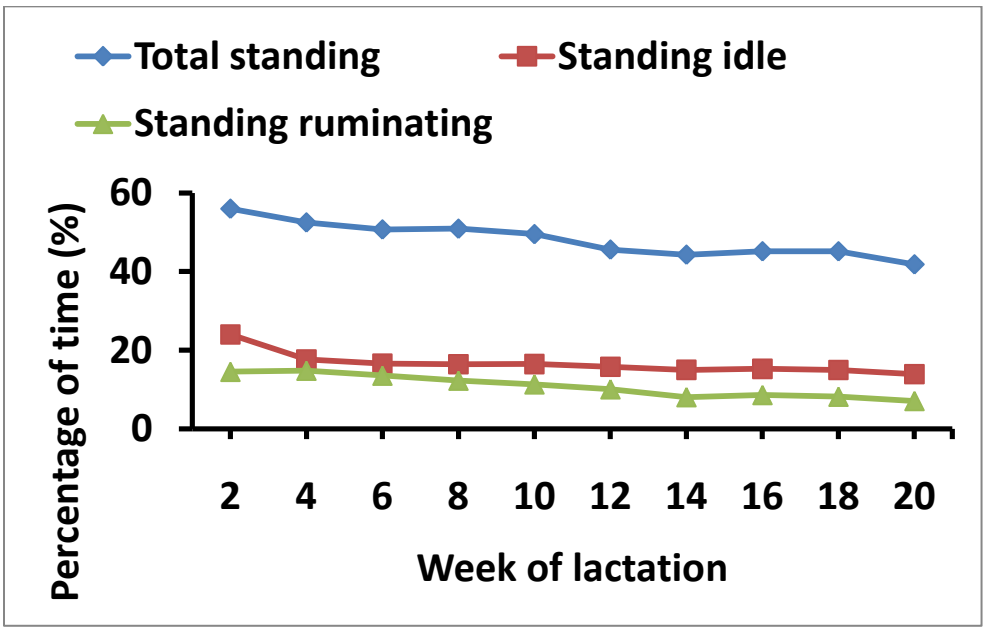


Figure 2.4: The effects of stage of lactation on the predicted means of total standing time, time spent standing idle and time spent standing ruminating

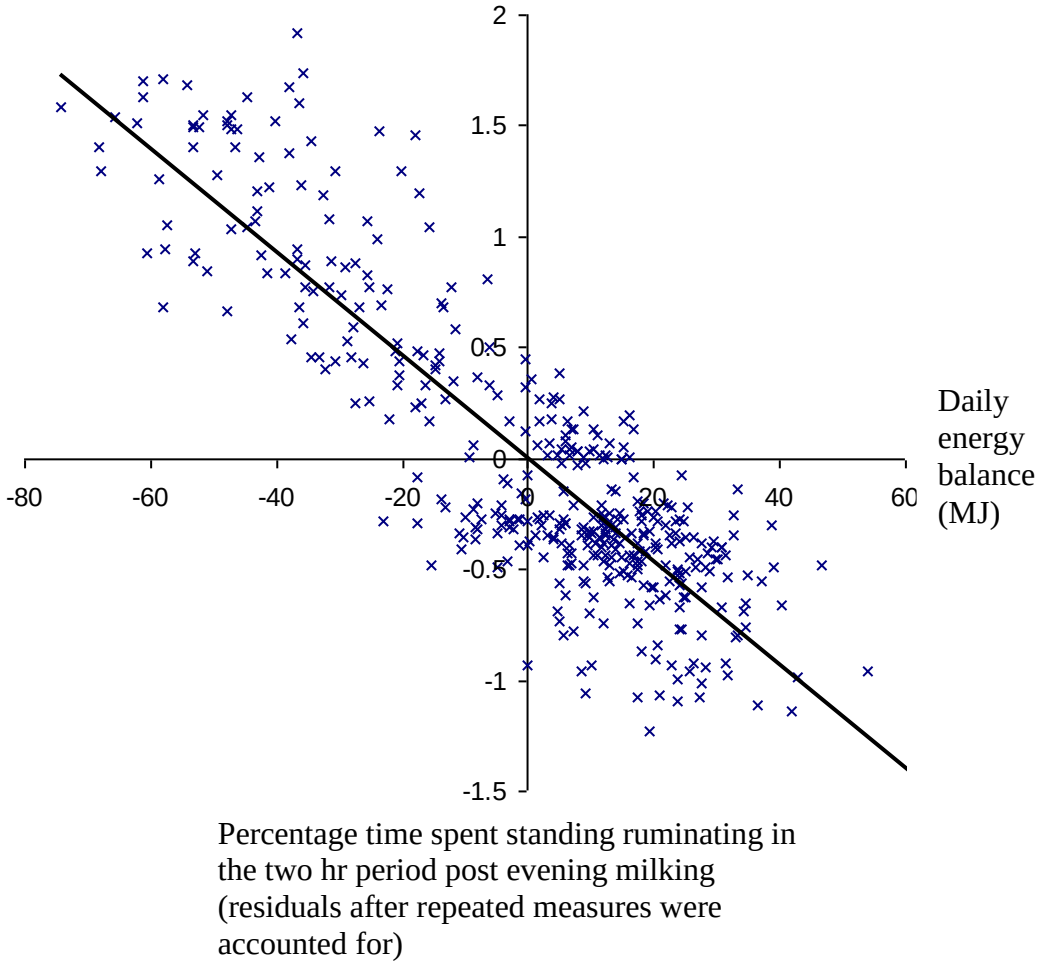


Figure 2.5: Regression of daily energy balance against the percentage time spent standing ruminating in the 2 hr period post evening milking, $r^2 = 0.748$

DISCUSSION

Dietary Effects on Maintenance Behavioural Activities

In the current study, postcalving dietary treatment had no significant effect on total lying time or total standing time, in agreement with work carried out by Bao and Giller (1991). However,

Nielsen et al. (2000) illustrated that cows on a low concentrate diet spent more time standing, which may result from an increase in the amount of time spent standing eating and standing ruminating. However, the observational period in the study by Nielsen et al. (2000) was only six hours during a 24 hr period, which may not portray an accurate time budget for the cow. Nevertheless, in the present study, animals on different dietary treatments were not housed in separate treatment groups; therefore, the behavioural activities observed on individual treatments may not be totally independent of each other.

A higher total ruminating time and time spent ruminating per unit DMI was observed in animals offered the low E postcalving diet and can be explained by the higher dietary forage content of the low E diet. High concentrations of cell wall constituents associated with a high dietary forage content will incur an increased particle retention time in the rumen (Doreau et al., 2004). For example, Allen (1996) reported an inverse relationship between ruminating time per unit DMI and total intake. However, contrary to results of the present study, Metz (1975) reported that daily food intake and total rumination time were positively correlated. The prioritization of ruminating behaviour in animals offered a low E postcalving diet will inevitably cause a reduction in the expression of other behavioural activities. In the current study, time spent lying idle, which has been deemed an essential activity in maintaining production and an acceptable level of welfare (Wierenga and Hopster, 1990), appears to have been be sacrificed to cater for the increased need to ruminate whilst lying in animals on the low E postcalving diet. The pattern of lying behaviour was also affected by postcalving diet. The increased number of lying bouts per d, of shorter duration, experienced by animals offered the high E postcalving diet may have been due to more discomfort in the form of increased udder pressure due to the increased milk yield. An increased frequency of positional changes (standing vs. lying) is one method of dealing with this discomfort. This is in agreement with Oésterman and Redbo (2000), who suggested that an increase in udder pressure will result in an increase in the number of lying bouts, on the basis that the cow experiences external udder pressure as well as internal pressure. When a lying bout has ended due to discomfort, the motivation for lying behaviour still remains. This will prompt the start of a new lying bout and consequently increase the number of bouts per d.

Dietary Effects on Feeding Behaviour

Animals offered the low E postcalving diet had a significant higher number of meals per d which may have been due to the lower energy density and high fibre content of the low E postcalving diet. High fibre diets have a greater bulk density than low fibre diets and therefore result in lower intakes of dry matter per meal. For animals offered a low E diet, physical factors are likely to limit feed intake more rapidly than for animals offered a high E diet, and cows respond by having a greater number of meals per day. Interestingly, Nocek and Braud (1985) stated that an increased number of meals per d (due to an increased feed allocation frequency), increases the efficiency of milk production as it facilitates more stable rumen fermentation patterns, causing a more constant rumen pH which will promote higher cellulolytic activity and improve digestion. The average meal duration was not significantly different across dietary treatments, which is contrary to previously published work. For example, Nielsen et al. (2000) and Friggens et al. (1998) both reported a longer meal duration in animals offered a low concentrate diet. A diet containing a high forage proportion (low concentrate diet) may require more mastication before being swallowed, therefore reducing the rate of ingestion of fresh feed. However, as all diets in the present study were thoroughly mixed in a feeder wagon and presented in a processed manner as a total mixed ration (TMR), any small differences in palatability would not be expected to influence feeding time.

Stage of Lactation and Parity on Behavioural Activities

Post calving, while the time spent ruminating in the standing position and the lying position was equal during the postcalving period, as lactation progressed there was an increased proportion of time spent ruminating whilst lying. In agreement with this, Nielsen et al. (2000) stated that the time spent standing ruminating decreases with time from calving. Furthermore, Öesterman and Redbo (2000) stated that cows prefer to ruminate whilst lying and that an increased frequency of ruminating whilst standing was an indication of discomfort. The purpose of ruminating whilst standing at the beginning of lactation is unclear and will be discussed later. An increase in the time spent lying with stage of lactation may be influenced by increased comfort levels due to the

reduction in udder pressure once peak lactation is surpassed. Nielsen et al. (2000) also stated that the number of lying bouts significantly decreased, and the duration of a lying bout significantly increased, with stage of lactation. The present study failed to identify a significant effect of stage of lactation on the average duration of a lying bout and despite identifying a significant influence of stage of lactation on the frequency of lying bouts, there was no obvious relationship.

Effect of Daily Energy Balance on Behavioural Activities

When cows experience negative energy balance it will inevitably result in metabolic stress. Stress has been deemed a causal factor for deviations from normal behaviour patterns. However, in identifying such deviations it is important that normal behaviour patterns (in a specific environment) are clearly defined. Within a given environment, cows display a clear and constant daily pattern of feeding and lying behaviour, producing a daily rhythm (Wierenga and Hopster, 1990). This daily rhythm will be influenced by environmental stimuli. For example, following a period of lying deprivation during milking, the motivation for lying behaviour will greatly increase. Metz (1985) stated that depriving cows from lying and eating for three hours (during milking) greatly increases their motivation to lie down, and to do so in preference to eating. This prioritization of lying over eating was also reported by Wierenga and Hopster (1990). Therefore, the absence of lying behaviour following milking could be construed as an indicator of the cow experiencing or being exposed to stress. Historically, such stresses would have been characterized as discomfort. Feddes et al. (1995) noted that a reduced total lying time and an increased time standing idle indicates a reduced level of comfort, and that cows will stand because it hurts to lie down or it's physically difficult to lie down. The results of the present study would suggest an alternative theory as to why the motivation for lying down behaviour is suppressed; displacement behaviour caused by severe NEB. When there are two conflicting motivations to express two separate behavioural activities, displacement behaviour can result. This is an alternative third behavioural activity for which no strong motivation is apparent. In the current study, a more severe NEB (metabolic stress) will increase the motivation to consume food which will cause a conflict of interest post milking when there is a high motivation to lie down. This conflict of interests appears to cause an increase in the expression of displacement

behaviour, which in this case is standing ruminating in the two hr period post milking. Munksgaard and Simonsen (1996) suggested that increased frequencies of specific behaviours such as eating or rumination may be displacement activities. Displacement behaviour is caused by stress which will inevitably be detrimental to the biological function of the animal.

The identification of a strong link between negative DEB and the expression of standing ruminating in the two hr period post evening milking provides evidence that behavioural abnormalities could prove useful in the identification of cows experiencing severe NEB. However, the results obtained in the current experiment are specific to the animals used and management system implemented. Therefore, variation in factors such as management system may influence the time budget of certain behavioural activities and so may not concur with the results of the current study. As a result, it is difficult to derive robust prediction models that can be employed on a commercial basis and further work needs to be undertaken to examine the effect of different management regimes before solid conclusions on the viability of using behavioural measurements as indicators of severe NEB.

CONCLUSIONS

The present study illustrated that a high E postcalving diet resulted in a more positive energy balance during early lactation, especially for cows managed on a low E precalving diet. Furthermore, diet energy level postcalving influenced the frequency and duration of behavioural activities. Significant associations between average DEB and behavioural activities highlighted potential areas for the development of indicators of energy imbalance. However, these results are specific to this study and further work is needed before solid conclusions can be made on the link between energy balance and dairy cow behavioural patterns.

CHAPTER 3: Effect of pre- and post-calving dietary energy content on the fertility of dairy cows during early and mid-lactation

ABSTRACT

Poor reproductive performance adversely impacts on the sustainability of modern high yielding dairy systems. Eighty Holstein animals (40 primiparous and 40 multiparous (mean parity, 3.2)) were allocated to one of four treatments comprising a 2x2 factorial design to compare the effects of a high and low energy density diet during both the pre- and post-calving periods. From day 80 until day 21 pre-calving, heifers on high and low pre-calving dietary treatments were offered high and low pasture allowances respectively. From day 21 pre-calving until calving, these animals were housed and offered access to diets on either an *ad libitum* (A) or restricted (R) basis (restricted to 6 kg DM per day). The pre-calving treatments for multiparous animals commenced 100 days prior to the predicted parturition date with diets offered *ad libitum.* Animals offered the low energy diet pre-calving were restricted (R) to 6 kg DM complete diet per day from day 42 pre-calving, while the high energy pre-calving diet continued to be offered *ad libitum* (A). The concentrate to forage (grass silage) ratios (DM basis) of the high and low energy density diets pre-calving were 36:64 and 17:83 respectively. Upon parturition the post-calving treatments (H and L) commenced and were offered *ad libitum* throughout. The concentrate to forage ratios (DM basis) of the high (H) and low (L) energy density diets post-calving were 70:30 and 28:72 respectively. Consequently, there were four treatments groups; AH, AL, RH and RL. The breeding period commenced 92 days from the start of the calving period, at a minimum of 42 days post-calving. Conception was defined by rectal palpation using an ultrasound scanner (Pd positive) at 100d post artificial insemination. Pre- and post-calving dietary treatment, and body condition score at calving, had no significant effect on any of the reproductive parameters. The average pregnancy rate to first service was low (25%), with the average number of services per conception being 2.8. The average 100d in calf rate from the beginning of the breeding period was 58% and overall, 86% of cows intended for breeding conceived during the six month breeding period. Post calving dietary treatment had a significant effect on average daily energy balance (ADEB) ($P \leq 0.001$). The range in ADEB for individual

animals in the first 21 days of lactation was +33 to -114 MJ/d. The interval to the commencement of luteal activity was negatively associated with cumulative EB ($P<0.05$) and a change in IGF-1 concentrations ($P<0.01$), and positively associated with weeks to energy nadir ($P<0.05$). An increase in ADEB significantly increased the duration of the luteal phase. There were no significant effects of plasma NEFA or liver TAG concentrations on serum IGF-1 concentrations.

INTRODUCTION

The continuing decline in reproductive performance of the contemporary high yielding dairy cow is placing the sustainability of this farming system in jeopardy. This decline has occurred over many years and reproductive failure has become the predominant reason for culling dairy cows (Mayne *et al.,* 2002). Elevated culling rates increases the need for replacements, therefore reducing the average age of the herd and subsequently reducing the herd's overall milk yield potential. There is a common consensus that negative energy balance (NEB) is one of the most important factors causing this decline in reproductive performance (Webb *et al.,* 1999). Most definitions of NEB assume that there is no requirement for body state change, and define NEB as the energy deficit required from body reserve mobilisation (BRM) in early lactation to meet the requirements of milk production. However, an animal has a genetic requirement to change body state depending on its physiological state, suggesting that this body reserve mobilisation is not entirely environmentally driven (Friggens *et al.,* 2004; Garnsworthy, 2006). Irrespective of the cause, an increase in post-calving BRM, commonly observed in cows which are over-conditioned at calving, causes the accumulation of tricylglycerols (TAG) in the liver (Butler, 2000), which have been associated with a longer interval to first ovulation and reduced fertility (Butler and Smith 1989; Rukkwamsuk *et al.,* 1999a). On commencement of BRM, non-esterified fatty acids (NEFA) from the adipose tissue are released into the bloodstream. An increase in the plasma NEFA concentration leads to an increase in hepatic uptake of NEFA. In the liver, NEFA are either esterified to form TAG or metabolised to acetyl CoA which will undergo oxidisation in the tricarboxylic acid (TCA) cycle, or be used as a substrate for ketogenesis (Rukkwamsuk *et al.,*

1999b). As expected, a positive relationship has been identified between NEFA uptake by the liver and the synthesis of TAG (Top *et al.*, 1995). Normally TAG are secreted by the liver in the form of very low density lipoproteins (VLDL) and transported to the mammary gland. However, this pathway is restricted by the limited supply of additional substrates needed for the conversion of TAG to VLDL, especially in early lactation (Rukkwamsuk *et al.*, 1999b). It has been suggested that the accumulation of TAG is detrimental to liver and reproductive function. An increase in liver TAG has been associated with reduced IGF-1 concentrations which have been implicated in the reduced production of important reproductive hormones: oestradiol and progesterone (Spicer *et al.*, 1990). However, as highlighted by Newbold (2006), this is not the only pathway through which NEB is detrimental to reproductive performance. NEFA in the bloodstream have been shown to act directly on follicular development, reducing cell proliferation and subsequently reducing the probability of a viable oocyte and ovulation (Jorritsma *et al.*, 2004).

The present experiment was initiated to examine the effect of two levels of energy in the diet, both pre- and post-calving and thus effects on cow energy status and reproductive performance.

MATERIALS AND METHODS

Animals and housing

Eighty Holstein animals (40 primiparous and 40 multiparous (mean parity, 3.2)) were used in the experiment (same animals as Experiment 1). Calving commenced on 27th August and ended on 21st December. Pre-calving, primiparous animals were housed 21 days before parturition and multiparous animals were housed for the duration of the pre-calving treatment. Following calving, all animals were housed as a single group in free stalls with concrete flooring. The cubicle to cow ratio was $\geq$ 1:1 at all times, meeting the recommendations of FAWC (1997). All cubicles had a bed measurement of 2.20 m long and 1.25 m wide, were fitted with rubber mats and bedded with sawdust thrice weekly. Concrete passageway floors were scraped a minimum of

four times daily, using an automated system. Cows were milked twice daily through a 50 point rotary parlour at 05.30 and 15.30 hours, with cows walking about 35 m to the milking parlour. Lights were left on at all times.

Experimental design, diets and feeding

The experiment was based on a 2x2 factorial design: with high or low energy density diets, both pre- and post-calving. From day 80 until day 21 pre-calving, heifers on high and low pre-calving dietary treatments were offered high and low pasture allowances respectively. These were based on a herbage allowance of 25 and 10 kg DM/cow/day assessed above 5cm cutting height. From day 21 pre-calving until calving, heifers were housed and those on high and low energy diets were offered diets either *ad libitum* (A) or restricted (R) to 6 kg DM per day. Dietary restriction was achieved by using electronic feeding gates which restrict access after the consumption of 6 kg DM per day. The pre-calving treatments for multiparous animals commenced 100 days prior to the predicted parturition date. These animals were housed and high and low energy diets were offered *ad libitum*. From day 42 pre-calving, multiparous animals receiving the low energy diet were restricted (R) to 6 kg DM complete diet per day, while those on a high energy pre-calving diet continued to be fed *ad libitum* (A). The concentrate to forage (grass silage) ratios (DM basis) of the high and low energy density diets pre-calving were 36:64 and 17:83 respectively. All treatments were balanced for parity, body weight and date of calving. Following parturition, the post-calving treatments commenced and the diets were offered *ad libitum* until the end of the experiment at 250 days post-calving. The concentrate to forage ratios (DM basis) of the high (H) and low (L) energy density diets post-calving were 70:30 and 28:72 respectfully, providing 12.5 and 11.7 ME per kg DM. Consequently, there were four treatments groups; AH, AL, RH and RL. The forage component was made up of grass and maize silages in a 1:1 ratio (DM basis). Diet composition and analysis is presented in Table 3.1. The concentrate component of the diet consisted of (DM g/kg): 260 soya bean meal; 162 barley; 162 wheat; 162 citrus pulp, 132 sugar beet pulp, 41 rapeseed meal, 34 dairy cow mineral/vitamin supplement, 25 molaferm (United molasses, Belfast) and 22 Megalac (Volac Ltd. Hertfordshire). Samples of grass and maize silage were taken weekly and analysed using near infrared reflectance spectroscopy (Park *et al.*, 1998),

while daily samples were analysed for oven dry matter. Fresh silage samples were also taken twice weekly for measurement of nitrogen and ammonia nitrogen using methods outlined by Steen (1989), and lactic and volatile fatty acids, ethanol and propanol using methods outlined by Porter and Murray (2001). The protein content of all diets was maintained at 180 g/kg DM (addition of soya to the low energy diet). Animals on the low energy density diet also received an additional 50 grams of dairy cow mineral per cow per day. Fresh diets were offered each morning between 10.00 and 11.00h (as a TMR) on an individual basis via electronic access to feeding boxes (1 box shared among 4 cows). The diets had a target excess of seven percent which was removed daily just before the allocation of fresh feed.

Table 3.1: Composition of high and low energy density post-calving diets

Treatment	Dietary constituent	Content (g/kg DM)	ADF (g/kg DM)	NDF (g/kg DM)	ME (MJ/kg)
High energy	Grass silage	150	284	467	11.3
	Maize Silage	150	202	381	10.8
	Concentrate	700			13.3
Low energy	Grass silage	360	284	467	11.3
	Maize Silage	360	202	381	10.8
	Soya	140			13.3
	Concentrate	140			13.2

Measurements

Progesterone concentration in milk was measured on Tuesday and Friday (AM milk samples) each week until the cow was confirmed in calf at day 30 post service. Each sample had a preservative tablet added (lactab Mark III, Thompson and Cooper Ltd, Runcorn, UK) and was stored at 4°C until analysis. Milk progesterone concentrations were determined using an enzyme-linked immuno-sorbent assay (ELISA) kit (Ridgeway Science Ltd, Lydney, UK) based on the

method of Sauer *et al.* (1986). Animals were inseminated at a minimum of 42 days post-calving, on the first observed standing heat following the commencement of breeding (30th November). All fertility events were recorded until six months after the commencement of breeding. Progesterone profiles were used to define irregular ovarian cycles and allow intervention. All interventions were recorded. Insemination details included: date, hour, bull and inseminator. Pregnancy was confirmed again at day 60 post insemination using rectal palpation and an ultrasound scan carried out by a veterinary surgeon. If reconfirmation was negative, milk progesterone sampling was restarted.

Progesterone Parameter Definitions

Full details of progesterone parameter definitions are provided by McCoy *et al.* (2006).

Commencement of luteal activity (CLA) was defined by the first of at least two consecutive progesterone concentrations ≥ 3 ng/ml in composite milk.

The luteal phase (LP) of an individual oestrous cycle was defined as the time period between the first elevated progesterone concentration measuring ≥ 3 ng/ml and the final consecutive milk progesterone concentration measuring ≥ 3 ng/ml.

Inter-ovulatory interval (IOI) was defined as the time period between the first progesterone rise of one cycle to the first progesterone rise of the next cycle.

Inter-luteal interval (ILI) was defined as the time period between the demise of one corpus luteum and the rise of the next. It is the interval from the first progesterone concentration < 3ng/ml to the last consecutive progesterone concentration < 3 ng/ml in composite milk.

Abnormal progesterone patterns

Delayed ovulation type I (DOVI) was defined as progesterone concentrations < 3 ng/ml in composite milk for ≥ 45 days (prolonged anovulation).

Delayed ovulation type II (DOVII) was defined as progesterone concentrations < 3 ng/ml in composite milk for ≥ 12 days after the commencement of luteal activity (prolonged inter-luteal interval).

Persistent corpus luteum type I (PCLI) was defined as progesterone concentrations ≥ 3 ng/ml for ≥ 19 days on the first luteal phase (delayed luteolysis of the corpus luteum during the first oestrous cycle).

Persistent corpus luteum type II (PCLII) was defined as progesterone concentrations ≥ 3 ng/ml for ≥ 19 days on subsequent luteal phases (delayed luteolysis of the corpus luteum during subsequent oestrous cycles).

Performance indicators included milk yield and composition, live weight, body condition score, locomotion score and food intake. Milk yield was recorded automatically at milking for each individual animal. A mean daily milk yield was calculated on a weekly basis. Milk analysis (fat, protein, lactose and somatic cell count) was carried out on a weekly basis (one AM and one PM sample, not mixed). Each sample had a preservative tablet added (lactab Mark III, Thompson and Cooper Ltd, Runcorn, UK) and was stored at 4°C until analysis. Milk composition was then determined using an infrared milk analyzer (IRMA). Body condition score (scale: 0-5) (Edmonson *et al.*, 1989) and live weight were measured weekly until drying off, and locomotion scores were assessed fortnightly using the method described by Manson and Leaver (1988). Individual feed intakes were recorded continuously via automatic feeding gates, from which a daily intake was calculated and then reduced to a weekly average daily intake.

Liver biopsies (approximately 20 mg wet weight per biopsy) were taken from four animals on each of the four treatments. Each animal was sampled at -13, 10, 22 and 56 days post-calving. Samples were obtained through an incision on the right hand side of the animal between the 10^{th} and 11^{th} rib where it crossed a line from the hip (tuber coxae) to the upper part of the right front leg (mid-humerus). Before taking the biopsies, a 25 cm^2 area was shaved and disinfected whereupon 10ml of local anaesthetic was given. Following a minimum of a 10 minute waiting period, a 0.5cm incision was made in the skin. Liver biopsies were taken from this incision using

a PRO-MAG biopsy instrument with a 14 gauge x 10cm needle (MDTEH, Florida, USA). The biopsies were immediately frozen in liquid nitrogen and stored at -80°C until analysis. Liver biopsies were homogenised, centrifuged and the supernatant was analysed for glycogen and triacylglycerol (TAG) content on an autoanalyser based on enzymatic colorimetric kits (Andersen *et al.*, 2002).

Blood samples were collected from the coccygeal vessel into vacutainer tubes between 0930 and 1130 on Tuesday mornings. Plasma (NEFA analysis) and serum (IGF-1 analysis) samples were centrifuged within two and 24 hours (refrigerated overnight) post collection, respectively. All samples were then stored at –20°C until analysed. Samples were taken weekly in the three week period prior to calving and in the first 100 days of lactation. Post 100 days they were taken fortnightly until drying off. Blood analysis of NEFA was carried out by automated chemistries. IGF-1 analysis was carried out on randomly selected multi- (7) and primiparous animals (5) from each of the four treatments. Five blood samples from each of these animals were analysed from weeks 2, 5, 8, 16 and 20 of lactation. Serum IGF-1 concentrations were determined by a double radioimmunoassay (RIA) after acid-ethanol cryo-precipitation of IGF-1 binding proteins (Wylie *et al.*, 1997). Radio-iodinated IGF-1 was purchased from Amersham (GE Healthcare, Bucks., UK). The primary antibody (AFP4892898) was a gift from Dr A Parlow (National Hormone and Pituitary Program, NIDDK) and was used at a final dilution of 1:400,000 and an incubation time of 64 hours.

Calculation of energy balance

The average daily energy balance for each animal was calculated per week of lactation using the equations described by Thomas (2004) [Energy balance = ME intake – ME requirement (-10 + (ME_{preg} + $ME_{maintmilk}$ * $Lwt^{0.75}$) + ((0.0013*Lwt)/K_m))]. Daily milk yields, daily DMI, weekly milk compositions, weekly live weights and feed compositions were all used in the calculations. Missing values were estimated from the week previous to and the week following the missing data. Less than 2% of the data were missing.

Statistical analysis

A repeated measures approach using the Residual Maximum Likelihood (REML) procedure available in GenStat (Payne et al. 1993) was used to analyse the data set with parity, pre-treatment, post treatment and stage of lactation (10 fortnightly periods from calving). This procedure can be used when data are categorised, unbalanced and subject to variation at different levels or strata. REML analysis can be thought of as a generalisation of ANOVA. If the data are perfectly balanced then the results of REML and ANOVA are exactly the same (Robinson 1987; Searle et al. 1992). Additionally the repeated measures analysis takes account of the correlations between observations on the same subjects (cows).

Multiple linear regression analysis was performed for several independent variables on all the reproductive parameters. Independent variables used in the multiple linear regression analysis were: milk yield, milk energy, daily energy balance, cumulative energy balance, average daily energy balance in the first three weeks of lactation, average daily energy balance in the first six weeks of lactation and weeks to energy nadir.

A logistic regression model was used to analyse the following data: pregnancy to first service, 100 day in calf rate, intervention, delayed ovulation types I and II, persistent corpus luteum types I and II and whether or not there was more than one abnormal pattern observed. The goal of logistic regression is to correctly predict the category of outcome for individual cases using the most parsimonious model (Hosmer and Lemeshow, 2000).

RESULTS

Effect of pre- and post-calving diets on body energy status

The statistical analysis indicated a significant effect of pre-calving treatment ($P<0.001$) on body condition score (BCS) at calving. Animals on the *ad libitum* high energy pre-calving diet, and

restricted low energy pre-calving diet had BCS (at calving) means of 2.68 and 2.49 (SED, 0.043) respectively. There was a significant (P<0.001) effect of parity on the condition score at calving. Multi- and primiparous animals, produced predicted means of 2.50 and 2.69 (SED, 0.052) respectively. Multiparous animals on the *ad libitum* high energy pre-calving diet, and restricted low energy pre-calving diet had BCS means of 2.62 and 2.38 (SED, 0.045) respectively. These were significantly different (P<0.001). Primiparous animals on the *ad libitum* high energy pre-calving diet, and restricted low energy pre-calving diet had BCS means of 2.74 and 2.60 (SED, 0.041) respectively. These were also significantly different (P<0.05).

Pre-calving treatment had no significant effect (P>0.05) on the average milk yield in the first 250 days of lactation. The high energy post-calving treatment significantly (P<0.001) increased milk yield, producing predicted yields of 31.7 and 26.5 (SED, 1.02) kg per day, for high and low energy post-calving diets respectively. Treatment effects are presented in Figure 3.1. There was a significant (P<0.001) effect of parity on the average milk yield; Primiparous (parity 1) and multiparous animals (parity 2+) had predicted milk yields of 27.2 and 31.3 (SED, 0.85) kg per day respectively. Similar trends were realised when energy corrected milk yield data were analysed.

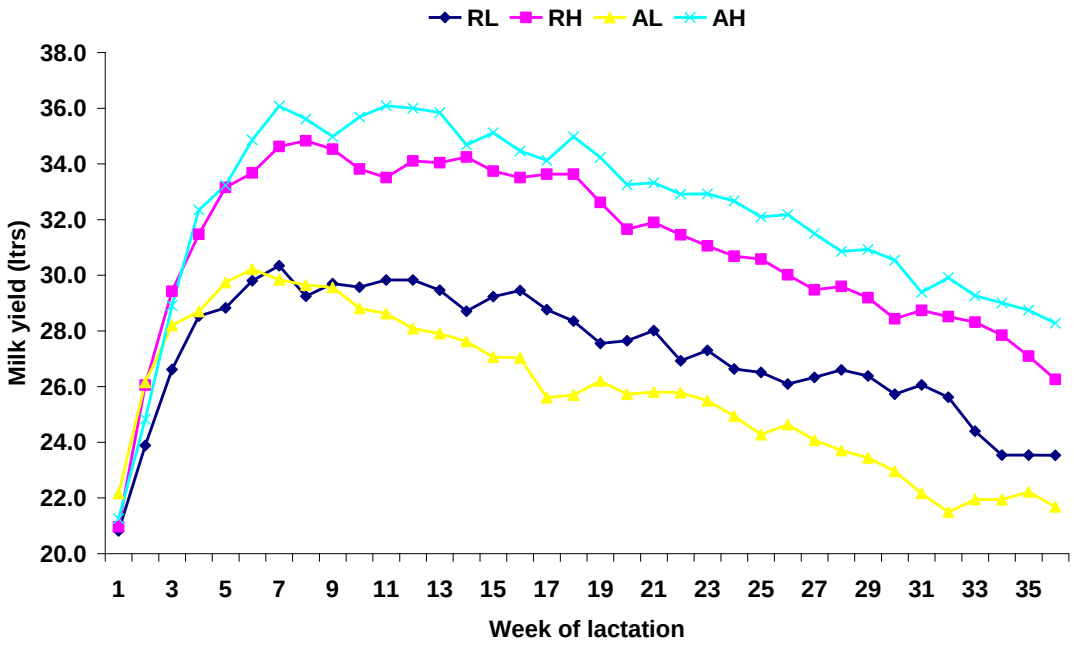


Figure 3.1: The predicted means of milk yield of animals offered a restricted (R) and *ad libitum* (A) diet pre-calving and a high (H) or low (L) energy density diet post-calving with weeks from parturition (n=80).

There was a significant effect of post-calving diet (P<0.001) on the daily and cumulative energy balance (Figures 3.2 and 3.3 respectively). Animals on the high energy diet post-calving achieved a positive daily and cumulative energy status more quickly than those on the low energy diet post-calving. There was a significant pre-/post-calving treatment interaction in both daily (P<0.001) and cumulative (P<0.01) energy profiles. The predicted means for the average daily energy status were of 10.3, -8.6, 25.7 and -26.6 (SED, 6.93) MJ per day for treatment groups AH, AL, RH and RL respectively over a 36 week period. The predicted means, for the average cumulative energy status, were 492, -2461, 2534 and -4345 (SED, 763) MJ for treatment groups AH, AL, RH and RL respectively over the 36 week post-calving period. There were no significant effects of parity on either DEB or CEB (P>0.05). A significant (P<0.001) interaction between stage of lactation and post-calving treatment on cumulative energy status was also apparent.

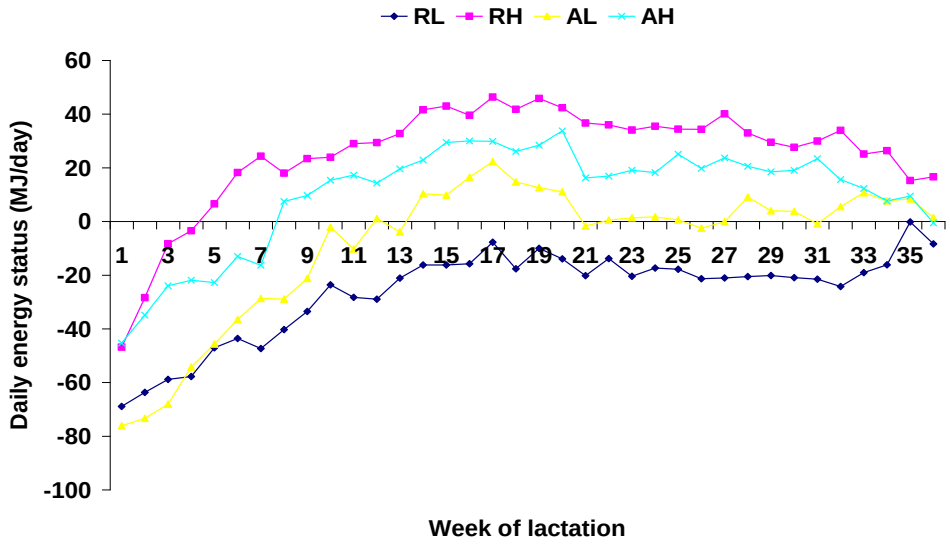


Figure 3.2: The predicted means of daily energy balance of animals offered a restricted (R) and *ad libitum* (A) diet pre-calving and a high (H) or low (L) energy density diet post-

calving with weeks from parturition (n=80). There was a significant pre-/post-calving treatment interaction (P<0.001)

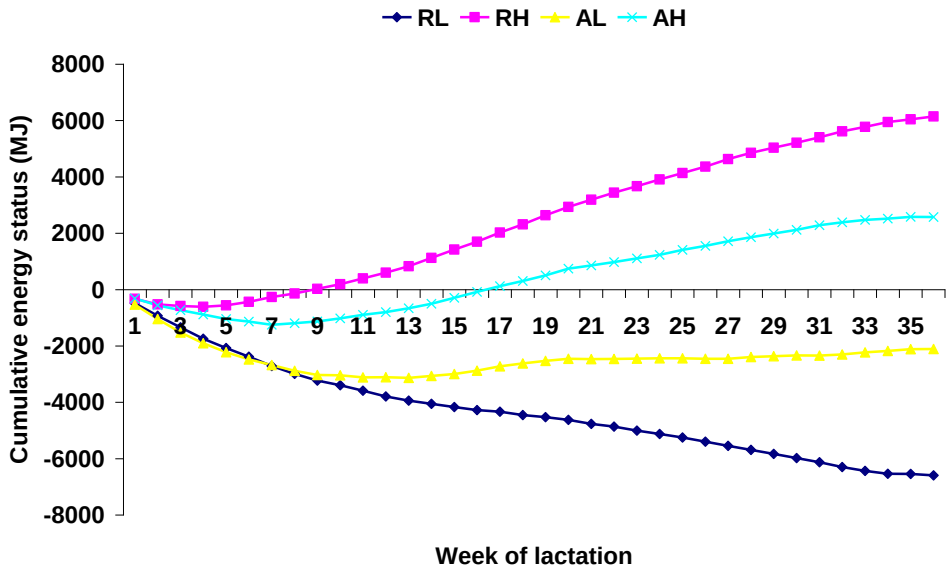


Figure 3.3: The predicted means of cumulative energy balance of animals offered a restricted (R) and *ad libitum* (A) diet pre-calving and a high (H) or low (L) energy density diet post-calving with weeks from parturition (n=80). There was a significant pre-/post-calving treatment interaction (P<0.01)

Effect of pre- and post-calving diets on blood and liver parameters

Treatment effects on blood and liver parameters during lactation are presented in Table 3.2. Pre-calving treatment had a significant (P<0.001) effect on plasma NEFA concentrations during lactation, with means of 0.43 and 0.35 (SED, 0.025) mmol/l for animals on the *ad libitum* high, and restricted low energy pre-calving treatments respectively. No significant post-calving treatment effect (P>0.05) on plasma NEFA concentration was apparent. There were no significant treatment effects (P>0.05) on liver triacylglycerol (TAG) concentrations. Following regression analysis (Table 3.3) it was observed that a more negative daily energy balance (DEB) significantly (P<0.001) increased plasma NEFA and liver TAG concentrations. Additionally, an

increase in plasma NEFA concentrations significantly (P<0.01) increased liver TAG concentrations. There was no significant effect (P>0.05) of parity on liver TAG concentration.

All IGF-1 samples were analysed in two assays with mean intra-assay coefficient of variation (CV) of 3.2% and 2.6% and a mean inter-assay CV of 10.9%. It was noted that 3.8% and 2.1% of the samples had a CV above 15% and 20% respectively, between duplicate samples. Post calving dietary treatment had a significant (P<0.001) influence on serum IGF-1 concentrations, producing predicted means of 113.9 and 102.9 (SED, 3.04) ng/ml for high and low treatments respectively. There was no significant effect (P>0.05) of parity on IGF-1. Following regression analysis (Table 3.3) it was observed that a more negative daily and cumulative energy balance significantly (P<0.01 and P<0.001 respectively) decreased serum IGF-1 concentrations. There was no significant association (P>0.05) between plasma NEFA or liver TAG concentration on serum IGF-1 concentrations. A change in serum concentrations (either a decrease or increase) of IGF-1 had a significant effect (P<0.01) on the days to CLA. This change was calculated by subtracting the highest or lowest IGF-1 concentration (depending on the trend), in weeks 5, 8, 16 and 20, from the first IGF-1 concentration in week 2. A large decrease in IGF-1 increased the time to CLA.

Table 3.2: Treatment effects on blood and liver parameters during lactation

Blood/liver sample[1]	**Treatment**[2]					**Significance**[3]	
	AH	**RH**	**AL**	**RL**	**SED**	**Pre**	**Post**
Serum IGF-1 (ng/ml)	110.3	119.2	103.7	101.5	4.28	ns	***
Plasma NEFA (mmol/l)	0.428	0.329	0.441	0.372	0.04	***	ns
Liver TAG (mg/g)	4.76	6.26	6.65	6.06	1.64	ns	ns

[1]IGF-1, insulin-like growth factor; NEFA, non-esterified fatty acid; TAG, triacylglycerol

[2] A, *ad libitum* diet pre-calving; R, restricted diet pre-calving; H, high energy density diet post-calving; L, low energy density diet post-calving; SED, standard error of the difference

[3] ns, P>0.05; ***, P<0.001

Table 3.3: Regression analysis of blood parameters with energy and fertility parameters.

Explanatory variable[1]	Response variable[2]	Relationship	Significance[3]	r^2
DEB	IGF-1	Positive	**	0.03
DEB	NEFA	Negative	***	0.09
DEB	TAG	Negative	***	0.36
CEB	IGF-1	Positive	***	0.08
NEFA	TAG	Positive	**	0.07
C-IGF-1	CLA	Negative	**	0.15

[1] DEB, daily energy balance; CEB, cumulative energy balance; NEFA, non-esterified fatty acid; C-IGF-1, Change in insulin-like growth factor from week 2 to a maximum or minimum value post week two

[2] TAG, triacylglycerol; CLA, commencement of luteal activity

[3] **, P<0.01; ***, P<0.001

Effect of pre- and post-calving diets on reproductive parameters

A significant (P<0.05) pre-/post-calving dietary treatment interaction was observed for the average peak concentration in progesterone (Table 3.4). There was also a significant (P<0.01) effect of parity on this parameter, with primiparous animals having a significantly higher peak progesterone than multiparous animals. No other significant dietary treatment effects (P>0.05) were obtained from the analysis of the remaining reproductive parameters, namely; calving difficulty, days to first service, pregnancy to first service, number of services per conception, days to conception, calving interval, interval between commencement of luteal activity and 1st artificial insemination, 100 day in calf rate (from commencement of breeding), days to commencement of luteal activity, average luteal phase, average inter ovulatory interval (IOI), average inter luteal interval (ILI), 1st progesterone concentration, number of ovulations, intervention, delayed ovulation type one (DOV I), delayed ovulation types two (DOV II),

persistent corpus luteum type (PCL I) one and persistent corpus luteum type two (PCL II). Predicted means for these reproductive parameters are presented in Table 3.5. The overall pregnancy rate to first service in this experiment was 25.0 percent, with a treatment range of 10.0 to 36.8%. The overall services per conception, 100 day in calf rate (post commencement of breeding) and calving interval were 2.8 services, 58.1 percent and 409 days respectively. Although not significant, there does appear to be a tendency for a restricted pre-calving treatment to reduce the calving interval and increase the 100 day in calf rate.

Table 3.4: Predicted pre-/post-calving interaction means for average peak concentration of progesterone

		Post-calving treatment[1]			
	Pre-calving treatment[1]	**H**	**L**	**Significance**[2]	**SED**[3]
Average progesterone concentration (ng/ml)	**A**	29.8	27.6	*	1.53
	R	28.7	32.4		

[1] A, *ad libitum* high energy diet; R, restricted low energy diet; H, high energy diet *ad libitum*; L, low energy diet, *ad libitum*

[2] ***,** P<0.05

[3] SED, standard error of the difference

Effect of energy status on reproductive parameters

Body condition score at calving had no significant effects (P>0.05) on the reproductive parameters analysed. Significant effects (P<0.05) of daily energy status on reproductive parameters were observed and these are presented in Table 3.6. It was observed that the interval from calving to energy nadir was positively associated (P<0.05) with the interval to commencement of luteal activity. Also, the average daily energy balance in the first 21 and 42

days was positively associated (P<0.05) with the duration of the luteal phase. However, when correlating the duration of the luteal phase of individual cycles, as opposed to cow averages, with the equivalent average daily energy value, no significant relationship was found. The average cumulative energy balance of individual animals was significantly (P<0.05) associated with the time to commencement of luteal activity. A more positive cumulative energy balance was associated with an earlier commencement of luteal activity. There was a strong negative correlation between cumulative energy balance and weeks to energy nadir (P<0.001; r^2, 0.61).

Table 3.5: Effect of dietary treatment on reproductive parameters

Treatment[1]	**AH**	**RH**	**AL**	**RL**	**SED**	**Overall**	**Significance[3]**
Pregnancy to first service (%)	10.0	36.8	30.0	23.5	9.81	25.0	ns
Services per conception	2.8	2.7	3.1	2.7	0.63	2.8	ns
100d in calf rate (%)[2]	50.0	65.0	52.6	64.7	11.0	58.1	ns
Calving interval (days)	415	405	415	399	19.2	409	ns
Calving difficulty	166	188	185	187	21.1	182	ns
Days to 1st service	71.8	71.0	67.4	66.7	5.92	69.2	ns
Days to conception	134	125	135	118	19.2	128	ns
Interval to CLA (days)	46.2	43.5	48.0	41.0	7.14	44.7	ns
CLA to 1st service (days)	25.2	26.7	27.5	25.7	7.97	26.3	ns
Average luteal phase (days)	17.5	15.7	14.5	16.1	1.55	16.0	ns
Average IOI	23.2	24.0	25.5	22.2	1.84	23.7	ns

(days)							
Average ILI (days)	7.9	7.3	9.4	8.9	1.54	8.4	ns
1st progesterone conc. (ng/ml)	20.7	22.1	19.7	20.9	3.79	20.9	ns
No. of ovulations	6.1	5.3	5.6	4.9	0.78	5.5	ns
Intervention (%)	57.1	52.4	47.6	64.7	11.1	55.5	ns
DOV I (%)	47.6	42.9	42.9	47.1	11.2	45.1	ns
DOV II (%)	33.3	14.3	23.8	29.4	9.6	25.2	ns
PCL I (%)	28.6	19.1	9.5	5.9	7.6	15.8	ns
PCL II (%)	52.4	33.3	33.3	41.2	10.9	40.1	ns

[1] A**,** *ad libitum* diet pre-calving; R, restricted diet pre-calving; H, high energy density diet post-calving; L, low energy density diet post-calving.

[2] 100 days post the commencement of the breeding period

[3] ns, P>0.05; (n=80)

Abnormal hormonal patterns

The mean and inter-quartile range values of some progesterone related parameters are presented in Table 3.7. There was no significant effect (P>0.05) of dietary treatment on the incidence of abnormal hormonal patterns following logistic regression analysis. In the current study 75% of the animals displayed one or more abnormal hormonal patterns in their progesterone profiles and 75% of these received intervention treatment during the breeding period. A total of 45% of animals had a prolonged anovulation (DOV I), 25% experienced at least one prolonged inter-luteal interval (DOV II), 16% had delayed luteolysis during their first cycle (PCL I) and 40% had at least one delayed luteolysis in their subsequent cycles (PCL II). Overall, 61% of animals that failed to conceive to their first service had at least one delayed luteolysis, either PCL I, PCL II or both. When assessing the incidence of abnormal progesterone profiles, from all the cycles recorded, it was found that: 24% of all cycles had prolonged luteal phases (either PCL I or PCL

II); 8% of all cycles had a prolonged inter-luteal interval (DOV II); and 42% of all cycles had an inter-ovulatory interval greater than or equal to 24 days.

Table 3.6: Regression of energy factors against reproductive parameters using the model: Y = Constant + Parity + X

X[1]	Y[2]	Probability[3]	$r^{2(4)}$	Relationship
Weeks to Energy Nadir	Weeks to CLA	*	0.07	Positive
ADEB (1-21 days) (MJ)	Average LP	*	0.07	Positive
ADEB (1-42 days) (MJ)	Average LP	*	0.08	Positive

[1] ADEB, average daily energy balance

[2] CLA, commencement of luteal activity; LP, duration of luteal phase

[3] *, $P<0.05$

[4] r^2, the proportion of variation in a data set that is accounted for by the statistical model

Table 3.7: Mean and inter-quartile range of progesterone related parameters

	CLA (days)[1]	LP (days)[1]	IOI (days)[1]	ILI (days)[1]	No. of cycles
Mean	42.8	16.0	23.7	8.4	3.7
Inter-quartile range	26.2 - 50.2	13.1 – 18.7	20.9 – 26.8	6.1 – 8.2	2.0 - 5.0

[1] CLA, commencement of luteal activity; LP, luteal phase; IOI, inter-ovulatory interval; ILI, inter-luteal interval

DISCUSSION

Dietary effects on body energy status

The difference in mean body condition score at calving, due to pre-calving plane of nutrition, was significant ($P<0.001$) although the actual difference was smaller than anticipated (2.62 vs. 2.38 for multiparous animals and 2.74 vs. 2.60 for primiparous animals). On average, animals had body condition scores at calving of 2.75 or less which is recommended as ideal by Garnsworthy (2006) who carried out an extensive review of the effects of body condition score, with particular focus on the modern Holstein dairy cow. The underlying basis of the recommendation of Garnsworthy (2006) was aimed at reducing the amount of tissue mobilisation in early lactation. Interestingly, the latter author stated that the Holstein breed has more internal fat relative to external (subcutaneous) fat when compared with other dairy and beef breeds. This would suggest that at the same body condition score, the modern Holstein cow will have a greater total fat content when compared to other dairy breeds (Garnsworthy, 2006). Therefore, a relatively small loss of body condition during early lactation could reflect a large depletion of internal fat reserves, which could predispose the Holstein cow to an increased risk of excessive NEB, in comparison with the same condition score loss in a non Holstein breed of dairy cow. This suggests that conventional methods of using body condition score change as an estimate of body energy usage, may considerably underestimate the actual amount of energy mobilisation in the Holstein cow. However, Garnsworthy (2006) also noted that the body condition score method is still the best system for monitoring changes in body fat reserves.

The effect of dietary treatment on animal (parity ≥ 2) energy status was discussed in chapter 2. However, in the present analysis, primiparous animals were added to the data set and the data were analysed over 36 weeks post-calving, as opposed to 20 weeks post-calving. The results of the present study indicate a highly significant ($P<0.001$) pre-/post-calving diet interaction, with a restricted low energy pre-calving treatment having a positive influence on the response in daily energy status to a high energy post-calving diet. In contrast, a restricted low energy pre-calving treatment resulted in a negative influence on the response in daily energy status to a low energy

post-calving diet. However, it would appear from the predicted means that this interaction was not apparent in the early stages of lactation. Cows on the *ad libitum*, high energy pre-calving, low energy post-calving diet had high levels of body tissue mobilisation in early lactation, which appeared to be unsustainable. An extremely high rate of mobilisation in this early period can cause an accumulation of TAG in the liver and impair liver function; which has been shown to be detrimental to many body functions including milk production (Top *et al.,* 1995). A reduction in milk production will reduce the requirement for mobilisation, allowing an improvement in body energy status to be achieved. It would appear that for animals on an *ad libitum* high energy pre-calving, low energy post-calving diet combination this sequence of events materialised. From weeks 5 to 36 the energy status of animals on this dietary regime improved dramatically due to a reduction in milk production (Figure 3.1). Animals on the restricted low energy pre-calving, low energy post-calving dietary regime did not respond in a similar manner and remained in severe negative energy balance throughout, despite both groups having similar energy deficits at the beginning of lactation (68.9 MJ/d for restricted low pre/ low post versus 76.1 MJ/d for *ad libitum* high pre/ low post). Animals on the restricted low pre-calving, high energy post-calving diet combination displayed a more positive energy trajectory than those on an *ad libitum* pre-calving, high post-calving diet combination, which was the opposite effect to that displayed in cows on the low post-calving dietary regimes.

Treatment effects on reproductive parameters

There was an observed pre-/post-calving interaction for the average peak concentration in progesterone (Table 3.4). These results will be examined in terms of post-calving treatment. Animals on the high energy post-calving diet (high energy intake) displayed no significant difference in average peak progesterone concentration which is most probably a result of a similar rate of metabolic clearance from the liver due to a high rate of dry matter intake (Newbold, 2006). However, animals on the low energy post-calving diet had lower dry matter intakes, therefore metabolic clearance from the liver would be expected to be lower. This would suggest that animals on the low energy post-calving diet would have higher progesterone concentrations. This was not the case for animals on the *ad libitum* high energy pre-calving, low

energy post-calving diet. These animals had severe negative energy balance in early lactation and are likely to have an impaired liver function due to an accumulation of TAG which will reduce progesterone production. Negative energy balance has been shown to reduce luteal progesterone concentrations (Spicer *et al.,* 1990) and an increase in the liver fat content has been shown to reduce progesterone synthesis (Newbold, 2006).

The results presented in Table 3.5 suggest that a restricted, low energy (R) pre-calving diet, when compared to an *ad libitum*, high energy (A) pre-calving diet, has beneficial effects on reproductive performance: e.g. the 100 day in calf rate (predicted means: R, 64.9%; A, 51.3%) and the calving interval (predicted means: R, 402 days A, 415 days), although these effects were not significant. This reproductive response is more likely to have been influenced by the physiological state generated by the restriction in feed intake during the dry period than to the actual condition score at calving. There was a considerable overlap in condition scores at calving between the pre-calving diets; the range of condition scores at calving of the restricted low energy and the *ad libitum* high energy groups were 1.88 to 3.25 and 2.25 to 3.25, respectively. Energy requirements pre-calving, especially in the last three weeks of pregnancy, will range from 90-120 MJ per day (energy requirements for maintenance and products of conception). Animals on the restricted diet only received circa 70 MJ per day. This ensured that all animals on a restricted diet were in a similar physiological state; lipolysis as opposed to lipogenesis. The mobilisation of body reserves in the dry period may have primed the cow for a period of food restriction and conditioned the liver to cope more efficiently with non-esterified fatty acids upon the commencement of lactation. Interestingly, Grum *et al.* (1996) suggested that the loss of body condition during the dry period reduced body reserve mobilisation at the start of lactation (indicated by reduced plasma NEFA) and subsequently reduced the predisposition of cows to fatty liver syndrome. In support of this, Friggens *et al.* (2004) stated that priming the cow via an increase in the concentration of circulating fatty acids during the dry period would allow her to better deal with the increased concentration of fatty acids post partum. In the current experiment, cows on a restricted pre-calving diet had significantly lower NEFA concentrations post partum (Table 3.2). It is thought that this early mobilisation of body energy reserves prepares the liver for increased oxidation of NEFA and a decrease in esterification, therefore liver function is better maintained in the early stage of lactation. Additional work by Douglas *et al.* (1998) illustrated

that a reduced intake in the dry period increased NEFA in the blood pre-partum and resulted in a lower liver lipid content postpartum. Cows receiving the *ad libitum* pre-calving diet, receiving circa 140 MJ per day, would have experienced an abrupt change from lipogenesis in the dry period to extreme lipolysis on the commencement of lactation. In this scenario, the liver will not have been conditioned to high NEFA levels and therefore is unlikely to cope with the required rate of metabolism and subsequent oxidation of non-esterifed fatty acids in early lactation. This will increase the esterification of non-esterified fatty acids and subsequently the accumulation of triacylglycerols in the liver. An accumulation of TAG in the liver will result in 'fatty liver syndrome', which is detrimental to liver function (Top *et al.,* 1996) and subsequent reproductive performance (Top *et al.,* 1995; Newbold, 2006). Interestingly, work by Gümen *et al.* (2005) illustrated that pregnant cows with no dry period prior to calving had fewer days open (shorter calving interval) than cows on a shortened or traditional dry period length. No dry period would restrict the energy available for lipogenesis, a similar effect to the restriction in intake pre-calving applied in the current study.

Energy effects on reproductive parameters

In a recent study, McCoy *et al*. (2006) reported that the mean interval to the commencement of luteal activity (CLA) was 36.1 days for Holstein cows. In the present study a mean interval of 42.8 days was recorded with 45 percent of animals showing an abnormal progesterone profile for CLA (CLA $\geq$ 45 days). It was observed (Table 3.6) that an increase in time to cumulative energy nadir significantly increased the time to the CLA (ovulation of a dominant follicle). Ferguson (1996) stated that the time to cumulative energy nadir (the point in time when ME intake equals ME requirement) was highly correlated with total cumulative energy balance in early lactation. Similarly, in the current study a strong negative relationship was observed between the time to cumulative energy nadir and the average cumulative energy balance over the first 250 days of lactation. Therefore a prolonged interval from calving to energy nadir will normally be associated with an increased amount of BRM. In the present study a larger amount of BRM was shown to significantly increase the concentration of NEFA in the blood and accumulation of triacylglycerols (TAG's) in the liver. As previously mentioned, an accumulation of TAG in the

liver is detrimental to liver function (Top *et al.*, 1996) and subsequent reproductive performance (Top *et al.*, 1995; Newbold, 2006). One possible pathway by which TAG can suppress reproductive performance is through the reduction of insulin-like growth factor 1 (IGF-1) production, which is essential for granulosa cell proliferation and steroidogenesis (oestradiol production) (Evans *et al.*, 2006). Oestradiol provides negative feedback in regulating FSH and a positive feedback to the hypothalamus thus stimulating the release of gonadotrophin-releasing hormone (GnRH), which triggers the pulsatile release of LH from the anterior pituitary, ultimately resulting in ovulation. In support of this hypothesis, Zurek *et al.* (1995) reported that the level of plasma IGF-1 was better correlated with the pulsatility of LH than with the interval to first ovulation. Despite no significant relationship between IGF-1 and liver TAG being identified, results of the current study suggest that a more negative daily and cumulative energy balance significantly reduced plasma IGF-1, which concurs with Diskin *et al.* (2003), who stated that the production of IGF-1 is positively associated with body condition and nutrient intake. The latter authors stated that the mechanism involved in IGF-1 production was centred around the combination of growth hormone (GH) and the growth hormone receptor type 1A (GHR1A). The GHR1A is very important in the liver, however it is affected by energy balance and insulin levels (insulin causes an increase in GHR1A). Newbold (2006), referring to work by Fenwick *et al.* (2006), stated that severe NEB, which is associated with an increase in liver TAG (Rukkwamsuk, 1999b), will reduce the gene expression of GHR1A, subsequently reducing IGF-1 synthesis. Webb *et al.* (1999) also referred to a decline in GH receptor capacity in underfed animals. Another important aspect of the IGF system is bioactivity and serum clearance rate of circulating IGF-1, both of which are controlled by insulin-like growth factor binding proteins (IGFBPs) (Thissen *et al.*, 1994). IGFBPs bind to IGFs making them inactive. The control of IGF-1 bioactivity is important as over-stimulation may be detrimental to oocyte development (Armstrong *et al.*, 2001). It is thought that an increase in dietary energy decreases the concentration of IGFBP-2 and -4 (causing an increase in the bioactivity of IGF-1) and increased the concentration of IGFBP-3 (Webb *et al.*, 2004). IGFBP-3 is thought to prolong the half life of IGF-1 in circulation and act as a storage pool for IGF-1 (Thissen *et al.*, 1994). In the absence of IGFBP-3, plasma IGF-1 clearance increases. Armstrong *et al.* (2001) also stated that IGF-1 has the potential to interact directly with the oocyte through the Type 1 IGF receptor. IGF receptors are present on all cell types. In the current experiment, there was a significant effect of the IGF-1

trajectory on interval to CLA. A large decrease in IGF-1 from week 2 onwards increased the interval to CLA. Ferguson (1996) stated that an increased interval to CLA will increase the interval to first service and subsequently the calving interval. However, it has been recently shown that very early CLA also has detrimental effects on conception rate (McCoy *et al.,* 2006). The latter author stated that the optimum time for CLA to achieve the highest conception rates is between 30 and 49 days post partum. Similar evidence was previously presented by Ferguson (1996) suggesting that if reproductive function was maintained during a period of severe weight loss or under feeding, then fertility will be reduced.

A significant positive relationship between the average daily energy balance in the first 42 days of lactation and the average duration of the luteal phase was obtained in the current study indicating that a more negative energy balance was associated with a shorter luteal phase. An average luteal phase of 16.0 days was observed, following correction for the underestimation made by twice weekly progesterone sampling. This mean value incorporates all luteal phases including those that were abnormal. A short luteal phase is defined by luteal regression prior to 10 days of the oestrous cycle (Salfen *et al.,* 1995). Normally luteal regression will result from pregnancy failure, which is dependent on the embryo's ability to develop normally and the mother's ability to recognise pregnancy. The bovine conceptus (embryo) releases interferon-τ (a product of the epithelium of the trophoblast) which modulates the release of the luteolytic hormone prostaglandin $F_2\alpha$, from the endometrium of the uterus (Roberts *et al.*, 1992). The interferon-τ will suppress stimuli (oxytocin receptor upregulation) which facilitate prostaglandin $F_2\alpha$ production and subsequently the normal regression of the corpus luteum, which occurs around day 16 of the oestrous cycle, if the oocyte has not been fertilised (Okunda *et al.,* 2002; Wathes and Lamming, 1995). Work by Salfen *et al.* (1995) suggests that the early release (around day 6 post oestrous) of prostaglandin $F_2\alpha$ will result in subnormal luteal function (oestrous duration of 7-10 days and decreased progesterone concentrations). Early synthesis of $PGF_2\alpha$ can be caused by the down regulation of PGE_2 (prostaglandin E_2 responsible for progesterone production). PGE_2 protects the corpus luteum from spontaneous or induced luteolysis and the existence of a positive correlation between PGE_2 and progesterone (Arosh *et al.,* 2004) would imply that if progesterone secretion decreases, PGE_2 synthesis would also decrease. A detrimental effect of NEB on luteal progesterone concentrations has been shown by

Spicer *et al.* (1990) and Butler (2000). In agreement with this, Newbold (2006) also reported findings implying that fatty liver reduced progesterone synthesis through the suppression of cholesterol production, which is a key precursor for the production of progesterone. The latter author also stated that additional clearance of progesterone, due to an increased metabolic rate, from the liver in high yielding cows (increased blood flow with an increased dry matter intake) will further reduce plasma progesterone concentrations. Work by Starbuck *et al.* (2000) illustrated that a delayed increase in corpus luteum progesterone production was associated with poor oestradiol secretion during the follicular phase leading up to ovulation in cows which were "at risk" of exhibiting delayed progesterone increases. This would imply that high IGF-1 concentrations during the follicular phase will be important in the early rise in progesterone concentrations post ovulation. In agreement with this, Robinson *et al.* (2006) stated that low IGF-1 would reduce the size and function (progesterone secreting ability) of the corpus luteum. In the current experiment, there was no effect of individual animal energy status on average peak progesterone concentrations. However, more specifically Mann and Lamming (2001) and Wathes *et al*. (2003) reported that a delay in progesterone increase in the early luteal phase (the 4/5 day progesterone concentration) in high yielding cows will reduce the capability of cows to become pregnant. Webb *et al.* (1999) referred to work by Mann *et al.* (1998) stating that maternal progesterone concentrations during the early luteal phase appear to affect embryonic development and its ability to inhibit luteolysis. A late rise in progesterone and lower luteal phase progesterone concentrations will be associated with a smaller embryo (Mann *et al.,* 1996) that is less likely to prevent luteolysis (Webb *et al.,* 1999).

Abnormal progesterone profiles

In the present study, 75 percent of animals displayed at least one abnormal progesterone profile which is high in comparison to the 44 percent and 41 percent reported by Royal *et al.* (2000a) and McCoy *et al*. (2006) respectively. The latter authors stated that there has been an increase in abnormal progesterone profiles in recent years, and that the increase in incidence of prolonged luteal phases has become particularly apparent. Despite showing no significant treatment or energetic effects, 57.5 percent of animals in this study experienced at least one case of delayed

luteolysis during lactation. Overall, 24.1 percent of oestrous cycles were prolonged due to delayed luteolysis. Post ovulation, the luteal phase is extremely important in maintaining regular reproductive cycles and a high reproductive performance. An extended luteal phase can occur in two situations; 1) when there is early embryo loss following maternal recognition of pregnancy, or 2) when there is delayed luteolysis in the absence of an embryo (pregnancy). A possible mechanism to distinguish between these two scenarios would be to measure interferon-τ concentrations 15 days post breeding. Elevated interferon-τ would indicate the presence of an embryo and subsequently the potential for pregnancy. An extended luteal phase in the absence of pregnancy, is detrimental to ovarian function (Opsomer *et al.,* 2000), and will increase the inter-ovulatory interval (IOI). Royal *et al.* (2000a) suggested that an IOI with a duration greater than, or equal to, 24 days would be associated with reduced fertility. The average IOI in this present study was 23.7 days, with 41.8 percent of all cycles being greater than or equal to 24 days. In more severe cases an extended luteal phase will result in long return intervals resulting in non pregnant cows not returning to oestrus within three weeks which, on a commercial basis, could indicate a false pregnancy. A luteal phase greater than or equal to 19 days is considered as an abnormal hormonal pattern which is caused by the delayed luteolysis of the corpus luteum. A possible explanation for this delay in luteolysis (in the absence of pregnancy) is a sub optimal uterine environment which can disrupt normal hormonal and luteolytic mechanisms (Kindahl *et al.,* 1999; Sheldon *et al.,* 2006a). In a more favourable uterine environment, prostaglandin $F_{2\alpha}$ acts on the corpus luteum and causes it to regress and cease progesterone production, removing the inhibitory effect on LH release and allowing the next ovulation to occur. Sheldon et al. (2006c) stated that in a sub optimal uterine environment (presence of bacterial pathogens) there is an increase in prostaglandin E_2 production, due to the stimulatory effect of lipopolysaccharides found on the uterine pathogen *E. coli*. Elevated prostaglandin E_2 has a luteotrophic effect which increases progesterone secretion and subsequently prolongs the luteal phase. Prostaglandin E_2 is important in the growth, development and maintenance of a normal corpus luteum (Arosh *et al.,* 2004). Interestingly, Lamming and Darwash (1998) reported that cows that have sub optimal uterine environments and experience persistent corpus lutea are more at risk of late embryonic loss once pregnant.

A sub optimal uterine environment has also been reported to affect the growth of ovarian follicles. Sheldon *et al.* (2002) stated that uterine infection will slow down ovarian follicle growth and cause a reduced production of oestradiol, which is important in ovulation. Uterine function is compromised in cattle by bacterial contamination. Failure to eliminate bacteria will cause infection, which if it persists may result in uterine disease (Sheldon *et al.,* 2006b).

CONCLUSIONS

Overall the results of this study suggest that the extent of negative energy balance in early lactation can be reduced by a nutritional strategy involving reduced energy intake pre-calving, followed by high levels of energy intake in early lactation. From a reproductive perspective, the high irregularity of ovarian cyclicity in this experiment is likely to be the main reason for the poor pregnancy rate to first service and the relatively poor 100 day in calf rates. A high inter-ovulatory interval (IOI) in the present study, which Royal *et al.,* (2000a) suggested would reduce fertility, and a high number of animals experiencing prolonged luteal phases in the absence of pregnancy, will inevitably reduce reproductive performance. On an individual cow basis, body energy status had a significant influence on some important reproductive parameters, with a more severe negative energy balance in early lactation resulting in a greater amount of irregularity in ovarian cyclicity. In the absence of statistically significant dietary treatment effects on these reproductive parameters, it could be suggested that these relationships are due to a high degree of variability in energy status between individual cows, irrespective of treatment. This indicates a strong genetic influence on the energetic trajectory of these animals, producing a lot of variation. However, the degree of negative energy balance in early lactation in high yielding Holstein dairy cows can be reduced by reducing the amount of body tissue mobilization in early lactation through improved pre-calving management, or by increasing the energy density of the diet post-calving. Furthermore, Garnsworthy (2006) has suggested a genetic strategy of selecting bulls which produce daughters with flatter BCS curves to reduce body reserve mobilization in early lactation and consequently reducing the deleterious effects of the products of excessive mobilization of body tissue on animal health and reproductive performance.

CHAPTER 4: The effect of nutritional strategy and individual animal management on the performance and fertility of Holstein Friesian dairy cows in early lactation

ABSTRACT

Long-term sustainability of their herds has been, and continues to be, a concern for many dairy farmers worldwide, thanks to a chronic decline in the fertility and longevity of the high-yielding dairy cow over more than thirty years. In the current study, 81 Holstein-Friesian dairy cows (30 primiparous and 51 multiparous of mean parity 2.1) were allocated to one of three experimental treatments: control (C), an individual cow management protocol (ICM) or a sequential glucogenic-lipogenic diet combination (GL) applied from calving until d 210 of lactation. Cows on the control treatment were offered a standard total mixed ration (TMR) containing 176 g of CP / kg DM. The ICM treatment was designed to keep individual cows within a target energy balance (EB) range by feeding, as appropriate, one of three diets of contrasting crude protein contents (174, 147 and 200 g of CP / kg DM) reflecting a standard, low and high protein content, respectively. The GL treatment entailed feeding a high-starch diet (177 g rising to 277 g of starch / kg DM) for the first 50 d of lactation and then switching to a high-fat diet (63 g fat / kg DM) from d 51 to d 120. There were no significant treatment effects on milk yield or milk composition (fat, protein, lactose and casein nitrogen). Milk urea nitrogen content was reduced by GL compared to C or ICM ($P < 0.01$, wk 1-30). Total DM intake was increased by ICM when compared to C or GL ($P < 0.01$, wk 1-30) and was a result, largely, of an increase in the DM intake of the forage component of the diet. Concentrate DM intake was not significantly affected by treatment. Both daily and cumulative EB were higher for cows on ICM than for cows on C or GL treatments ($P < 0.05$ and $P < 0.05$ wk 1-30, respectively), but live weight and body condition score were unaffected by treatment. Plasma BHB ($P < 0.05$, wk 24-32) and urea ($P < 0.01$, wk 14-20) concentrations were significantly lower on the GL treatment. Blood glucose levels were unaffected by treatment. Cow fertility measures were largely unaffected by treatment with only 'd to 1st observed heat' increased by ICM ($P < 0.05$) when compared with C or GL cows. In the current study results indicated that production and fertility characteristics were

unaffected by diet or nutritional strategy. However, adjusting dietary CP concentration is a useful tool with which to regulate the energy balance of individual cows in early lactation.

KEYWORDS

Dairy cow, fertility, nutrition, individual cow management

INTRODUCTION

Over the past 20-30 years, the increase in milk yield that has been achieved by increasing the proportion of Holstein genes in the dairy cattle population (Sondergaard et al., 2002) has been associated with more unsustainable dairy herds. For a period following parturition, the high-yielding dairy cow is unable to consume sufficient food to meet the energy demand associated with maintenance and the rapid increase in milk yield that occurs over this time. This often results in periods of quite severe negative energy balance (NEB) that necessitate the mobilisation of body fat reserves and, to a lesser extent, body protein reserves. Prolonged NEB in early lactation can lead to acute metabolic stress and long-term problems of cow fertility and cow health (Ingvartsen et al., 1999; Pryce et al., 2004).

Holstein-Friesian cow fertility problems and their consequences have been well documented in recent years. Royal et al. (2000) reported an ongoing decline in pregnancy rate to first service of one percent per annum for the previous 20 years while Mayne et al. (2002) reported average conception rates of only 37% for dairy cows in Northern Ireland. However, Pryce et al., (2002) found that conception rates in maiden heifers have remained high (70-80%) indicating that genetics alone cannot be solely responsible. If this rate of decline in cow fertility continues, Maas et al. (2008) have predicted that the Holstein-Friesian cow population will become unsustainable within as few as 10 years due to a failure to breed enough replacement females. Accordingly, it is vital that strategies be found to improve fertility and health of the high-yielding Holstein-Friesian cow in order to protect its longevity and maintain farm profitability.

Various nutritional interventions have been applied, experimentally, in an effort to minimise the degree of NEB that develops in the modern Holstein-Friesian cow (Garnsworthy et al., 2008a).

One approach, that of reducing dietary crude protein concentrations, was shown by Law et al. (2009) to be an effective technique by which to alleviate NEB in early lactation; however, this has a negative impact upon milk production. An alternative approach has been to offer either glucogenic or lipogenic diets, or a sequential combination of both, in an effort to improve cow health and reproductive status in early lactation (van Knegsel et al., 2007a; Garnsworthy et al., 2008c). In ruminants, lipogenic nutrients originate directly from dietary fat or, indirectly, from dietary fibre through ruminal production of acetate and butyrate. They may also be derived from body fat reserves. The inclusion of dietary fat may also be beneficial to blastocyst growth rate in lactating dairy cows (Fouladi-Nashta et al., 2007). However, on a cautionary note, supplemental fat in excess of 50-60g / kg DM may reduce feed intake (Schauff and Clark, 1992). Glucogenic nutrients originate, mostly, from dietary starch that escapes rumen degradation and is, instead, digested post-ruminally, or they can arise by hepatic gluconeogenesis (van Knegsel et al., 2007a) increasing circulating insulin concentrations which, in turn, are associated with an early return to cyclicity postapartum (Gong et al., 2002).

The aims of the current study were to assess animal performance (including a range of fertility measures) in Holstein-Friesian dairy cows subjected to two contrasting nutritional strategies. These were (i) the manipulation of dietary protein content so as to regulate EB and limit the extent of NEB in individual animals in early lactation and (ii) biphasic alteration of dietary starch and dietary fat contents in early-to-mid lactation to encourage, initially, the resumption of ovarian cyclicity and, subsequently, embryo survival.

MATERIALS AND METHODS

Animals and housing

Eighty-one Holstein-Friesian dairy cows (30 primiparous and 51 multiparous) were allocated to one of three treatments (n = 27 cows per treatment), applied over the first 210 d of lactation. Cows had a mean predicted transmitting ability (PTA_{05}) for milk yield, percent fat and percent protein of + 404 kg, + 0.04 % and + 0.05 %, respectively. For 8 wk prior to calving, cows were

offered a total mixed ration (TMR) containing concentrates, soyabean meal and grass silage (10:10:80 on a DM basis) restricted to a metabolisable energy (ME) intake of approximately 90 MJ ME / d. All cows also received 100 g of dry cow minerals per d. A restriction of 6 kg DM / d plus 3 kg DM of chopped straw was placed on each individual animal. The target body condition score (BCS) at calving was 2.25-2.75 units. Live weight (LW) and BCS were monitored weekly through the dry period.

Immediately after calving, cows were allocated to treatment and housed as a single unit in free stalls, with a cubicle:cow ratio of ≥ 1:1 at all times, in accordance with FAWC guidelines (1997). Multiparous cows (mean parity = 2.1; n = 17 per treatment) were balanced for milk yield in the previous lactation, and by parity, LW and BCS at calving. Primiparous cows (n = 10 per treatment) were balanced by LW and BCS at calving. Mean calving date (all) was October 7th 2007.

Treatments

The dietary treatments were: control diet (C), individual cow management (ICM) and a sequential glucogenic-lipogenic diet (GL). For the C and GL treatments, a TMR was formulated to contain a concentrate-to-forage ratio of 60:40. For the ICM treatment, the TMR was formulated to contain a concentrate-to-forage ratio of 45:55 to allow for an additional 5 kg DM of concentrates to be offered via the parlour. The forage component of all three TMRs was a grass silage and maize silage mixture (60:40, DM basis). Published values for energy and protein concentrations of feed ingredients (AFRC, 1993) were used when calculating the proportions used in the overall ration (Table 4.1). Diets were formulated according to "Feed into Milk", the current UK feed rationing scheme for dairy cattle (Thomas, 2004). The control (C) diet was formulated so as to supply 200g starch / kg DM and 180 g total diet crude protein (CP) / kg DM.

All TMRs were prepared fresh daily between 10.00am and 1200 noon, using a mixer wagon and then deposited into a series of feed boxes. Access to each feed box was controlled via a Calan

gate operated by an automatic cow identification system which also enabled automated recording of individual intakes (Forbes et al., 1986). The TMR portion of each diet was offered *ad libitum* to a target excess of 10 percent, and uneaten feed was removed twice weekly. Cows on the C and GL treatments were offered 0.5 kg DM of concentrates via the parlour at each milking (AM and PM). Treatments were applied for the first 210 d of lactation.

Table 4.1 Ingredient composition of concentrate feedstuffs offered during the first 30 weeks of lactation (g/kg fresh).

	Treatment					
	Control	Individual cow management			Glucogenic/Lipogenic	
		Base	High protein	Low protein	Glucogenic (days 35-50)	Lipogenic (days 51-120)
Barley	185	185	25	270	315	50
Wheat	185	185	25	270	315	50
Citrus pulp	115	165	30	120	60	245
Soya hulls	115	160	25	120	60	230
Soya bean meal (Hi pro)	160	110	410	75	100	150
Rape meal	160	110	410	70	95	155
Megalac[1]	20	30	-	-	-	63
Trace minerals and vitamins	5	5	5	5	5	5
Salt	4	4	4	4	4	4
Limestone	13	13	13	13	13	13
Calcined magnesite	5	5	5	5	5	5

Molaferm	30	30	30	30	30	30

[1] Volac Ltd., Orwell, Hertfordshire, UK

Individual cow management feeding strategy

A target weekly average daily EB (DEB) was selected for cows and heifers based upon data from a previous study at this institute (Law et al., 2009). A deviation of + / - 10 MJ / d was applied to each weekly target DEB value to dictate the range over which an individual animal's EB was allowed to fluctuate without triggering a diet change (Figure 4.1). From calving, all cows on this treatment were offered a dietary protein concentration of 175g CP / kg DM for at least the first three wk of lactation. Decisions to alter dietary protein content, or not, thereafter, were made on the basis of weekly average DEB and according to a fixed protocol (see below) that incorporated a 3-wk rest period following adjustment in early lactation (wk 1 – 10 of lactation) or a 2-wk rest period following adjustment in mid-lactation (wk 11 – 30 of lactation). During a rest period no further diet change was permitted (Figure 4.2 – decision tree). Adjustment of dietary protein content was achieved by selecting one of three supplementary concentrates (150, 256 and 363 g CP / kg DM offered as two 2.5 kg DM / d portions at the AM and PM milkings) enabling three levels of total dietary protein (150, 175 and 200 g CP / kg DM) to be provided. The protocol was applied rigidly except, and only, if animal health considerations dictated otherwise.

Individual Cow Management protocol: in any wk after wk 3, if the calculated weekly average DEB fell within its target range (Figure 4.1), the protein content of the diet was left unchanged. However, if the weekly average DEB fell below the lower limit of the target range, then the total diet protein content was reduced by one increment. Conversely, if the weekly average DEB breached the upper limit of the target range, then the total diet protein content was increased by one increment.

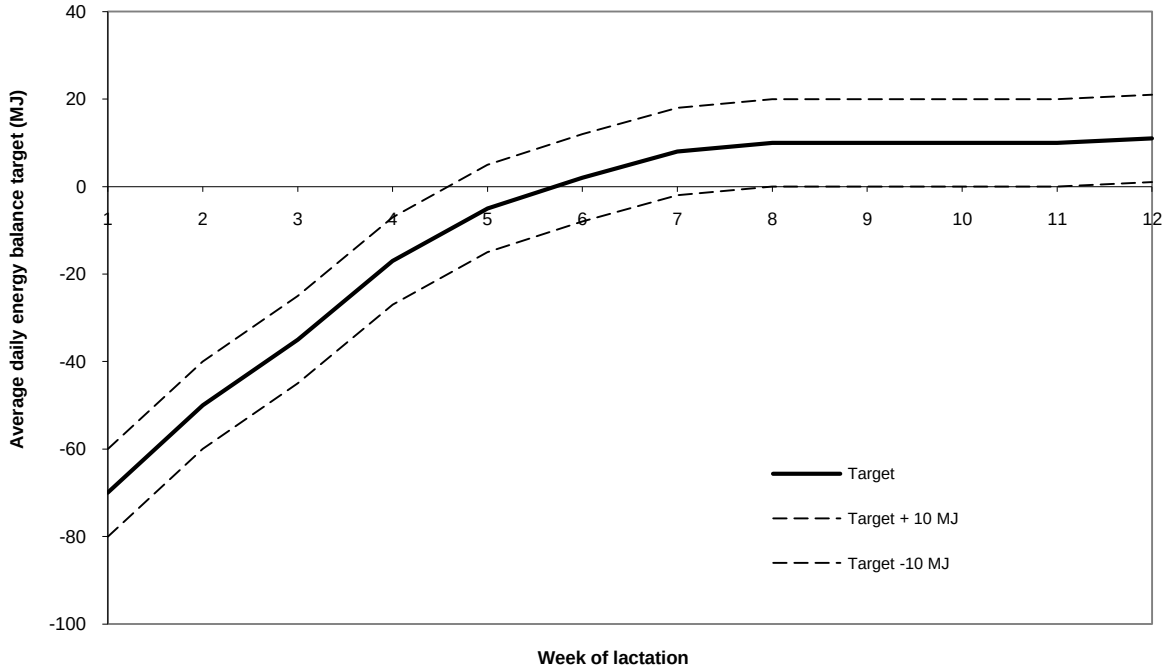


Figure 4.1: The individual cow management target energy balance curve for cows over the first 12 wk of lactation. This curve was used as a tool to determine the dietary protein level offered and subsequent dietary adjustments required on an individual animal basis for each wk of lactation. Solid line shows target average daily energy balance, dashed lines (--) shows the energy balance range within which no dietary adjustment would be required.

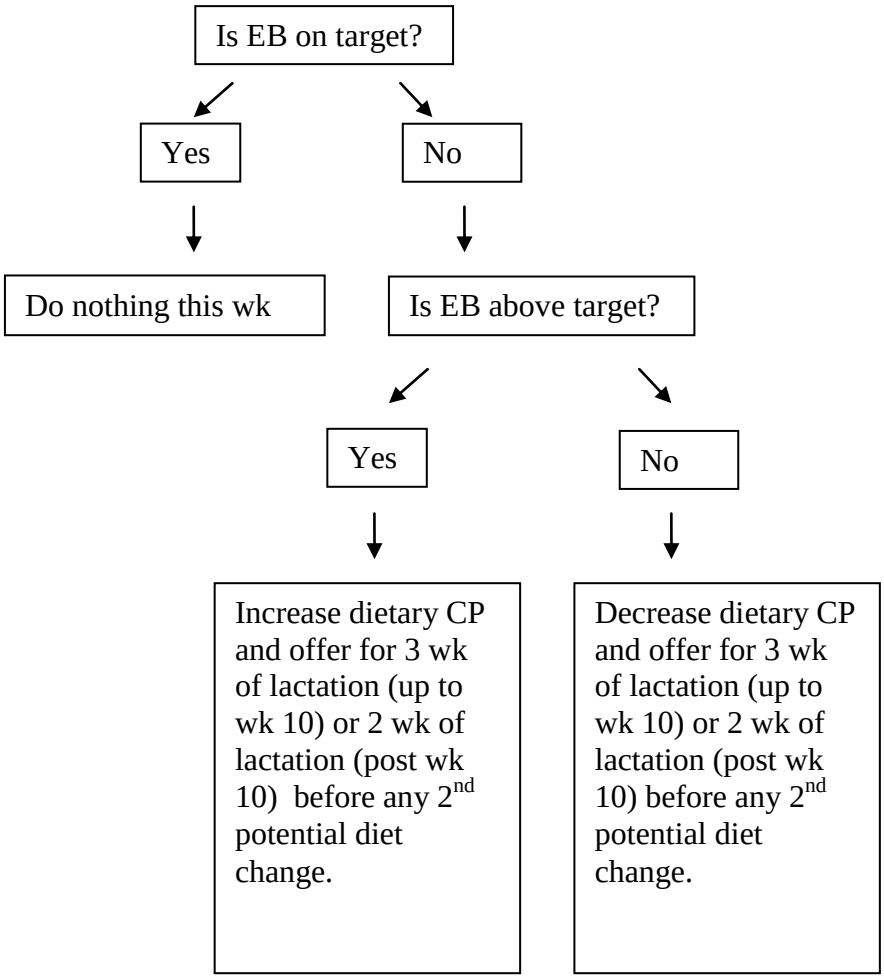


Figure 4.2: Individual cow management weekly decision tree (from wk 3 of lactation)

Glucogenic-lipogenic diet

Cows on this treatment were offered a high-starch diet for the first 50 d post partum (200 and 300 g starch / kg DM during d 1-35 and 36-50 respectively) to maximise the likelihood of a return to oestrus following parturition. From d 51 to d 120 post partum, the diet was changed to one that was high in protected fat (35.9g / kg DM) and low in starch (100 g starch / kg DM). From d 120 to d 210 , the GL diet was replaced by the control diet.

Measurements

Cows were milked twice daily at approximately 06.00 and 17.00. Milk yields were recorded automatically and an average daily yield for each wk of lactation was calculated. Milk composition was determined weekly for fat, protein, lactose, casein and milk urea nitrogen (MUN) contents on two consecutive AM and PM milk samples, using an infrared milk analyser (IRMA, Foss UK Ltd. Warrington, UK).

Intakes of grass silage, maize silage and concentrates for each animal were recorded daily throughout the experiment and used to calculate an average daily DM intake for each wk of lactation. Animal LW (kg) was recorded twice daily at milking, and used to calculate an average daily LW for each wk of lactation. Body condition score (Edmondson et al., 1989) was assessed weekly using a five point scale from 0 (thin) to 5 (fat).

Blood samples were taken from the coccygeal vein between 0930 and 1130, at weekly intervals up to wk 12 of lactation, then fortnightly to wk 20, and every 4 wk thereafter until wk 30. Blood samples were taken into heparin-coated and fluoride oxalate coated, evacuated tubes (Becton Dickinson, Oxford, UK). Plasma was recovered by centrifugation and stored at -20°C prior to analysis for glucose (fluoride oxalate tubes), total protein, albumin, globulin, β-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA) and urea (heparin-coated tubes) using a clinical analyser (Olympus UK Ltd, Middlesex, UK). Non-esterified fatty acid concentrations were determined using a standard kit (Wako Chemicals GmbH, Neuss, Germany).

Samples of grass and maize silage were collected and analysed daily for dry matter (85°C for 18 h). Weekly samples were scanned by near infrared spectroscopy (Park et al., 1998) for estimation of metabolisable energy (ME) content (MJ / kg DM) and, twice weekly, fresh samples of maize and grass silage were analysed for gross energy (GE) (Porter, 1992) and pH. The same samples were also analysed for nitrogen and ammonia nitrogen concentrations as described by Steen (1989), and for lactic acid and volatile fatty acids, ethanol, and propanol concentrations as described by Porter and Murray (2001). Dried silage samples were prepared twice weekly and milled (0.8mm screen), a composite 2-wk sample was analysed for ADF, NDF and ash as

described by Cushnahan and Gordon (1995). Twice weekly samples of maize silage were dried at 60°C and bulked for analysis of starch according to McCleary et al. (1994) using a commercial kit (Megazyme, Megazyme International Ireland Ltd., Bray, Ireland).. Each batch of concentrate was sampled and composite 2-wk samples were analysed for oven DM content (100°C for 24 h except startch (60°C for 48 h)). Dried samples were analysed for nitrogen (Steen, 1989), ADF, NDF and ash concentrations (Cushnahan and Gordon, 1995) and were bulked for starch analysis (Megazyme kit).

Average DEB was calculated for each animal in each wk of lactation using the equations described by Thomas (2004) [Energy balance = ME intake – ME requirement (-10 + (ME_{preg} + $ME_{maintmilk}$ * $Lwt^{0.75}$) + ((0.0013*Lwt) / K_m))]. Average daily milk yield, average daily DM intake, weekly milk composition, weekly LW and feed composition data were used in the calculations. For control and GL treatments, missing values (fewer than 2% of all data) were estimated from the wk previous to and the wk following.

During the first 210 d of lactation, a total of 3 cows and 4 heifers were removed from the trial. One cow was removed due to persistent mastitis, one due to digestive problems and one due to infertility. Two heifers were removed because of poor (incomplete) milk let-down from all four quarters while another heifer was removed due to lameness and one further heifer removed due to fertility problems.

Statistical analysis

Data were analysed in Genstat, Version 6.1 (Payne et al., 1993). A repeated measures approach using the Residual Maximum Likelihood (REML) procedure was undertaken to analyse the weekly data over the first 210 d of lactation. The model used fitted wk of lactation, treatment, parity (multiparous verses nulliparous). Analysis of variance was used to analyse data from wk 1-30, 1-15 and 16-30, with pre-calving LW used as a covariate in the analysis of post-calving LW and DM intakes.

RESULTS

Forage quality

The grass silage offered throughout the experiment had an average DM of 293g / kg fresh weight and average CP and ME contents of 156 g / kg DM and 11.9 MJ / kg DM respectively (Table 4.2). The maize silage had an average DM of 305 g / kg fresh weight, with CP and starch contents of 78 and 273 g / kg DM respectively and an ME content of 11.3 MJ / kg DM. The diets offered had starch contents ranging from 94 – 277 g / kg DM, with protein contents ranging from 147 – 200 g / kg DM (Table 4.3). Metabolisable energy (ME) contents for the rations offered were similar across treatments (12.4-12.6 MJ / kg DM) except for the lipogenic diet which had a higher ME content (13.1 MJ / kg DM). Overall, the diets differed from the target composition in terms of CP and starch content of the total diet by less than 1.3% (on a DM basis).

Animal performance

Repeated measures analysis identified significant effects ($P < 0.001$) of treatment on DMI (Figure 4.3), grass silage DM intake and maize silage DM intake. There was no treatment effect on concentrate DM intake ($P > 0.05$). ANOVA analysis (Table 4.4) revealed significant treatment effects in all time periods measured (e.g. wk 1-30, 1-15 and 16-30) for grass silage DM intake ($P < 0.001$) and maize silage DM intake ($P < 0.001$), but only in wk 1-15 and 1-30 for average total DM intake ($P < 0.01$). Total DM intake was significantly higher ($P < 0.01$) in cows on the ICM treatment in wk 1-15 and wk 1-30, but not during wk 16-30. Mean total DM intakes in the first 30 wk of lactation were 20.3, 21.5 and 20.1 kg / d (± 0.33 kg / d SEM) for control, ICM and GL treatments respectively. Concentrate DM intake did not differ significantly ($P > 0.05$) between treatments in any time period. Over the first 30 wk of lactation, maize silage DM intake was on average 0.5 kg / d (± 0.06 kg / d SEM) greater and grass silage DM intake was 0.7

kg / d and 0.6 kg / d (± 0.09 kg / d SEM) higher for ICM than for C and GL treatments, respectively.

Table 4.2 Chemical composition of grass silage, maize silage (g/kg volatile corrected dry matter, unless otherwise stated) and concentrate feedstuffs (g/kg oven dry matter, unless otherwise stated) offered during the first 30 weeks of lactation.

	Forage		Concentrate					
				Individual cow management			Glucogenic/Lipogenic	
	Grass silage	Maize silage	Control	Base	High protein nut	Low protein nut	Glucogenic	Lipogenic
Oven DM (g/kg)	279	305	863	865	869	864	853	871
Volatile corrected DM (g/kg)	293	322						
Crude protein	156	78	210	175	363	150	175	189
Neutral detergent fibre	564	432	253	268	248	257	228	318
Acid detergent fibre	359	243	142	159	138	148	127	203
Ash	92	69	75	81	95	72	70	95
Gross energy (MJ/kg DM)	19.2	19.1						
pH	3.75	3.74						
Ammonia-N (g/kg total N)	64	77						

Lactic acid	119.5	60.9						
Acetic acid	14.3	19.3						
Starch	ND	282	220	225	46	324	386	81
ME	11.9	11.3	13.0	13.1	12.5	12.7	12.8	13.5

ND = not determined

Table 4.3 Chemical composition of diets offered during the experiment.

	Treatment						
		Individual cow management			Glucogenic/Lipogenic		
	Control	Base + 2.5 H + 2.5 L	Base + 5 LP	Base + 5 HP	Days 0-34 (Base)	Days 35-50 (Glucogenic)	Days 51-120 (Lipogenic)
Dry matter (g/kg)	634	630	629	630	634	628	638
Crude protein (g/kg of DM)	176	174	147	200	176	155	163
Starch (g/kg of DM)	177	168	203	133	177	277	94
Fat (g/kg DM)	12.0	13.5	13.5	13.5	10.5	10.4	37.8
Metabolisable energy (MJ/kg of DM)	12.6	12.5	12.5	12.5	12.6	12.4	13.1

Table 4.4 Effects of treatment on food intake during the first 30 weeks of lactation (kg/DM/cow/day).

Average DMI kg/day	Treatment			SEM[2]	Significance[1]
	Control	Individual cow management	Glucogenic/ Lipogenic		
Weeks 1-15					
Grass silage	4.4[b]	5.2[a]	4.4[b]	0.11	***
Maize silage	3.0[b]	3.5[a]	2.9[b]	0.08	***
Concentrate	11.9	12.3	11.9	0.23	NS
Total (weeks 1-15)	19.3[b]	21.1[a]	19.2[b]	0.41	**
Weeks 16-30					
Grass silage	4.9[b]	5.6[a]	4.8[b]	0.09	***
Maize silage	3.3[b]	3.7[a]	3.2[b]	0.06	***
Concentrate	13.2	12.7	13.0	0.18	NS
Total (weeks 16-30)	21.3	22.0	21.0	0.33	NS
Weeks 1-30					
Grass silage	4.7[b]	5.4[a]	4.6[b]	0.09	***
Maize silage	3.1[b]	3.6[a]	3.1[b]	0.06	***
Concentrate	12.5	12.5	12.4	0.19	NS
Total (weeks 1-30)	20.3[b]	21.5[a]	20.1[b]	0.33	**

[1] NS, P>0.05; *, P<0.05; **, P<0.01; ***, P<0.001

[2] SEM – standard error of the mean

[a,b] Values within a row with no common superscript differ (P<0.05)

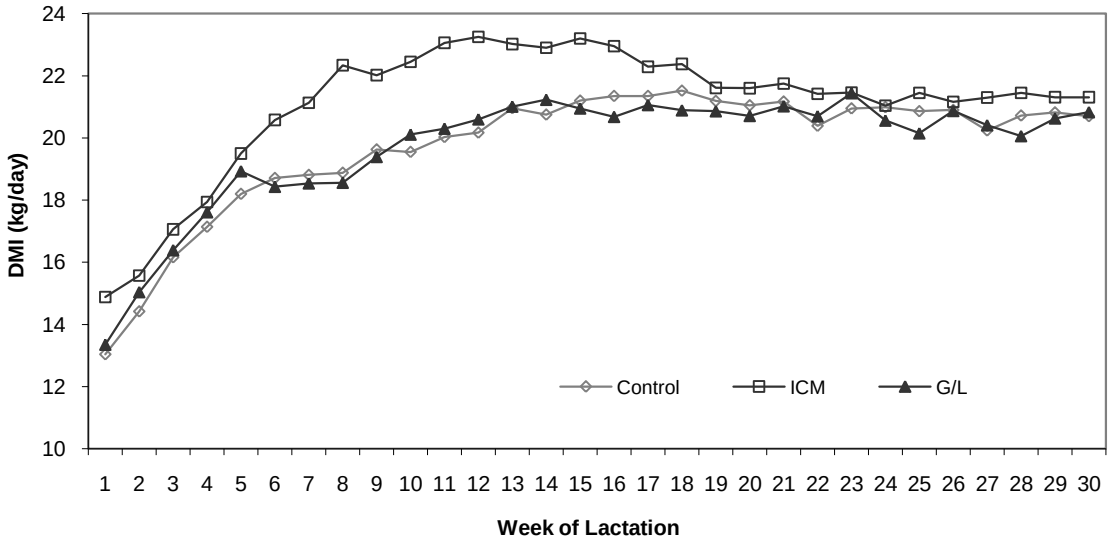


Figure 4.3: Average weekly dry matter intakes (kg / d) for each treatment. Effect of early lactation feeding regime (Control (◊), Individual Cow Management (□) and Glucogenic Lipogenic (▲)) on average weekly food intake (kg DM / d) during the first 210 d of lactation.

A more detailed exploration of the data for the ICM treatment revealed that the first dietary adjustment was a reduction in dietary protein content for 13 cows and an increase in dietary protein content for another 13 (one cow having been removed for health reasons). On average, the first dietary adjustment was made in wk 5 (4.92 ± 2.30 (SD) and 4.92 ± 1.71 (SD) wk for the first dietary change to be a reduction in dietary protein or an increase in dietary protein, respectively). Cows that moved to the low protein diet remained on this diet for an average of 7.0 wk (49 d ± 34 d), while cows that moved onto the high protein diet remained on this for an average of 20.3 wk (142 d ± 54 d). At the point of the first dietary change, DM intakes were reduced by an average of 3.8kg DM / d in the cows which had their dietary protein content reduced (17.3 ± 3.75 (SD) kg DM / d), compared to cows which had their dietary protein content increased (21.2 ± 2.96 (SD) kg DM / d), Figure 4.4).

Over the first 210 d of lactation there was no significant effect ($P > 0.05$) of treatment on either milk yield (Figure 4.5) or milk composition (fat, protein, lactose or casein N). Average milk yields over the first 210 d of lactation were 34.2 kg / d, 33.7kg / d and 34.7 kg / d (± 1.13 kg / d SEM) for the control, ICM and GL respectively (Table 4.5). Milk urea nitrogen was significantly lower for the GL treatment when compared to both C and ICM in wk 1-15 ($P < 0.05$), wk 16-30 ($P < 0.01$) and wk 1-30 ($P < 0.01$). Repeated measures analysis gave similar outcomes with no significant effect on milk yield or any milk components ($P > 0.05$) except MUN ($P < 0.001$). At the point of the first dietary change, milk yield was on average 4.9 kg higher in the ICM cows that required their first dietary adjustment to be a reduction in the dietary CP offered (36.3 kg ± 8.1 kg milk / d versus 31.4 ± 6.3 kg milk / d in the ICM cows that had their first dietary adjustment to be a reduction in dietary CP compared to an increase in dietary CP, Figures 6a and b). Both repeated measures analysis and ANOVA showed that LW and BCS were not significantly affected by treatment at any stage over the first 210 d of lactation ($P > 0.05$, Table 4.6).

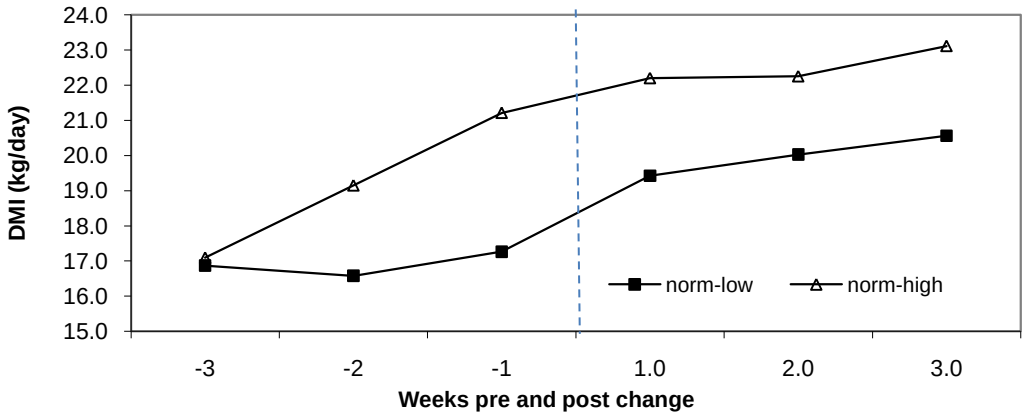


Figure 4.4: Individual cow management dry matter intake 3 wk pre and post 1st dietary change (diet change at -1). Norm-low (■) and norm-high (△) indicates subgroups of animals that had their dietary protein contents reduced (norm-low) or increased (norm-high) at their first dietary change.

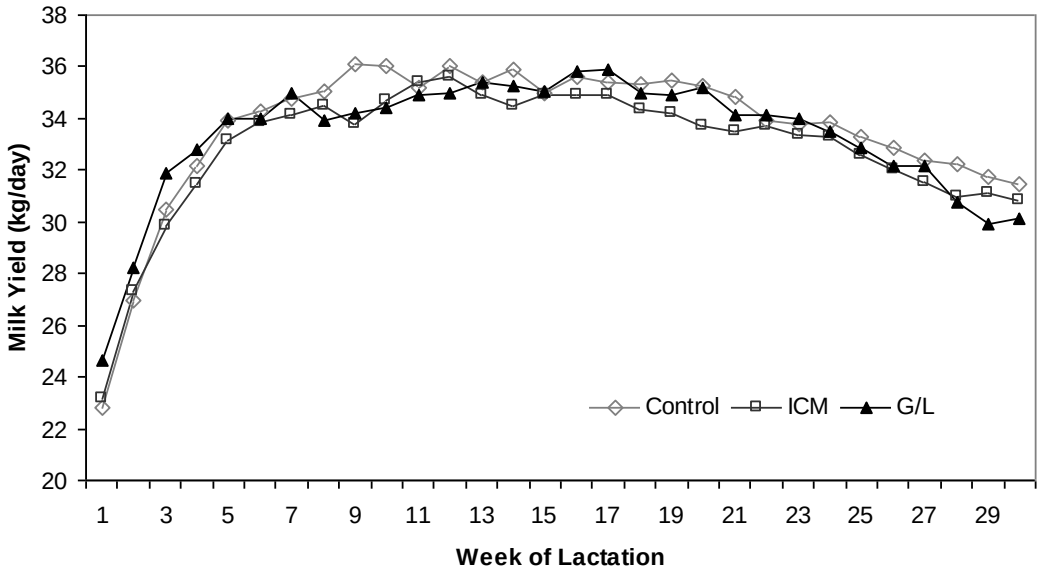


Figure 4.5: Average weekly milk yields for each treatment. Effect of early lactation feeding regime (Control (◊), Individual Cow Management (□) and Glucogenic Lipogenic (▲)) on average weekly milk yield (kg DM / d) during the first 210 d of lactation.

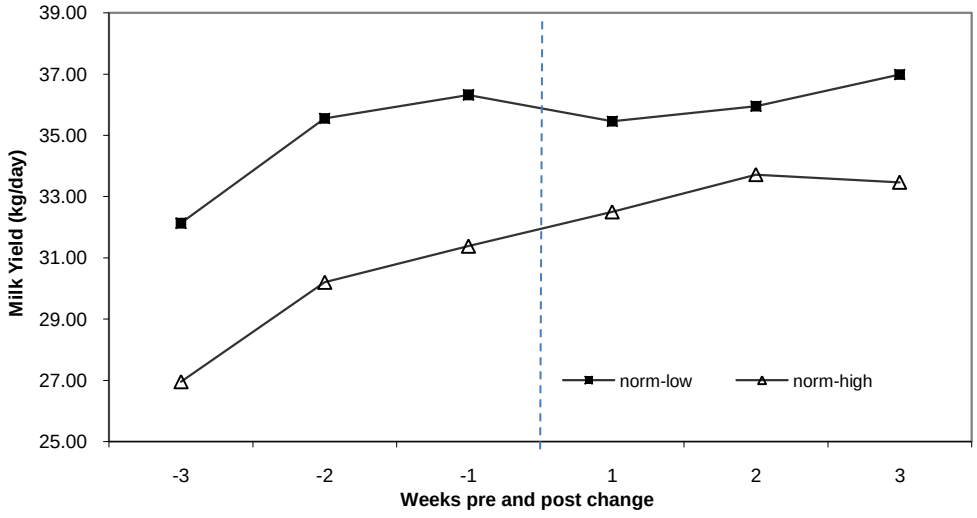


Figure 4.6a: Individual cow management milk yields 3 wk pre and post 1st dietary change (diet change at -1). Norm-low (■) and norm-high (▲) indicates subgroups of animals that had their

dietary protein contents reduced (norm-low) or increased (norm-high) at their first dietary change.

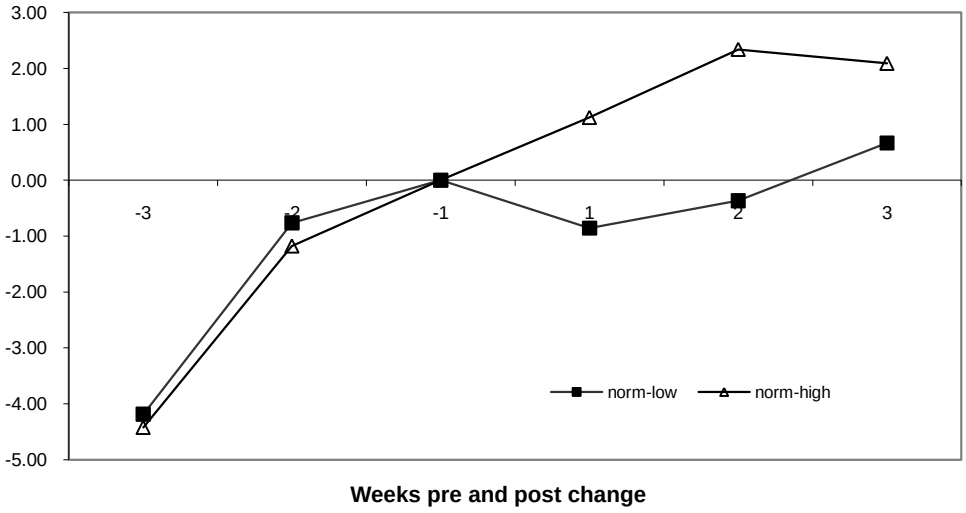


Figure 4.6a: Difference in Individual cow management milk yields 3 wk pre and post 1st dietary change (diet change at -1). Norm-low (■) and norm-high (▲) indicates subgroups of animals that had their dietary protein contents reduced (norm-low) or increased (norm-high) at their first dietary change.

Nutrient Utilisation

Milk N output and nitrogen utilisation efficiency for milk production (Table 4.7) were unaffected by dietary treatment ($P > 0.05$), whereas mean dietary N intake (g / d) was significantly affected by treatment ($P < 0.01$). Nitrogen intake was lower in C cows than in cows on either ICM or GL treatments (407.6 vs. 435.8 vs. 434.6 g / d respectively ± 9.13 g / d SEM).

Total ME intake (MJ / d) and ME requirement (MJ / d) were not affected by treatment ($P > 0.05$). Daily EB (MJ / d) and cumulative EB (MJ) over the first 210 d of lactation were both affected by treatment ($P < 0.05$, Table 4.7). Daily EB (MJ / d) and cumulative EB (MJ) were both significantly higher for cows on the ICM compared to control or GL cows. Daily EB was

31.7 MJ / d for ICM compared to 16.6 MJ / d and 18.7 MJ / d for C and GL treatments respectively. Cumulative EB over the first 210 d of lactation was 6470 MJ for the ICM compared to 3240 MJ and 3803 MJ for C and GL treatments respectively. Within the ICM treatment strategy, differences in both daily and cumulative EB were observed between cows that had their dietary protein concentration reduced at the first dietary change compared with cows that had their dietary protein content increased (Figure 4.7). At the point of the first dietary change, daily EB for these groups were, on average -35.8 MJ / d and 45.5 MJ / d, giving a difference of 81.3 MJ / d (Figure 4.7). Cumulative EB was 5224 MJ versus 7887 MJ for these same respective subgroups.

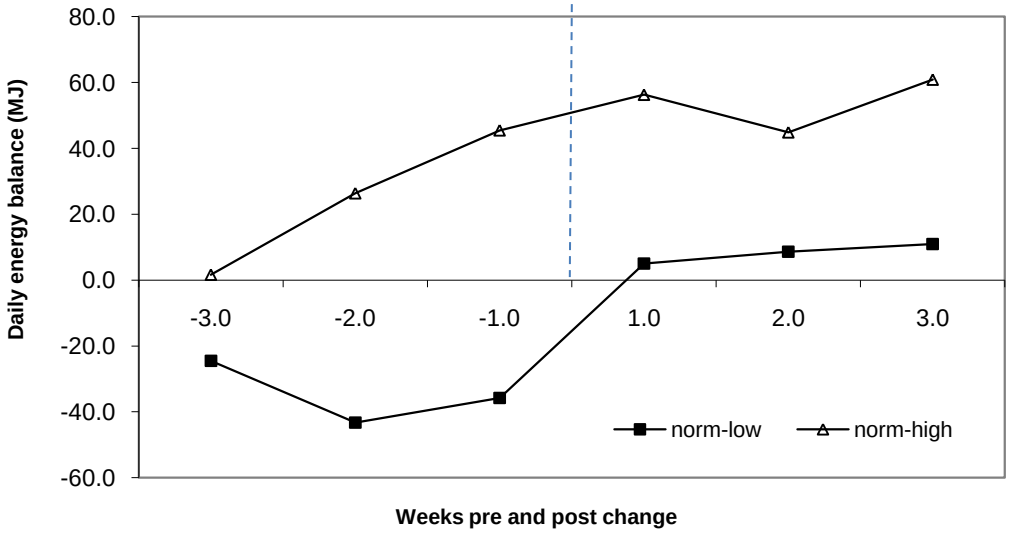


Figure 4.7: Individual cow management daily energy balance 3 wk pre and post 1st dietary change (diet change at -1). Norm-low (■) and norm-high (△) indicates subgroups of animals that had their dietary protein contents reduced (norm-low) or increased (norm-high) at their first dietary change.

Table 4.5 Effects of treatment on milk production and composition during the first 30 weeks of lactation.

	Treatment

	Control	Individual cow management	Glucogenic /Lipogenic	SEM[2]	Significance[1]
Weeks 1-15					
Milk yield (kg/day)	34.4	33.8	35.0	1.31	NS
Milk constituents (g/kg)					
Fat	37.9	38.7	39.1	0.77	NS
Protein	33.6	33.4	33.4	0.33	NS
Lactose	46.4	46.4	46.1	0.27	NS
Casein Nitrogen	26.9	26.6	26.5	0.27	NS
Milk urea (mg/kg)	165.1[a]	173.5[a]	157.1[b]	3.81	*
Milk constituent yield (kg/day)					
Fat	1.30	1.30	1.37	0.05	NS
Protein	1.15	1.13	1.17	0.04	NS
Fat + protein	2.45	2.42	2.54	0.09	NS
Weeks 16-30					
Milk yield (kg/day)	33.9	33.6	34.3	1.51	NS
Milk constituents (g/kg)					NS
Fat	37.8	39.4	37.6	0.96	NS
Protein	33.9	34.3	33.8	0.35	NS
Lactose	46.1	46.2	46.2	0.29	NS
Casein Nitrogen	26.8	27.2	26.6	0.31	NS
Milk urea (mg/kg)	162.7[a]	169.9[a]	154.1[b]	3.42	**
Milk constituent yield (kg/day)					
Fat	1.27	1.31	1.28	0.04	NS
Protein	1.14	1.15	1.16	0.03	NS
Fat + protein	2.41	2.46	2.44	0.07	NS
Weeks 1-30					
Milk yield (kg/day)	34.2	33.7	34.7	1.13	NS
Milk constituents (g/kg)					

Fat	37.7	39.0	38.4	1.15	NS
Protein	33.8	33.9	33.6	0.31	NS
Lactose	46.2	46.3	46.2	0.27	NS
Casein N	26.9	26.9	26.6	0.27	NS
Milk urea (mg/kg)	163.9[a]	171.7[a]	155.6[b]	3.32	**
Milk constituent yield (kg/day)					
Fat	1.28	1.30	1.32	0.04	NS
Protein	1.15	1.14	1.16	0.04	NS
Fat + protein	2.43	2.44	2.49	0.07	NS

[1] NS, P>0.05; *, P<0.05; **, P<0.01; ***, P<0.001

[2] SEM – standard error of the mean

[a,b] Values within a row with no common superscript differ (P<0.05)

Table 4.6 Effects of treatment on a number of key liveweight and body condition score parameters during the first 30 weeks of lactation.

	Treatment strategy				
	Control	Individual cow management	Glucogenic/ Lipogenic	SEM[2]	Significance[1]
Liveweight (kg)					
Post calving	559	559	564	6.39	NS
At week 15	574	560	565	6.9	NS
At week 30	601	597	598	7.04	NS
Mean (weeks 1-30)	575	565	570	5.98	NS

Change weeks 1-15	15.5	1.2	0.2	6.13	NS
Change weeks 15-30	28.7	36.4	31.4	5.69	NS
Change weeks 1-30	43.6	36.8	32.8	7.78	NS
Body condition score					
Post calving	2.7	2.6	2.6	0.04	NS
At week 15	2.5	2.4	2.4	0.05	NS
At week 30	2.7	2.6	2.6	0.25	NS
Mean (weeks 1-30)	2.5	2.4	2.5	0.21	NS
Change weeks 1-15	-0.17	-0.24	-0.25	0.44	NS
Change weeks 15-30	0.20	0.19	0.23	0.04	NS
Change weeks 1-30	0.03	-0.05	-0.02	0.07	NS

[1] NS, P>0.05; *, P<0.05; **, P<0.01; ***, P<0.001

[2] SEM – standard error of the mean

Table 4.7 Effects of treatment on energy and nitrogen utilisation efficiency during the first 30 weeks of lactation.

	Treatment				
	Control	Individual cow management	Glucogenic/ Lipogenic	SEM[2]	Significance[1]
Weeks 1-30					
ME requirement (MJ/d)	239.7	236.8	241.1	6.0	NS

ME intake (MJ/d)	256.3	268.6	259.2	5.2	NS
Daily energy status (MJ/d)	16.6[b]	31.7[a]	18.7[b]	3.8	*
Cumulative energy status (MJ)	3240[b]	6470[a]	3803[b]	806.8	*
Dietary nitrogen intake (g/day)	407.6[b]	435.8[a]	434.6[a]	9.13	**
Milk nitrogen output (g/day)	124.4	124.1	123.2	1.69	NS
Efficiency of nitrogen use for milk production	0.31	0.30	0.29	0.02	NS

[1] NS, P>0.05; *, P<0.05; **, P<0.01; ***, P<0.001

[2] SEM – standard error of the mean

[a,b] Values within a row with no common superscript differ (P<0.05)

Analysis by ANOVA showed that plasma NEFA and glucose concentrations (Table 4.8) were not significantly ($P > 0.05$) affected by treatment over the time periods analysed (wk 1-15, 16-30 and 1-30). Plasma urea concentrations were significantly lower ($P < 0.01$) for cows on the GL treatment compared with those on either the C or ICM treatments in wk 14-20 of lactation. However, no significant ($P > 0.05$) difference in plasma urea levels was found in wk 1-12 or 24-32 of lactation. Plasma BHB levels were significantly lower for cows on the GL treatment compared to cows on the C or ICM treatment in wk 24-32 of lactation ($P < 0.05$), but there was no difference through wk 1-12 or 14-20 ($P > 0.05$). Repeated measures analysis identified significant effects of dietary treatments on plasma BHB ($P < 0.05$), NEFA ($P < 0.005$) and urea concentrations ($P < 0.05$), with NEFA concentrations significantly higher for the GL treatment compared to C or ICM (0.71 versus 0.63 ± 0.02 (both C and ICM) mg / ml SED).

Table 4.8 Effects of treatment on blood metabolite concentrations during the first 30 weeks of lactation.

	Treatment				
	Control	Individual cow management	Glucogenic/ Lipogenic	SEM[2]	Significance[1]
Plasma NEFA (meq/l)					
Weeks 1-12	0.67	0.68	0.75	0.3	NS
Weeks 14-20	0.56	0.55	0.62	0.03	NS
Weeks 24-32	0.48	0.46	0.49	0.03	NS
Plasma glucose (mmol/l)					
Weeks 1-12	3.14	3.13	3.16	0.05	NS
Weeks 14-20	3.24	3.20	3.25	0.03	NS
Weeks 24-32	3.19	3.22	3.26	0.04	NS
Plasma urea (mmol/l)					
Weeks 1-12	5.56	5.37	5.10	0.18	NS
Weeks 14-20	5.90[a]	5.77[a]	5.16[b]	0.17	**
Weeks 24-32	5.52	5.35	5.51	0.17	NS
Plasma BHB (mmol/l)					
Weeks 1-12	0.57	0.59	0.58	0.02	NS
Weeks 14-20	0.52	0.59	0.52	0.02	NS
Weeks 24-32	0.60[a]	0.62[a]	0.52[b]	0.03	*

[1] NS, P>0.05; *, P<0.05; **, P<0.01; ***, P<0.001

[2] SEM – standard error of the mean

[a,b] Values within a row with no common superscript differ (P<0.05)

Fertility

There was no difference ($P > 0.05$) between treatments in d to 1st service, conception rate to 1st service, conception rate to 1st and 2nd service combined, 100 d in-calf rate and in-calf rate at the end of the breeding period (Table 4.9). In ICM cows, conception rate to 1st AI was 15% in cows that had their dietary protein increased as the first diet change compared to 31% in cows that had their dietary protein content decreased as the first diet change. There was a significant difference in d to display 1st observed heat ($P < 0.05$) with cows on ICM taking, on average, 62.7 d to 1st observed heat compared with 46.3 d and 45.1 d (±5.11 SEM) for cows on the control and GL treatments respectively. No significant difference ($P > 0.05$) in calving interval was found between the C, ICM or GL treatments.

Table 4.9 Effects of treatment on fertility during the first 30 weeks of lactation.

	Treatment				
	Control	Individual cow management	Glucogenic/ Lipogenic	SEM[2]	Significance[1]
Days to 1st observed heat	46.3[b]	62.7[a]	45.1[b]	5.11	*
Days to 1st service	76.7	78.7	87.2	3.56	NS
Conception to 1st AI (proportion)	0.35	0.23	0.39	0.09	NS
Conception to 1st and 2nd AI (proportion)	0.61	0.65	0.61	0.10	NS
Mean number of services/cow	2.6	2.4	2.6	0.33	NS

100 day in calf rate (from the start of the breeding season)	0.74	0.77	0.78	0.08	NS
Proportion of cows in calf at end of breeding season	0.95	1.00	0.91	0.03	NS
Calving interval (days)	385	399	400	8.84	NS

[1] NS, P>0.05; *, P<0.05; **, P<0.01; ***, P<0.001

[a,b] Values within a row with no common superscript differ (P<0.05)

[2] SEM – standard error of the mean

DISCUSSION

The current study was designed to compare the effects of two, strategically different, nutritional interventions (ICM and GL) applied in early lactation, on the performance and fertility of high-yielding dairy cows. As part of the ICM treatment, the energy balance of individual cows was calculated weekly and dietary protein levels were then reduced, increased or left unchanged so as to either alter or maintain the EB of individual cows within a pre-defined EB range that changed as lactation progressed. In previous studies, reducing the crude protein intake of cows in early lactation has been observed to reduce milk yield and to improve (i.e. make less negative) their DEB (Law et al., 2009). Alternatively, the GL treatment used a sequential combination of high starch (glucogenic) diet followed by a low-starch, high fat (lipogenic) diet from calving until d 120 of lactation in order, firstly, to stimulate a return to cyclicity, and subsequently, to favour embryo survival. The ICM and GL were both designed with the common objective of improving dairy cow fertility.

Animal performance

Dry matter intake was significantly greater amongst cows on ICM, despite that the possibility that when cows had their dietary crude protein content reduced in this treatment a reduction in DM intake may also have occurred. The increase in DM intake over the first 105 d after calving was approximately 1.8 kg / d higher than for cows on C or GL and was largely due to an increase in forage intake. The scale of this increased intake of the forage component of the diet is not readily explicable but one contributory factor may be that, as the study progressed, an increasing number of cows were moved onto the higher protein diet in order to maximise milk yield and thereby constrain EB to within the pre-defined limits. Increased dietary protein concentration has been a well documented driver of DM intake, provided that dietary energy content is not limiting (Oldham, 1984; Faverdin et al., 2003) and this increase in DM intake as dietary protein was increased may explain the overall significant increase in intake in the ICM treatment. Broderick (2003) clearly documented a linear increase in DM intake with increases in dietary CP concentration from 15.1% to 18.5%. Although Broderick et al., 2003 found an increase in DM intake at 18.5% compared to 16.7%, no significant increase in milk yield or milk components was identified beyond 16.7%. In addition, Broderick et al., (2003) identified only marginal increases in milk yield and composition of 1.1 kg / d and 1.5 kg / d of milk and fat corrected milk respectively when the CP was increased from 15.1% to 16.7%. In the current study, in the subgroup of animals where the dietary protein content of the diet was reduced in early lactation from 174 g / kg to 147 g / kg DM, no significant reduction in DM intake was observed. This was similar to the study Law et al. (2008) in which DM intake was unaffected in early lactation (d 1-150) when the dietary protein content offered was either 144g / kg or 173g / kg.

The GL treatment focussed on the effects of changes in dietary starch and fat contents. The lipogenic diet contained 6.35 % fat while the glucogenic diet contained only 3.49% fat. Both were within the range of fat contents recognised as not significantly affecting DM intake. For example, Dixon and Stockdale (1999) showed that fat could be incorporated into cattle diets at between 4 and 7% inclusion without adverse effects on fibre digestion or intake. Chamberlain and Wilkinson (2005) recommended starch and sugar levels of between 20-25% of the total diet increasing to a maximum of 30% inclusion in diets for well-managed, high-yielding, dairy cows.

Average 210 d milk yields did not differ significantly at 7182 kg, 7077 kg and 7287 kg respectively for the control, ICM and GL treatments. An increase in average overall milk yield might have been expected with ICM as almost 72% of the cows on this treatment had moved to the high protein diet by wk 13 of lactation. However, on the high protein diet, dietary CP levels (200g / kg), may have been in excess of that needed to generate a milk yield response. Apart from MUN, milk composition was unaffected by treatment. Milk composition might also have been expected to be affected by ICM since Leonardi and Armentano (2003) and M'Hamed et al. (2001) each found that milk fat concentration increased in response to increased dietary CP (from 161 to 189 g CP / kg DM and from 141 to 179 g CP / kg DM respectively). However, no change in milk fat was noted in the current work and Lunquist et al, (1986) similarly found no response in milk fat content when they increased dietary protein concentrations from 125 through 155 to 180 g / kg DM.

As expected, there was no effect of the GL treatment on milk yield, which was consistent with the results of similar investigations by Van Knegsel et al. (2007) and Garnworthy et al. (2008). But while these latter studies did report significant effects on milk fat yield, there was no significant effect of GL on milk fat yield in the current study. Milk urea nitrogen was significantly reduced throughout the 210 d of lactation in GL cows when compared to those on the ICM and control treatments. The reduction in MUN may be partly associated with the lower dietary protein content of the GL diets which was, on average, 1% lower than that of the control diet from d 50 of lactation (2% lower between d 35 and d 50) and except for a short period in early lactation at least 1% lower than that of the ICM (between 3.7 and 4.5% depending on the stage of lactation and dietary CP level offered to each individual cow in the ICM treatment).

Nutrient Utilisation

Neither BCS nor LW were significantly affected by treatment, despite a significant difference in DEB. Differences in BCS and LW may have been anticipated due to the diversity of the diets offered. Average LW at wk one post-calving and wk 30 (210 d) post-calving only differed by 5kg and 4kg, respectively, across the three treatments, whilst BCS differed by less than 0.1 of a

BCS unit at the same time points (i.e. post calving wk one and wk 30). Although no difference amongst treatments were identified, cows on the ICM treatment that had their dietary protein content reduced showed a difference in LW and BCS when compared with cows that had their dietary protein content increased as the first dietary change (data not shown). Daily EB was significantly greater in ICM cows when compared to GL cows or C cows at 31.7, 18.7 and 16.6 MJ / d respectively over the first 210 d of lactation. Given this significant increase in daily EB in ICM cows, an increase in LW and / or BCS might reasonably have been expected. On average, cumulative EB was 2949MJ greater in cows on ICM, equating to 1.65 units of BCS change (AFRC 1993) or 126kg of LW change (AFRC, 1990). Law et al. (2009) showed similar improvement in daily and cumulative EB but failed to find an effect on BCS or LW over the course of lactation and concluded that the discrepancies in EB may, in part, be a result of two assumptions made in the calculation of energy requirements: that the maintenance ME requirement is identical for all levels of production and that the net efficiency of energy utilisation for lactation (k_1) does not fall below 0.59 (Law et al., 2009) despite partial scaling to the level of intake (Thomas, 2004).

The complexity of the interrelationship between DM intake, milk yield and daily EB was highlighted by several observations from the current study. Firstly, animals that moved to a lower protein diet at their first diet change had considerably lower DMI at the point of intervention (wk -1) than those that moved to a higher protein diet at their first diet change (Figure 4.4). The impact on EB of the difference in DM intake (mean: 4kg / d) is likely to have been compounded by the substantially higher milk yields (mean: +5kg / d) prior to intervention, in cows moved to the lower protein diet (Figure 4.5). It is likely that these animals are mobilising more body tissue, which will suppress DMI and in combination with higher milk yield exacerbates the extent of NEB (estimated at -80 MJ / d at the point of intervention; Figure 4.7). This suggests that such animals are experiencing more metabolic stress Than their counterparts who are consuming more energy and producing less milk. Veerkamp et al. (1994) illustrated a similar relationship, with high yielding cows having a high mobilisation of body reserves in early lactation.

Significant dietary effects on plasma NEFA, BHB and urea concentrations were identified in the current study. Changes in plasma NEFA, BHB and urea levels are traditionally associated with a move into negative EB or with considerable changes in nutritional status. Plasma glucose levels might also have been expected to change, (particularly in cows on the GL treatment) but were unaffected. A study by Cabrita et al. (2007) showed reduced concentrations of glucose and insulin in plasma when starch levels were reduced from 23.7% starch to approximately 15% starch per kg DM in diets for dairy cows. In the study by Cabrita et al. (2007) there was a decrease in plasma glucose of approximately 5.3 mg / dl to a level of 56.0 mg / dl in the low protein / low starch treatment. In the current study, dietary starch levels ranged from below 10% to 30%, however no significant difference in plasma glucose levels were detected, with average treatment plasma glucose levels ranging from 3.19 mmol / L to 3.23 mmol / L (57.42 and 58.23 mg / dL respectively). These levels are not dissimilar to the lower levels quoted by Cabrita et al. (2007). Detection of a change in plasma glucose level is potentially problematic as plasma glucose concentrations are under tight homeostatic control and rarely change except in extreme circumstances (Chamberlain and Wilkinson, 2005). The reduced plasma urea levels in the GL cows compared to control cows could be a result of reduced CP content of the diet or could be due to the glucogenic / lipogenic nature of the diet as Garnsworthy et al. (2008 b) found a similar association with plasma urea and dietary starch levels, (although overall levels were higher in this particular study). In addition a similar decrease in blood urea with a decrease in starch content was identified.

Fertility

The fertility of dairy cows is subject to multifactorial influences. Treatments applied in the current study did not significantly affect any measure of fertility except 'd to 1st observed heat' but it is not clear why this parameter was increased amongst cows on the ICM treatment and not in those on either GL or control treatments. More detailed fertility observations from this study were reported by Gilmore et al. (2011). It was hypothesised that reductions in the extent of the early-lactation NEB in cows in which EB was closely monitored and managed (ICM treatment)

would facilitate an earlier return to oestrus and lead to improved fertility. However, a contrary outcome was realised.

The GL treatment, was designed to improve fertility by favourably manipulating the circulating concentrations of hormones (notably, insulin) associatedwith reproductive function through changing the character of the diet from glucogenic to lipogenic. However, the GL treatment did not influence fertility in the current study compared to the other regimes (C or ICM). Garnsworthy et al. (2008 b c) concluded that a dietary starch content greater than 160g / kg DM and a dietary fat content less than 44g / kg (approx 15g / kg DM Megalac) are optimal when seeking to positively influence ovarian function. The control diet offered in the current study closely complied with this recommendation, with a fat content of 43-45 g / kg and a starch content of 177g / kg, whereas the GL diets contained supplementary starch (up to 277g / kg in the glucogenic diets) and fat (35.9g / kg Megalac, in the lipogenic diet (approx 63.5 g / kg fat)). Garnsworthy et al. (2008 c) concluded that dietary total fat concentration for cows should be < 50g / kg DM in order not to depress plasma insulin concentrations at the start of the breeding period. Plasma insulin levels increase with increasing dietary starch and decreasing fatty acid concentrations. Garnsworthy et al. (2008 c) reported a maximum plasma insulin: glucagon concentration ratio in cows offered 15g Megalac per kg diet DM and insulin levels decreased as Megalac inclusion rose above 15g / kg DM.

CONCLUSIONS

The present study assessed the effects of two innovative nutritional strategies on the performance and fertility of high-yielding dairy cows over the first 210 d of lactation. Significant effects of nutritional regime (ICM or GL) were noted on DM intake and estimated EB but neither nutritional regime significantly affected fertility, milk yield, liveweight or condition score.

The current study has shown that altering dietary crude protein concentration is an effective approach through which to lessen the severity of the negative EB in dairy cows in early lactation

without detriment to milk yield in the long term. It was also clear that not all animals needed dietary intervention in order to remain within a daily EB target. Further investigation of this approach with larger numbers of animals may prove useful in developing future nutritional management strategies to minimise NEB in the critical early lactation period. Ultimately, fertility problems in the high-yielding Holstein Friesian are multi-factorial in origin, with nutrition being only one contributor. Further work is required with larger numbers of animals before a definitive nutritional impact on Holstein Friesian dairy cow fertility can be identified.

CHAPTER 5: An evaluation of the effect of altering nutrition and nutritional strategies in early lactation on reproductive performance and oestrous behaviour of high yielding Holstein-Friesian dairy cows

ABSTRACT

Reproductive performance in the high yielding dairy cow has severely declined in the last 40 years. The aim of this study was to compare the effectiveness of four nutritional strategies in improving the reproductive performance of high-yielding dairy cows. It was hypothesized that offering cows a high starch ration in early lactation would enhance the onset of luteal activity, and that reducing the severity of negative energy balance in the early postcalving period would improve reproductive parameters. Nutritional regimes aimed at improving fertility were applied to 96 Holstein-Friesian dairy animals. Upon calving, animals were allocated in a balanced manner to one of four dietary treatments. Primiparous animals were balanced according to liveweight, body condition score and calving date. Multiparous animals were balanced according to parity, previous lactation milk yield, liveweight, body condition score and calving date. Treatment 1 was based on an industry "best practice" diet (Control) to contain 170g CP/kg dry matter (DM). Treatment 2 was an "individual cow feeding strategy" whereby the energy balance (EB) of individual animals was managed so as to achieve a predetermined target daily EB profile (± 10 MJ/d). Treatment 3 was a high-starch / high-fat combination treatment whereby an insulinogenic (high-starch) diet was offered in early lactation to encourage cyclicity and followed by a lipogenic (low-starch, high-fat) diet to promote embryo development. Treatment 4 was a low protein diet, containing 140g CP/kg DM, supplemented with protected methionine at an inclusion level of 40g per animal per d. The nutritional strategies implemented in this study had no statistically significant effects on cow fertility measures which included the onset of luteal activity, conception rate, in-calf rate and the incidence of atypical cycles. The individual cow feeding strategy improved EB in early lactation but had no benefit on conception rate (CR) to first insemination. However, CR to second insemination, 100 d pregnancy rate (from the commencement of breeding), and overall pregnancy rate tended to be higher ($P > 0.05$) in this

group. The high-starch / high-fat treatment tended to decrease the proportion of delayed ovulations and increase the proportion of animals cycling by day 50 postcalving. Animals that failed to conceive to first insemination had a significantly longer luteal phase in the first cycle postpartum and a longer inter-ovulatory interval in the second cycle postpartum. With regards to oestrous behaviour, results indicate that as the size of the sexually active group increased, the intensity of oestrous and the expression of mounting or attempting to mount another cow also increased. Furthermore, cows that became pregnant displayed more intense oestrous behaviour than cows that failed to become pregnant ($P < 0.001$).

Key Words: nutrition, fertility, reproductive performance, high yielding dairy cows.

INTRODUCTION

Reproductive performance in the modern high-yielding Holstein-Friesian dairy cow has declined at such a rate, and to such an extent, over the last forty years that it is predicted that this high-performance production system will become unsustainable by 2020 if the current rate of decline continues (Maas *et al*., 2008).

It is considered unlikely that this decline in reproductive performance has a direct genetic origin as conception rates in non-lactating Holstein-Friesian heifers have remained high (at 70-80%) during a period when milk production has increased by 218% (Beam and Butler, 1999). However, in general, the increase in milk energy output has not been matched by a proportionate increase in energy intake (EI) resulting in a negative energy balance (NEB), which forces, and is counterbalanced by, the mobilization of body reserves. Negative energy balance has been determined as an underlying causal factor of poor reproductive performance in high-yielding dairy cows (Jorritsma *et al*., 2003) and has been associated with a delay in the onset of luteal activity (OLA; Jolly *et al*., 1995), an extended interval to first service (Butler *et al*., 1981) and decreased conception rates (Domecq *et al*., 1997). Specifically, NEB impairs ovarian function through a reduction in the maximum diameter of ovarian dominant follicles (Lucy *et al*., 1991; Mackey *et al*., 1999). Smaller dominant follicles produce less estradiol, suppressing the pulsatile

secretion of luteinizing hormone (LH; Butler, 2001), and reducing ovarian responsiveness to LH (Butler, 2001). Collectively, these events increase the proportion of follicles that fail to ovulate (Mackey *et al*., 1999).

A number of diet components have the potential to influence the extent of the postpartum NEB as well as the circulating concentrations of specific blood metabolites and hormones. For example, dietary crude protein (CP) content has been shown to affect milk output and thereby influence NEB (Law *et al*., 2009a). The latter authors reported reduced milk production and improved EB in cows offered a diet of 114 g CP/kg DM (low CP) compared to those offered 173 g CP/kg DM (high CP). Diets high in CP also increase blood urea concentrations (Law *et al*., 2009a), and Butler *et al*. (1996) argued that blood urea concentrations above 19 or 20 mg/dL would result in a 20% decrease in pregnancy rate post-AI. The inclusion of protected fat in dairy cow rations increases the energy density of the diet and also has the potential to reduce the extent of the NEB (Van Knegsel *et al*., 2007a). However, supplemental fat in excess of 50–60 g/kg DM may reduce feed intake (Schauff and Clark, 1992) and offset any potential beneficial impact on NEB. Nonetheless, the inclusion of supplemental fat is often preferred to starch inclusion, as a means of increasing the energy density of the diet, as high dietary starch levels can be detrimental to digestion, milk composition and cow health (Staples *et al*., 1998). Importantly, however, diets high in starch content increase the supply of glucogenic precursors and can increase circulating insulin concentrations (Van Knegsel *et al*., 2007b) which, in turn, are associated with enhanced cyclicity early postpartum (Gong *et al*., 2002). Supplementation of the diet with fat can increase cholesterol concentration (Grummer and Carrol, 1988) which serves as a precursor for progesterone synthesis (Staples *et al*., 1998). The inclusion of dietary fat has also proved beneficial to blastocyst growth rate in lactating dairy cows (Fouladi-Nashta *et al*., 2007). More recently, it has been suggested that the type of fat offered to dairy cows is more important than the quantity of fat with particular emphasis on the effects of omega-3 and omega-6 fats on reproductive performance (Childs *et al*., 2008). However, much of the published literature has focused on the effects of including of calcium salts of palm fatty acids on dairy cow fertility (Garnsworthy *et al*., 2008a, b; Staples *et al*., 1998).

Despite the key importance of nutrition, poor reproductive performance remains multifactorial in origin with management factors also making a significant contribution. For example, there has been a decline in the observed expression, intensity and detection of animals in oestrus (Van Eerdenburg *et al*., 1996; Kerbrat and Disenhaus, 2004) and poor heat detection is a major contributor to reduced reproductive performance in modern high-yielding dairy cows (Reimers *et al*. 1985) due to shorter and less intense oestrous expression. (Lopez *et al*., 2004). Lyimo *et al*. (2000) found that maximum estradiol concentrations, which are influenced by NEB, were related to total oestrous expression.

The aim of the current study was to evaluate the effect of four distinctive nutritional regimes or strategies on the reproductive performance of contemporary high-yielding Holstein-Friesian cows.

The hypotheses attached to the use of the nutritional regimes applied in the current study were as follows:

1. Adjusting the protein content of individual cow diets upwards or downwards can be used to more closely align cows to an optimal and more uniform energy balance trajectory.
2. Offering a high-starch diet postcalving reduces the interval from calving to the onset of luteal activity.
3. Switching cows to a low-starch / high-fat diet at 50 days postcalving improves reproductive parameters.
4. Offering a reduced protein ration supplemented with protected methionine reduces plasma urea concentrations in dairy cows whilst sustaining milk production and improving reproductive parameters.

MATERIALS AND METHODS

Animals and housing

The experiment involved 108 Holstein-Friesian animals (40 primiparous and 68 multiparous; mean parity 2.1), calving between 24th August and 19th December 2007. Following calving, animals were housed as a single group in free stalls with concrete flooring. The cubicle to cow ratio was >1:1 at all times, meeting the recommendations set by FAWC (1997). All cubicles had a rubber mat measuring 2.20 m long and 1.25m wide and sawdust bedding was renewed three times weekly. Concrete passageway floors were scraped at least 4 times daily by an automated system. Lights were left on at all times. Cows were milked twice daily through a 50 point rotary parlor, commencing at 05.30 and 15.30 hours, with cows walking approximately 35m to the parlor.

Experimental design, diets and feeding

Immediately after calving, animals were allocated to one of four dietary treatments in a balanced manner. Primiparous animals were assigned to treatments based on their live weight (LW), body condition score (BCS) and calving date. Multiparous animals were assigned to treatments according to parity, previous lactation milk yield, LW, BCS and calving date. Treatment 1 (control) reflected an industry “best practice” diet. It was formulated using the UK feed rationing programme ‘Feed into Milk’ (Thomas, 2004) and contained 170g CP/kg dry matter (DM) and 12.5 MJ metabolizable energy (ME)/kg DM. Treatment 2 was an "individual cow feeding strategy" whereby the calculated EB of individual cows was manipulated in order to maintain a target daily energy balance (DEB) profile (± 10 MJ/d) based on that observed in cows offered 150 g CP/kg DM (Law *et al*., 2009c; Figure 5.1). To achieve this, cows were offered 5.22 kg DM of one of three concentrates differing in CP (via the parlor feeder), so as to achieve total dietary CP concentrations of 200g CP / kg DM (high), 170g CP/kg DM (medium) or 140g CP/kg DM (low). After calving, all animals were initially offered 170g CP/kg DM (medium) for 3 weeks, after which, the CP content of the ration was altered weekly (if required) to dictate milk yield and subsequent DEB, until d 210 of lactation. For example, if after 3 wks the energy balance was below the target energy balance for that specific wk of lactation, the CP content of the diet was changed from high to medium or from medium to low depending on which diet was currently being offered. Similarly, if energy balance for the previous 3 wks was above the target, the CP

content of the diet was increased from low to medium or medium to high depending on which diet was currently being offered. Treatment 3 (high-starch / high-fat), a "fertility improver" ration, was based on previous work by Gong *et al*. (2002) that had shown improved reproductive performance by feeding an insulinogenic or lipogenic diet at different stages of the reproductive cycle. In the current study, an insulinogenic (high-starch) diet was offered for 50 d after parturition in order to encourage renewed luteal activity. Then, to prevent detrimental effects of high insulin levels on oocyte quality (Fouldi-Nashta *et al*., 2005), a lipogenic (low-starch / high-fat) diet, supplemented with 750 g of protected fat per d (as fed basis) ['Megalac', calcium salts of palm fatty acids; Volac International, Royston, UK], was offered between d 51 and d 120 of lactation. Treatment 4 was a low protein diet, containing only 140g CP/kg DM but supplemented with protected methionine ('MetaSmart', Kemin UK, Ltd.) at an inclusion level of 40g per animal per d. This treatment was designed to assess the effects of offering a reduced CP ration supplemented with protected methionine (the first limiting amino acid for milk production; Newbold, 2006) on the nitrogen efficiency of milk production and reproductive performance.

Table 5.1. Formulations (g/kg) of the concentrates used in preparing the treatments

	Treatment						
		Individual Cow			High-Starch / High-Fat		
	Control	Base	High Protein	Low Protein	High	Low	Low Protein
Barley	186	185	25	280	314	50	198
Wheat	186	185	25	278	314	50	197
Citrus pulp	116	163	30	120	60	245	170
Soya hulls	115	160	25	120	60	230	170
Soya bean meal (Hi Pro)	160	110	420	75	100	150	125
Rape meal	160	110	418	70	95	155	60
Megalac[1]	20	30	-	-	-	63	20
Trace minerals and vitamins	5	5	5	5	5	5	5

Salt	4	4	4	4	4	4	4
Limestone	13	13	13	13	13	13	13
Calcined Magnesium	5	5	5	5	5	5	5
Molasses	30	30	30	30	30	30	30
Protected Methionine	-	-	-	-	-	-	4
ME (MJ/kg of DM)	13.0	13.1	12.5	12.7	12.8	13.5	13.0
CP (g/kg of DM)	221.0	180.0	419.0	157.0	178.0	205.0	176.0
Starch (g/kg of DM)	252.0	249.0	58.0	370.0	416.0	78.0	264.0

[1]Volac Ltd., Orwell, Hertfordshire, UK.

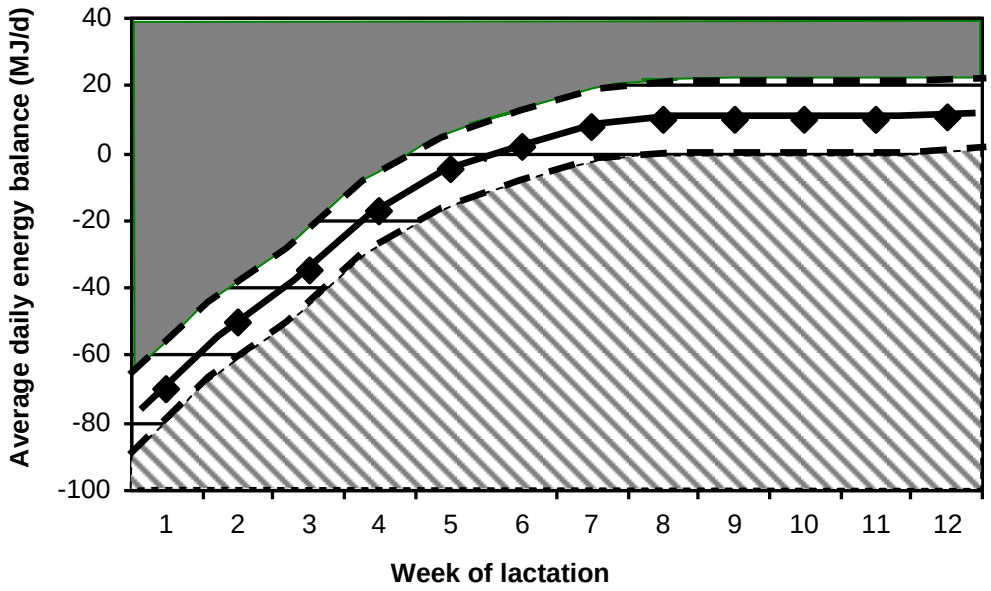


Figure 5.1: Example of the energy balance (EB) profile used to determine the dietary protein level offered to animals on the 'individual cow treatment' (based on Law *et al*., 2009a). The solid line (◆) illustrates the target average daily EB for each week of lactation; the dashed line (– –) illustrates the upper and lower thresholds for each week of lactation. The diagonally striped zone illustrates EBs below target whilst the solid grey zone illustrates EBs above target.

All diets were offered as a total mixed ration (TMR) but, irrespective of treatment, all animals were offered 1 kg of their concentrates allowance in the milking parlor each day. The concentrate

to forage ratio of the TMR was 60:40 (DM basis) in the control, high-starch / high-fat and low protein treatments. The individual cow TMR had a concentrate to forage ratio of 45:55 (DM basis) as an additional 6 kg (as fed) of concentrate was offered in the milking parlor daily (to facilitate the alteration of dietary CP content). The formulations of each of the concentrates used are presented in Table 5.1.

The forage component of all diets consisted of 60% grass silage and 40% maize silage (dry matter basis). The TMR diets were offered *ad libitum* and were freshly mixed each day between 1000 and 1100h. Individual DMI's were recorded continuously by using feed boxes placed on computer-linked load cells with access to the boxes controlled by a gated system with electronic identifiers (Calan Inc., Northwood, NH, USA). Feed allocation was calculated on a daily basis and included an excess of 10% of the total feed consumed on the previous day. An average daily intake was calculated for each animal for each week of lactation.

Measurements

Oestrous behaviour was observed during one 30-minute period every 12 h at 0900 and 2100 h for the first 150 d of the breeding period (commencing 30th November 2007). Individual behavioural activities were defined according to a scoring system developed by Van Eerdenburg *et al*. (1996; Table 5.2) involving nine key activities previously illustrated as the main behaviours expressed during oestrus (Van Eerdenburg *et al*., 1996), each allocated an associated number of points (score). After each 30-min observational period, the total number of points scored was calculated. A total score greater than or equal to 50 points during a single period or consecutive observational periods (an aggregate score for a particular cycle) was taken to indicate an animal in oestrus (Van Eerdenburg *et al*., 1996).

Table 5.2. The scoring scale used for observed symptoms of oestrus (Van Eerdenburg *et al*., 1996)

Symptoms of oestrus	Score[1]
Mucous vaginal discharge	3
Cajoling	3
Restlessness	5
Sniffing the vagina of another cow	10
Chin-resting	15
Mounting (or attempting to mount) other cows	35
Mounting headside of another cow	45
Mounted but not standing immobile	10
Standing immobile on being mounted	100

[1]The symptom scores are cumulative.

Milk samples for progesterone analysis were collected on Mondays, Wednesdays and Fridays (all AM) from parturition until 100 d into the breeding period, or until confirmation of pregnancy (whichever was sooner). Milk samples were taken aseptically and to each was added a preservative tablet (Lactab Mark III, Thompson and Cooper Ltd, Runcorn, UK). Samples were stored at 4 ^{o}C until analysis. Milk progesterone concentration was determined using a competitive enzyme–linked immuno-sorbent assay (ELISA) kit (Ridgeway Science Ltd, Gloucestershire, UK). The assay is based on the method of Sauer *et al*. (1986). Pregnancy was confirmed via an ultrasound scan carried out by a veterinarian at approximately d 30 post insemination and by elevated milk progesterone concentrations. Animals on the control, individual cow and low protein treatments were inseminated as required beginning 56 d postcalving. Those on the high-starch / high-fat treatment were inseminated from no earlier than 71 d postcalving to allow at least one oestrous cycle to occur on the high-fat diet before insemination. Breeding commenced no earlier than 56 or 71 d postcalving (according to treatment) and did not occur prior to 30th November to maintain a compact autumn-calving pattern. All inseminations were performed following the first observed oestrus after the

commencement of breeding. All fertility-related events were recorded. Artificial insemination was carried out 12 hours after an observed oestrus by a trained technician.

Progesterone parameter definitions

A full description of the methodology used to interpret and analyze the progesterone data was provided by McCoy *et al*. (2006). Briefly,

- the onset of luteal activity (OLA) is indicated by the first of at least two consecutive progesterone concentrations ≥ 3 ng/ml in whole milk.
- the luteal phase (LP) of an individual oestrous cycle is defined as the period between the first progesterone concentration ≥ 3 ng/ml and the last consecutive milk progesterone concentration ≥ 3 ng/ml in whole milk.
- the inter-ovulatory interval (IOI) is defined as the period between the first progesterone rise (above 3ng/ml) of one cycle to the first progesterone rise (above 3 ng/ml) in the next cycle
- the inter-luteal interval (ILI) is defined as the period between the demise of one corpus luteum and the rise of the next, and is the interval from the first milk progesterone concentration < 3ng/ml to the last consecutive milk progesterone < 3 ng/ml in whole milk.

Abnormal progesterone patterns

Progesterone data was assessed according to Lamming and Darwash (1998) to characterize abnormal progesterone profiles which were:

- delayed ovulation type I (DOV I) was defined as progesterone concentration < 3 ng/ml in whole milk for ≥ 45 d (prolonged ovulation).
- delayed ovulation type II (DOV II) was defined as progesterone concentrations < 3 ng/ml in whole milk for ≥ 12 d after the OLA (prolonged inter-luteal interval).

- persistent corpus luteum type I (PCL I) was defined as progesterone concentrations ≥ 3 ng/ml for ≥ 19 d on the first luteal phase (delayed luteolysis of the corpus luteum during the first oestrous cycle).
- persistent corpus luteum type II (PCL II) was defined as progesterone concentrations ≥ 3 ng/ml for ≥ 19 d on subsequent luteal phases (delayed luteolysis of the corpus luteum during subsequent oestrous cycles).

Several measures of reproductive performance were calculated for each cow. These included conception rate to first, second and combined inseminations. Additionally, 'in-calf' rates at 100 d and at the end of the breeding period, were calculated.

Milk yield (MY) and dry matter intake (DMI) were recorded daily for each cow and average daily MY and DMI were calculated on a weekly basis for each animal. Milk composition, live weight and body condition score (BCS) on a scale from 0 to 5 (Edmundson *et al*., 1989) were recorded weekly. Milk composition (fat, protein and lactose content and somatic cell count) was determined, weekly, in refrigerated preserved samples (Lactab Mark III) collected during two consecutive milkings (AM and PM) from each animal. The a.m and p.m samples were analyzed separately for milk fat, protein and lactose content by infrared milk analyzer (Milkoscan FT 120, Foss UK ltd., Warrington, UK) and milk somatic cell count was determined using a Fossmatic 360 (Foss Electric, Hillerod, Denmark). A weighted milk composition was calculated for each week.

Samples of grass silage and maize silage were analyzed daily for oven dry matter. Weekly samples were subjected to near infrared reflectance spectroscopy (Park *et al*., 1998) for estimation of ME (metabolizable energy) content. Twice weekly, fresh samples of maize silage and grass silage were analyzed for gross energy (Porter, 1992) and pH. The same samples were also analyzed for nitrogen and ammonia nitrogen concentrations as described by Steen (1989), and for lactic acid and volatile fatty acids, ethanol, and propanol concentrations as described by Porter and Murray (2001). Dried silage samples were prepared twice weekly and a composite 2-wk sample was analyzed for neutral-detergent fiber (NDF), acid-detergent fiber (ADF), and ash contents as described by Cushnahan and Gordon (1995). Samples of maize silage were dried at

60 ^{o}C twice weekly and composited 2-wk samples were analyzed for starch using the Megazyme kit; K-TSTA (procedure detailed at http://www.megazyme.com/booklets/KTSTA.pdf). Each batch of concentrate was sampled and composite 2-wk samples were analyzed for oven DM content (at 60^{o}C). Dried samples were analyzed for nitrogen (Steen, 1989), ADF, NDF and ash concentrations as described by Cushnahan and Gordon (1995) and were bulked for starch analysis by Megazyme kit.

Blood samples were collected weekly between 0930 and 1130 h from the coccygeal vein of all cows using heparin-coated evacuated tubes (Becton Dickson, UK) from calving until d 100 of lactation, and then every 2 weeks thereafter. Plasma was recovered and stored at -20 ^{o}C pending urea analysis using an Olympus AU640 autoanalyser (Olympus Life and Materials Science, Hamburg, Germany).

Calculation of energy balance

The average DEB for each animal was calculated for each week of lactation according to Thomas (2004) as below:

DEB = ME intake – ME requirement [-10 + (ME_{preg} + $ME_{maintmilk}$ * $LW^{0.75}$)] + [(0.0013*LW) / Km)] where $ME_{maintmilk}$ is the combined ME requirement for maintenance and milk production and Km (efficiency of energy use for maintenance) = 0.35 x ME / GE + 0.503.

MY, DMI, milk composition, LW and feed composition data were all used in the calculations. Missing values (fewer than 2 percent of all data) were estimated from data for the week prior to, and the week following.

Statistical analysis

All statistical analysis was performed using Genstat (Payne *et al*., 2007). Data were analyzed by a repeated measures approach using the Residual Maximum Likelihood (REML) procedure. The model fitted fixed effects for parity, postcalving treatment, and week of lactation for each parameter and included all second and third- level interactions between these variables. Within

the model, individual animals represented subjects and week of lactation represented time points which were equally spaced and the same for all animals. As the correlations between successive measurements on the same subject were assumed to decrease with time with the interval between successive time points being 1 week, an auto-regressive error correlation model of order 1 was used.

Linear regression analysis was performed to examine the effects of production and metabolic parameters (independent variables) on all reproduction-related variables. The independent variables used in the multiple linear regression analysis were MY, milk energy concentration, daily EB, cumulative EB, average daily EB in weeks 1-3 of lactation, average daily EB in weeks 1-6 of lactation, and the number of weeks to the EB nadir. A logistic regression model was used to analyze CR to first service, CR to second service, CR to first and second services, 100 d in-calf rate, DOV types I and II, PCL types I and II, and whether or not there was more than one abnormal progesterone profile observed. When assessing treatment effects, voluntary waiting period was included as a covariate in the analysis. Animals on the control, individual cow and low protein treatments had a voluntary waiting period of 56 d postcalving whilst animals on the high-starch / high-fat treatment had a voluntary waiting period of 71 d.

Oestrous behaviour data were not normally distributed and therefore a square root transformation was applied prior to analysis to normalise the data.

In total, 16 animals were removed from the study over the duration of the experiment due to ill health, injury or death.

RESULTS

Nutritional composition

The nutritional composition of the diets offered in the current study are presented in Table 5.3. The control diet had a starch content of 177g and a protein content of 185 g CP/kg DM. The starch contents of the basal (0-35 d postpartum), high (35-50 d postpartum) and low starch diets offered as part of the high-starch / high-fat treatment were 177, 273 and 97 g starch/kg DM respectively. The dietary protein contents of the rations offered to animals on the individual cow treatment were 205, 181 and 156 g CP/kg DM for high, medium and low protein rations. The CP content of the low protein diet was 163 g/kg DM and the starch content was 187 g CP/kg DM. Metabolizable energy (ME) contents for the rations were similar across all treatments (12.2-12.5 MJ/kg DM).

Table 5.3. Key nutritional components of the total diet for each nutritional treatment[1]

	Treatment							
		Individual Cow			High-Starch / High-Fat			
	Control	High	Medium	Low	Basal	High	Low	Low Protein
Starch (g/kg of DM)	177.0	136.2	168.7	201.3	177.3	273.7	97.3	187.1
CP (g/kg of DM)	185.7	205.6	181.1	156.6	186.0	165.7	169.6	164.0
ME (MJ/kg of DM)	12.5	12.3	12.3	12.4	12.2	12.5	12.2	12.2
Fat (g/kg DM)	48.9	38.1	53.0	38.1	48.9	38.1	71.8	48.9

[1] the treatment diets refer to the total mixed rations and include any concentrates fed in the parlor

Production performance

Animals allocated to the individual cow treatment had higher average DMI ($P < 0.001$; SED, 0.32), total ME intake ($P < 0.001$; SED, 0.08), grass silage DMI ($P < 0.001$; SED, 0.05) and maize silage DMI ($P < 0.001$; SED, 3.69) compared to those allocated to the other treatments (Table 5.4). Mean concentrates DMI was not different between treatments ($P > 0.05$). Milk yield, milk fat and milk protein concentrations were not affected ($P > 0.05$) by dietary treatment.

Across all treatments, average milk yield was 32.8 kg/d, milk fat was 38.4 g/kg and milk protein was 33.7 g/kg during the first 210 d of lactation. Neither LW nor BCS during the first 210 d of lactation were affected by dietary treatment ($P > 0.05$). A statistically significant ($P = 0.015$; SED 0.675) interaction was observed between stage of lactation and treatment effects for milk protein content, however there were no statistically significant relationships identified for other production variables.

Plasma metabolites

Milk urea concentrations were lower in animals offered the low protein treatment ($P < 0.001$) compared to those offered the control, individual cow, and high-starch / high-fat treatments (133.6 g/kg vs. 164.3, 174.0 and 158.2 g/kg respectively). Mean plasma urea concentrations differed ($P < 0.001$) between the low protein treatment (11.0 mg/dL) and control, individual cow management and high-starch / high-fat treatments (14.1, 14.1 and 14.7 mg/dL respectively).

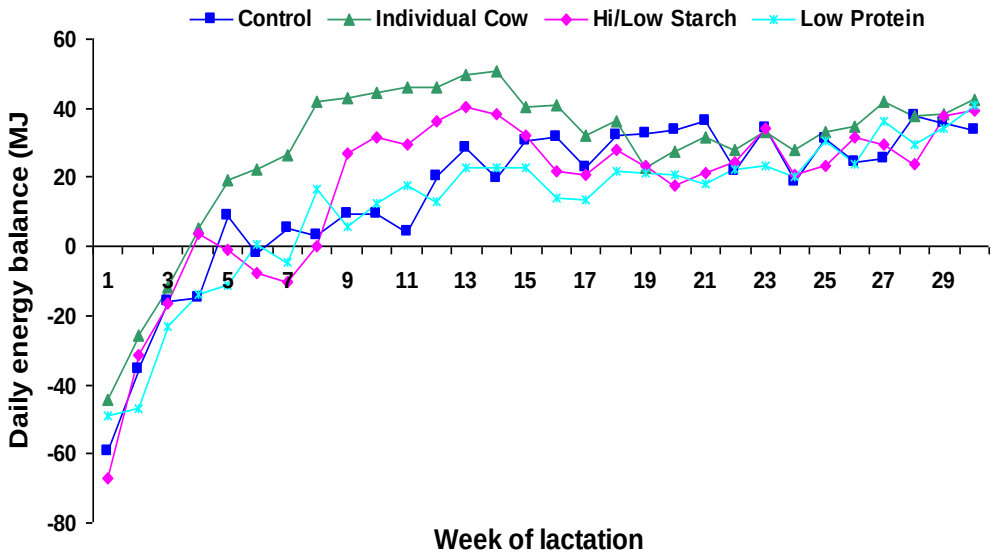


Figure 5.2: Effects of treatments on daily energy balance between calving and 210 days of lactation (P < 0.001, SED 3.62).

Table 5.4. Treatment effects on milk production and energy balance between 1 and 210 d of lactation.

	Treatment					*P* value[1]	
	Control	Individual Cow	High-starch/ High-Fat	Low Protein	SED	Treat	Treat*DIM[2]
Milk yield (kg/d)	32.8	32.7	33.3	32.6	1.14	0.707	0.108
Milk fat (g/kg)	37.7	39.4	38.6	38.1	0.72	0.198	0.494
Milk protein (g/kg)	33.8	33.9	33.6	33.7	0.38	0.984	0.015
Milk Urea (mg/kg)	164	174	158	134	2.9	0.001	0.285
Mean liveweight (kg)	563	557	565	566	15.4	0.861	0.959
DMI (kg/day)	19.7	21.0	19.7	19.3	0.32	0.001	0.752
Concentrate DMI (kg/d)	12.2	12.3	12.2	11.9	0.17	0.458	0.495
Grass silage DMI (kg/d)	4.5	5.3	4.5	4.4	0.82	0.001	0.166
Maize silage DMI (kg/d)	3.0	3.5	3.0	3.0	0.05	0.001	0.166
Total ME (MJ/d)	249	265	251	244	3.7	0.001	0.717
DEB (MJ/d)	18.4	31.7	18.8	13.1	3.62	0.001	0.429

[1] Statistical significance.

[2] Interaction between days in milk (DIM) and treatment effects

Energy status

Overall, mean daily EB during the first 210 d of lactation was higher ($P < 0.001$) in animals allocated to the individual cow treatment (31.7 MJ/d) than in cows offered the control, high-starch / high-fat or low protein treatments (18.5 MJ/d, 18.8 MJ/d and 13.1 MJ/d respectively) (Figure 5.2). Animals allocated the individual cow treatment had established a positive EB by week 4 postpartum, whilst animals on the control, high-starch / high-fat and low protein treatments entered positive EB only in weeks 5, 8 and 8 postpartum respectively. Cumulative EB was higher ($P = 0.001$) in animals allocated to the individual cow treatment (6657 MJ) than in those allocated the control, high-starch / high-fat or low protein treatments (3874 MJ, 3958 MJ and 2740 MJ, respectively).

Fertility parameters

Conception rate (CR) to first or second, or first and second service combined was not affected ($P < 0.05$) by nutritional treatment (Table 5.5) at 30.5% (SED, 9.5), 41.7% (SED, 12.2) and 58.8% (SED, 10.2) respectively. Animals allocated to the individual cow treatment had a lower ($P > 0.05$) CR to first insemination (24%) than did those receiving the control, high-starch / high-fat and low protein diets (33.3, 36.0, and 28.5% respectively). Conversely, the CR to second service of animals allocated to the individual cow treatment was higher ($P > 0.05$) than in those allocated to the other treatments (52.6 vs. 42.9, 31.3 and 40.0% respectively). There was no statistically significant correlation between EB and CR in the current study. Voluntary waiting period had no significant effect on any of the parameters analyzed.

Nutritional treatment had no effect on CR during the first 100 d of the breeding period or throughout the entire 6-month breeding season (both $P > 0.05$; Table 5.5). However, animals offered the individual cow treatment had slightly improved '100 d in-calf' rate and higher CR at the end of the breeding season, though neither of these variables were statistically significant. The '100 d in-calf rate' and overall CR of animals offered the individual cow treatment, the

control treatment, the high-starch / high-fat treatment and the low protein treatments were 79.2 and 95.8%, 71.4 and 85.7%, 68.0 and 88.0 %, and 71.4 and 81.0 % respectively.

Luteal activity characteristics (by milk progesterone concentrations)

Nutritional treatment had no effect ($P > 0.05$) on the interval to the OLA, or on the durations of the luteal phase, inter-ovulatory interval or inter-luteal interval (Table 5.5). However, although not statistically significant, the low protein and high-starch / high-fat treatments had the greatest proportions of animals cycling by 30 d postpartum (81.0% and 73% respectively), whilst the control treatment and individual cow treatment achieved only 54% and 67% respectively. By 40 d postpartum, 85.7 and 84.6% of cows on the low protein and high-starch / high-fat treatments were cycling compared to 75.0 and 77.8% of those on the control and individual cow treatments.

Parity had a statistically significant effect on the duration of the IOI with multiparous animals having a longer IOI than primiparous animals (24.2 vs. 21.3 d; $P < 0.05$). However, parity did not affect ($P > 0.05$) either the interval to OLA, or the duration of the LP or ILI. The LP was shorter ($P < 0.001$) in the first cycle postpartum than in either the second or third cycles (10.9 d vs. 14.2 and 14.8 d) but was unaffected by parity or nutritional treatment (Table 5.6). The IOI and ILI were not different between cycles ($P > 0.05$).

In the first cycle postpartum, the duration of the LP was greater ($P < 0.05$) in animals that did not become pregnant to either the first or second services when compared to animals that became pregnant (12.6 vs. 9.5 d; SED, 1.43). The length of the second cycle postpartum was greater ($P < 0.05$) in animals that did not become pregnant to the first AI postpartum than in those that became pregnant (25.3 vs. 21.3 d, respectively; SED, 2.70). The length of the second cycle postpartum tended to be greater ($P = 0.062$) in animals that were not pregnant by 100 d after the commencement of breeding than in those that were pregnant (26.4 vs. 22.9 d, respectively; SED, 2.62). In the third cycle postpartum, the ILI was greater ($P < 0.05$) in animals that were not pregnant by 100 d after the commencement of breeding than in animals that were pregnant (6.0 vs. 4.6 d, respectively; SED, 0.68). Compared to pregnant animals, those that were not pregnant

by the end of the breeding period had a longer third cycle IOI (26.3 vs. 22.3 d; $P < 0.05$; SED, 3.78) and ILI (5.8 vs. 4.7 d; $P < 0.01$; SED, 1.30).

Table 5.5. Treatment effects on conception rate and oestrous cycle characteristics.

	Treatment					
	Control	Individual Cow	High-starch / High-fat	Low Protein	SED	*P* value[2]
Voluntary waiting period (d)	56	56	71	56	-	-
Interval from calving to conception (d)	134.0	127.9	150.0	142.0	13.5	0.548
Conception rate to 1st insemination (%)	33.3	24.0	36.0	28.5	9.5	0.805
Conception rate to 2nd insemination (%)	42.9	52.6	31.3	40.0	12.2	0.641
Conception rate to 1st and 2nd inseminations (%)	61.9	60.0	56.0	57.1	10.2	0.977
In-calf rate at 100days of the breeding period (%)	71.4	79.2	68.0	71.4	9.3	0.841
In-calf rate at the end of the breeding period (%)	85.7	95.8	88.0	81.0	6.9	0.430
Onset of luteal activity (d)	31.5	26.1	21.7	25.5	6.54	0.648
Proportion of animals cycling by 30 d postpartum	54.2	66.7	73.1	81.0	18.3	0.756
Proportion of animals cycling by 40 d postpartum	75.0	77.8	84.6	85.7	15.4	0.648
Proportion of animals cycling by 50 d postpartum	87.5	81.5	92.3	85.7	12.9	0.715
Luteal phase (d)	12.6	14.1	14.3	12.4	1.21	0.252
Inter-ovulatory interval (d)	22.6	23.9	22.3	22.2	1.74	0.552
Inter-luteal interval (d)	6.2	5.8	5.0	5.8	1.55	0.595
Delayed ovulation type I[1] (%)	12.5	18.5	8.0	17.0	8.58	0.979
Delayed ovulation type II[1] (%)	25.0	15.4	8.0	16.7	8.01	0.170
Persistent corpus luteum type I[1] (%)	4.2	26.9	16.7	17.6	5.61	0.089
Persistent corpus luteum type II[1] (%)	25.0	46.2	44.0	22.2	8.93	0.819
Animals with 1 or > atypical cycles (%)	58.3	81.5	57.6	47.6	18.5	0.281

[1] Percentage of cycles that were atypical

[2] Statistical significance of treatment effects.

Table 5.6. Effects of cycle number postcalving on oestrous cycle parameters

	Cycle number postcalving				
	1	2	3	SED	*P* value[1]
Luteal phase (d)	10.94	14.4	14.78	0.911	0.001
Inter-ovulatory interval (d)	21.07	24.16	23.04	1.725	0.177
Inter-luteal interval (d)	6.08	6.05	5.01	1.169	0.476

[1] Statistical significance of cycle number effects

Atypical progesterone profiles

Across all treatments, 62.2% (SEM 14.0) of animals displayed at least one atypical progesterone profile (Table 5.5). Within this average figure, animals subjected to the individual cow treatment had the highest proportion displaying one or more atypical profiles (81.5%) while 57.6, 58.3, and 47.6% of cows on the high-starch / high-fat, control and low-protein treatments respectively displayed one or more atypical profiles but treatment did not significantly affect the proportion of animals exhibiting at least one atypical cycle.

There was no effect of treatment on the incidences of DOV I or DOV II ($P > 0.05$), which were observed in 12.5 and 16.3% of cycles, respectively, across all nutritional treatments. The incidences of PCL I or PCL II were not affected ($P > 0.05$) by nutritional strategy and occurred in 16.4 and 34.4% of cycles respectively (Table5).

Oestrous behaviour

In the current study, an oestrous detection rate of 85.5% was achieved when recording commenced at the start of breeding and continued to 150 d of lactation. Dietary treatment had no statistically significant effect on the expression of individual oestrous behaviours or the total oestrous score for each animal (Table 5.7). An increase in the size of the sexually active group (the number of animals in oestrus simultaneously) increased ($P < 0.01$) the total oestrous score (163, 356, 307, 381 and 445 for sexually active group sizes of 1, 2, 3, 4 or 5 or more animals respectively; SED, 77.1; Table 5.8). There was no statistically significant effect of nutritional treatment on the size of the sexually active group. The size of the sexually active group had an effect on the incidence of mounting or

attempting to mount other cows ($P < 0.05$; SED, 1.95) and the incidence of standing immobile on being mounted ($P < 0.01$; SED, 2.47). Chin-resting and sniffing the vagina of another cow were expressed most frequently during the observation periods (89.0% and 88.1% respectively). The behaviour of mounting, or attempting to mount, another cow was expressed in 81.6% of all heats. Standing immobile on being mounted was observed in only 47.4% of all heats.

Table 5.7. Treatment effects on the expression of oestrus behaviours and total score

	Treatment				SED	*P* value[1]
	Control	Individual Cow	High-Starch / High-Fat	Low Protein		
Chin-resting	6.575	8.061	7.501	8.226	1.158	0.660
Mounting or attempting to mount another cow	6.661	6.488	8.07	6.984	1.277	0.420
Sniffing the vagina of another cow	5.61	6.483	5.078	5.778	0.8729	0.403
Standing immobile on being mounted	4.485	6.964	7.963	6.183	2.236	0.381
Total Score	241.6	372.7	380.7	325.5	67.91	0.170

[1] Statistical significance of treatment effects.

Table 5.8. Effects of the size of the sexually active group on the expression of oestrous behaviours (following a square root transformation)

Behavioural activity	Size of the sexually active group					SED	*P* value[1]
	1	2	3	4	5+		
Chin-resting	6.563	7.264	6.599	9.383	8.144	1.32	0.086
Mounting (or attempt) other cows	3.993	6.894	8.019	7.255	9.094	1.952	0.023
Sniffing the vagina of other cow	6.509	5.426	5.345	5.826	5.579	0.959	0.406
Standing immobile on being	0.795	8.344	7.408	6.996	8.452	2.467	0.007

mounted							
Total score	162.9	356.0	306.7	380.7	444.5	77.07	0.006

[1] Statistical significance of the effects of the size of the sexually active group.

Table 5.9. Effects of total oestrous score on conception

		Pregnant	Not Pregnant	SED	*P* value[1]
	All	406.4	201.3	39.88	0.001
Parity	Primiparous	329.9	199.6	54.65	0.062
	Multiparous	483.0	203.1		

[1]Statistical significance of total oestrous score effects

A parity x nutritional treatment interaction was observed for standing immobile on being mounted ($P < 0.01$; SED, 1.22). Standing immobile on being mounted was expressed more frequently by primiparous animals in the high-starch / high-fat and low protein treatment groups than in either the control or individual cow management groups. However, standing immobile on being mounted was expressed more frequently in multiparous animals offered the control and individual cow treatments compared to the high-starch / high-fat and low protein treatments.

The total oestrous score was associated with conception success ($P < 0.001$) (Table 5.9) with animals that conceived having a higher total oestrous score than animals that did not conceive (406 vs. 201; SED, 39.9) and there was a tendency for multiparous animals to achieve higher total oestrus scores than primiparous animals ($P = 0.062$).

Logistic regressions were performed on variables including daily EB, cumulative EB and blood metabolite concentrations against conception rates but there were no statistically significant differences.

DISCUSSION

The aim of the current study was to assess the effectiveness of four nutritional strategies in improving the fertility of high-yielding dairy cows.

High-starch / high-fat (treatment 2)

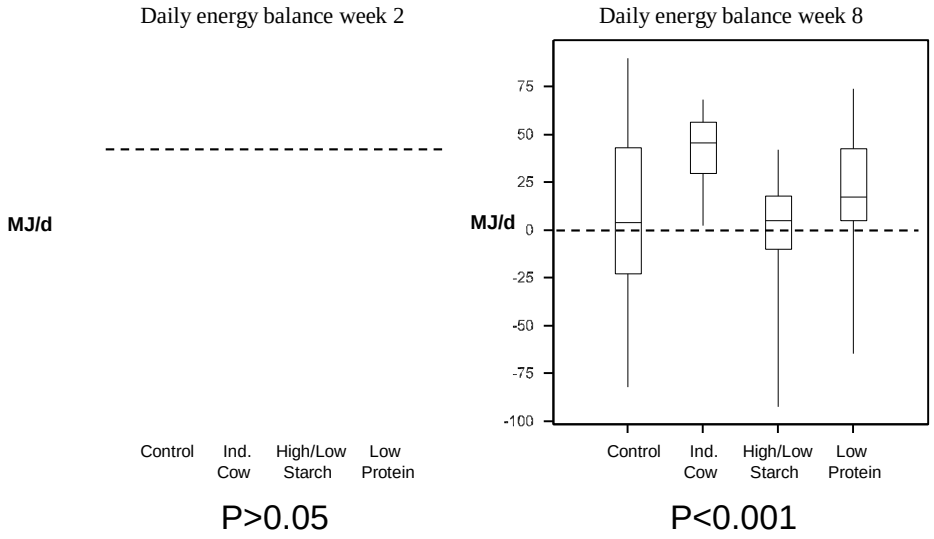
The high-starch/high-fat treatment was designed to influence plasma insulin concentrations such that, in early lactation (calving to 50 d postcalving), the high-starch option would favor elevated insulin, thereby enhancing ovarian follicle development and reducing the interval to OLA. Gong *et al*. (2002) illustrated that including 260 g starch/kg diet DM (high-starch) increased the proportion of dairy cows ovulating by 50 d postpartum and reduced the interval from calving to first ovulation. Van Knegsel *et al*. (2007a) also found that multiparous cows offered a high-starch diet tended to have an earlier OLA than animals offered diets with lower starch contents. However, Garnsworthy *et al*. (2009) reported a delay in OLA in cows offered a high-starch diet (182 g starch/kg DM) compared to those offered a low-starch diet (98 g starch/kg DM) although, in this study, the time difference was not statistically significant. In the current study, the low-protein and high-starch/high-fat treatments tended to have the greatest proportions of cows cycling by 30 d postpartum (0.81 and 0.73 respectively). In the current study also, the average interval to OLA was 33 d in animals offered the high-starch/high-fat treatment, which is comparable to the values of 31.6 d reported for multiparous cows fed high-starch diets by Garnsworthy *et al*. (2009). Van Knegsel *et al*. (2007a) reported an interval to OLA of 20.4 and 31.7 d in multiparous and primiparous cows, respectively while Gong *et al*. (2002) reported an interval to OLA of 41.4 and 27.5 d in cows of high genetic merit and low genetic merit respectively.

High plasma insulin levels are detrimental to oocyte quality in cows (Fouldi-Nashta *et al*., 2005) and heifers (Adamiak *et al*., 2005, 2006), explaining the reason for the switch from a high-starch (insulinogenic) formulation to a low-starch (non-insulinogenic) formulation within treatment 2. The low-starch formulation was made 'lipogenic' by supplementation with 750 g/d of protected fat in an effort to further encourage follicular development and corpus luteum formation between d 50 to 120 of lactation, as suggested by Gong *et al*. (2002). A reduction in oocyte quality will suppress the potential for oocyte development and reduce the blastocyst count post-fertilization (Fouladi-Nashta *et al.,* 2005). Recently, Garnsworthy *et al*. (2009) reported higher pregnancy rates by d 120 postpartum in cows offered a high-starch diet until the time of the first rise in milk progesterone and then switched to a low-starch diet than in cows offered a low-starch diet

followed by a high-starch diet, or a high-starch diet throughout, or a low-starch diet throughout. In the current study, the high-starch/high-fat treatment had no effect on conception rate to first service, or '100 d in-calf rate' or the incidence of atypical progesterone profiles. One explanation for the different results between studies could be that a high-starch diet was continued after the OLA in the current experiment which may have had detrimental effects on the potential for oocyte development post insemination.

In the current study there was no statistically significant effect of treatment on conception rate to first service when data from multiparous animals were analyzed separately. However, there was a tendency for multiparous animals on the high-starch/high-fat treatment to have a higher conception rate to first service (52.9%) relative to controls (25.0%). This was greater also than the 38% conception rate to first service observed by Garnsworthy *et al*. (2009) for a similar experimental treatment. In the current study, treatment groups consisted of 27 cows. Due to reasons unrelated to experimental procedures, 16 animals were removed at various stages during the study. Using the calculations of Morris (2002) for the analysis of continuous data with a normal distribution, and assuming a co-efficient of variation of 17%, 26 animals per treatment would be required to detect a significant difference of 12% between treatments (at the 95% confidence level). However, for data with a binomial distribution, the assessment of treatment effects would have required 267 animals per treatment in order to detect a 12% difference in CR at the 95% confidence level and with an experimental power of 0.8 (Thrusfield, 2005). Such numbers of cows were beyond the resources available for the current study.

Figure 5.3. Average daily energy balances during week 2 and week 8 postpartum



Individual cow management (treatment 3)

In the individual cow treatment, the variation in EB within treatment was significantly decreased between wk 2 and wk 8 postcalving (Figure 5.3). In wk 2 postpartum, there was no difference in average DEB between diets, however, by wk 8 postpartum, all animals on this treatment had reached positive DEB and the degree of variation in DEB within the treatment was much less than that in other treatments resulting in a more energetically uniform group of animals.

Overall, animals on the control treatment had the greatest variation in EB. Although the effect was not statistically significant, cows on the individual cow treatment tended to have better reproductive performance (conception rate to 1st and 2nd service and 100d in-calf rate) which may have been due to a higher proportion of animals being in positive EB earlier in lactation. It is hypothesized that the developmental and steriodogenic competence of follicles is reduced by exposure to a metabolically challenging period such as that presented by NEB during the long period of follicular growth (Britt, 1992; Roth *et al*., 2001). Furthermore, the oocytes within such follicles, which ovulate 60-80 d later, may be inferior (Leroy *et al*., 2009). However, animals on the individual cow treatment tended to have a lower conception rate to first insemination despite their generally improved energy status. The observed tendency for an improved conception rate to second insemination in animals on the individual cow treatment may be a reflection of

successful ovulation of a good quality oocyte which has been recruited during a period of increasing EB. Animals allocated to the individual cow treatment attained a positive EB at wk 4 postpartum, whereas those allocated to the control, high-starch / high-fat and low-protein treatments attained a positive EB in wk 5, wk 8 and wk 8 respectively. Beam and Butler (1997, 1999) suggested that follicles emerging after, rather than before, the EB nadir are more likely to ovulate because of the increase in LH pulse frequency which is evident after the EB nadir (Diskin *et al*., 2003). Despite the apparent advantages and benefits, it is acknowledged that managing cows on an individual basis in order to improve EB may prove difficult to implement in practice on-farm.

Onset of luteal activity

There were no statistically significant treatment effects on the interval to OLA in the current study. However, the mean interval (35.3 d) was longer than previously reported: 27.0 d by Darwash *et al*. (1997); 27.9 d by Royal *et al*. (2000); 30.1 d by McCoy *et al*. (2006) and 32.2 d by Law *et al*. (2009c). This chronological progression suggests that the interval to OLA is continuously increasing. Increases in time to OLA may reflect the inability of the ovulatory follicle to produce sufficient estradiol to facilitate ovulation in the early postpartum period and may be related to the degree of negative DEB (as previously stated). Short intervals from calving to OLA increase the potential number of oestrous cycles preceding insemination and improves conception rate (Butler, 2003). Conception rate to first service in the current study (mean 30.5%) was lower than reported previously. Lamming and Darwash (1998), found a CR of 60.9% in cows with normal progesterone profiles and 43.7% in those with atypical profiles. Royal *et al*. (2000) reported a CR of 39.7% while Mayne *et al*. (2002) reported a value of 37.1%. The current value was close to that of 30.7% reported by Law *et al*. (2009c). Trends observed between 1998 and 2009 suggest a continuation in the decline in conception rate to first service of 1% per year as calculated by Royal *et al*. (2000).

Progesterone profiles

In the current study, 62.2% of cows exhibited at least one atypical progesterone profile. This figure is higher than that (42.1%) reported by McCoy *et al*. (2006) but similar to the figure of 62% reported by Law *et al*. (2009c) Similarly, Royal *et al*. (2000) reported that the proportion of cows exhibiting at least one atypical cycle had increased from 31.7% to 43.7% between two databases collated for the periods 1975-1982 and 1995-1998 respectively. Delayed ovulation occurred in 28% of all cycles as reflected in a prolonged ILI (Royal *et al*., 2000). In the current study, the incidence of DOV I (12.5%) was lower than reported in many recent studies but within the range of values reported since 1998 e.g. 11% by Lamming and Darwash (1998); 12.9% by Royal *et al*. (2000); 17.4% by McCoy *et al*. (2006) and 20.7% by Law *et al*. (2009c). The incidence of DOV II (16.3%) was comparable to previously published values of 13% by Lamming and Darwash (1998), 16% by Royal *et al*. (2000) and 28% by Law *et al*. (2009c). Delayed ovulation may be caused by a delay to, or failure of, the surge in circulating concentrations of luteinizing hormone (LH) which causes thinning and subsequent rupture of the follicle wall, leading to ovulation. In their review, Peter *et al*. (2009) attributed DOV II to the insensitivity of the hypothalamus to positive feedback by estradiol or to altered follicular responsiveness to gonadotropic support, mediated via metabolic hormones such as IGF-1 and insulin (Beam and Butler, 1999). Delayed ovulation type II may occur also where there is incomplete luteolysis of the corpus luteum from the previous cycle or where there is a continuing luteinized ovarian structure (Lee *et al*., 1988; Sirois and Fortune, 1990; Silvia *et al*., 2002; Sartori *et al*., 2004).

The incidence of PCL I in the current study was 16.4% which is comparable to values of 16.0, 19.0 and 19.0% from Taylor *et al*. (2003), McCoy *et al*. (2006) and Law *et al*. (2009c) respectively. The incidence of PCL II (34.4%) was unexpectedly high when compared to previously published work which showed values of 6.35% (Lamming and Darwash, 1998), 6.4% and 16.8% in 1975-1982 and 1995-1998 respectively (Royal *et al*., 2000), 11% (Taylor *et al*., 2003), 11.9% (McCoy *et al*., 2006) and 20.7% (Law *et al*., 2009).

Persistent corpora lutea are mostly associated with three occurrences: 1) when there is embryonic loss following maternal recognition of pregnancy; 2) when there is delayed luteolysis in the absence of pregnancy and 3) when an estradiol-producing dominant follicle is absent at the time

of luteal regression (Peter *et al*., 2009). Delayed luteolysis may occur as a result of a sub-optimal uterine environment that disrupts normal hormonal and luteolytic mechanisms (Kindhal *et al*., 1999; Sheldon *et al*. 2006). Sheldon *et al*. (2006) illustrated that uterine infection can increase PGE_2 as a result of a stimulatory effect of lipposaccharides found on the uterine pathogen *Escherichia coli*. Elevated PGE_2 has a luteotrophic effect, which increases progesterone secretion and subsequently prolongs the luteal phase. Under normal, optimal uterine conditions, prostaglandin F2α from the uterus acts on the corpus luteum and causes it to regress and cease progesterone production, removing the inhibitory effect on LH release and allowing the next ovulation to occur (Law *et al*., 2009c). The absence of an estradiol-producing dominant follicle at the time of luteal regression results in the suppression of the pulsatile release of PGF2α and, subsequently, luteolysis (Thatcher *et al*., 1989; McCracken *et al*., 1999; Knickerbocker *et al*., 1986).

Extended IOI is evident in animals with cycles exhibiting PCL II and/or DOV II. In the current study, there were no statistically significant treatment effects on the incidence of IOI and the overall mean was 22.7 d. However, the mean IOI in animals allocated to the individual cow management treatment was 23.9 d. According to Royal *et al*. (2000), IOIs outside the range 19-23 d are associated with reduced fertility. In the current study, 36% of cycles had an IOI greater 23 d while 27% were shorter than 19 d.

In the current study, cows failing to conceive to first or second inseminations had a longer luteal phase in the first cycle postpartum. The luteal phase is generally shorter in the first cycle than in subsequent cycles due to the premature release of prostaglandin F2α (Peter *et al*., 1989) in response to the increased oestradiol produced from formation of the post-ovulatory dominant follicle (Crowe, 2008). Furthermore, animals failing to conceive to the first insemination and/or not pregnant by 100 d after commencement of breeding, had a longer IOI in the second cycle postpartum and a longer ILI in the third cycle postpartum. Using the IOI limits set by Royal *et al*. (2000), 63% of the cycles in the current study were associated with reduced fertility. In concurrence, the results from the current study confirm that irregularities in oestrous cycles have detrimental effects on fertility.

The expression and detection of oestrous behaviour

Efficient oestrous detection is essential to increasing the reproductive performance of dairy cows. In the current study, treatments had no statistically significant effect on behavioural expression during oestrous or on the total oestrous score calculated for successive observations. The most commonly expressed oestrous behaviours were chin-resting (89.0%), sniffing the vagina of another cow (88.1%) and mounting or attempting to mount another cow (81.6%) (Table 5.10). The most reliable oestrous behaviours when expressed were 'standing immobile upon being mounted by another cow' (100.0%), 'mounting or attempting to mount another cow' (87.6%), chin-resting (85.2%) and 'sniffing the vagina of another cow' (84.6%).

Table 5.10. Dependability of oestrous behaviours

	Sniffing	Chin-resting	Mounting	Standing Heat
Reliability[1]	84.6	85.2	87.6	100.0
Percentage expression[2]	88.1	89.0	81.6	47.4
Dependability[3]	7456	7587	7149	4736

[1] Percentage of cows that expressed this behaviour and were judged to be in oestrus according to their milk progesterone concentration profile.

[2] Percentage of oestrous cycles in which behaviour was expressed.

[3] Function; reliability x percentage expression.

Van Vliet and Van Eerdenburg (1996) conducted 30 minutes of observation of cows every 2 h and found that only 37% of oestrous cycles were characterized by standing immobile on being mounted. Using the same protocol, Lyimo *et al*. (2000) observed that 53% of oestrous cycles were characterized by standing immobile on being mounted. Standing immobile on being mounted was expressed in 47.4% of cycles in the current study when observed twice daily for 30 minutes. This is lower than previously reported (51.7%) by Law *et al.* (2009b) who also observed animals twice daily for 30 minutes. Standing immobile on being mounted has long been recognized as the primary and most reliable sign of oestrous and the best indicator of the cow's preovulatory state (Hafez *et al*., 1969). However, the expression of standing immobile on being mounted appears to have decreased in the modern high yielding dairy cow and may no

longer be a dependable behaviour in defining oestrous on modern dairy herds where labor and time resources are limiting (Law *et al*., 2009b). In the current study, dependability was calculated according to Law *et al*. (2009b). Our data suggest that, the most dependable behaviours as indicators of oestrous are chin-resting and sniffing the vagina of another cow, while standing immobile on being mounted was ranked only 4th in terms of dependable behaviour.

The number of animals in oestrus simultaneously (the sexually active group) is known to affect the expression of oestrus behaviour (Van Eerdenburg *et al*., 1996; Law *et al*., 2009b). In the current study, as the size of the sexually active group increased, the expression of mounting or attempting to mount another cow, and the total oestrous score, also increased. The behaviour of standing immobile on being mounted was expressed significantly more when two or more animals were in oestrus simultaneously. Roelofs *et al*. (2005) found that chin-resting and mounting or attempting to mount another cow was expressed less frequently when one animal was in oestrus compared to when two or more animals were in oestrus simultaneously.

The expression of oestrus is positively correlated with, and is largely controlled by, plasma estradiol concentration (Lyimo *et al*., 2000). Larger pre-ovulatory follicles have been associated with higher estradiol concentrations which potentially increase the intensity of oestrous expression (Lyimo *et al*., 2000). However, Lopez *et al*. (2004) reported that high-yielding dairy cows had lower estradiol concentrations compared to lower yielding dairy cows despite having larger preovulatory follicles. All animals in the current study were high-yielding dairy cows and ovarian activity was not measured. However, animals that became pregnant had a significantly higher total oestrous score in the cycle of conception and may be a consequence of increased estradiol concentrations and the subsequent LH surge, inducing ovulation which may have been delayed in animals that failed to become pregnant. Parity had no statistically significant effect on the total oestrous score, but multiparous cows tended to achieve higher total oestrous scores than did primiparous cows.

CONCLUSION

None of the nutritional regimes or interventions applied in the current study had a statistically significant effect on the reproductive performance of the high-yielding Holstein-Friesian dairy cows. However, with larger numbers, significance may have been achieved and verified the observed trends; improved ovarian activity in animals offered the high/low starch diet and improved energy balance and subsequent fertility in cows managed on an individual basis. Managing the nutrition of cows so as to adjust individual milk yields resulted in a more energetically uniform group of cows at the commencement of breeding. The results reinforce the need for attention to detail in detecting oestrus in high-yielding dairy cows as standing heat (the most commonly used pre-ovulatory indicator) was expressed in only 47.4% of the oestrous cycles. Secondary behaviours such as sniffing the vagina and chin-resting were more frequently expressed and we suggest that these behaviours should be incorporated into heat detection protocols. The findings from this study re-emphasize the complex and multifactorial nature of the reproductive cycle in modern high-yielding Holstein-Friesian cows.

CHAPTER 6: The effect of offering additional concentrates from either week-2, 6 or 10 of lactation on the performance of Holstein Friesian dairy cows

INTRODUCTION

Until recently, genetic selection programmes within most Holstein-Friesian populations focused largely on milk production traits, resulting in cows with a high capacity for milk volume output. However, the increased milk production potential of these higher yielding cows can be attributed in part to their increased ability to mobilise fat and muscle to support milk synthesis, especially during early lactation (Wathes *et al*., 2007). This increased mobilisation of tissue reserves reflects the fact that these cows often experience severe and prolonged periods of negative energy balance, with Patton *et al*. (2007) having observed higher yielding cows to be genetically predisposed to increased negative energy balance. Negative energy balance, and the associated mobilisation of body reserves, is associated with changes in blood metabolite and hormone profiles which may in turn have a detrimental effect on health and fertility (Pryce *et al*., 2001). Indeed, work by many authors, including Goff and Horst (1998), have shown that a high proportion of health disorders occur during the period immediately pre and post calving, a reflection of the significant metabolic and physiological stress which occurs at this time.

In order to sustain these higher milk outputs in early lactation, and replenish mobilised body reserves in mid and late lactation, increased nutrient intakes are required (Ferris *et al*., 2001). One option by which this can be achieved is to increase concentrate feed levels, and this will increase both the nutrient density of the diet and total dry matter intakes. However, when diets which contain high concentrate proportions are offered in early lactation, the extra concentrates may actually increase milk yield at a time when intakes have not yet reached their peak, thus increasing tissue mobilisation and negative energy balance. In addition, offering diets containing a high proportion of concentrates in early lactation can increase the risk of rumen disorders, which can have a long term negative impact on performance. Alternatively, delaying concentrate inclusion in the diet until after this critical period my delay the rise to peak milk yield, allow feed intakes to increase in synchrony with milk yield and reduce the extent of

negative energy balance in early lactation. However, the impact on cow performance of delaying concentrate introduction to the diet needs to be considered as concentrates are the most expensive component of the diet of dairy cows, and an economic response must be achieved.

The effect of offering additional concentrates at different stages of lactation was examined by Broster *et al*. (1969). Offering diets containing higher levels of concentrates increased milk yield and milk solid yield in early lactation, while a lower response to additional concentrates was achieved in mid and late lactation. However the primiparous cows involved in this experiment were low yielding and physiologically very different from those on most farms today. Nevertheless, the impact of delaying concentrate inclusion in the diet does not appear to have been examined previously with higher yielding Holstein-Friesian cows. Thus the objective of the current study was to examine the effects on food intake, milk production, body tissue reserves and fertility parameters of introducing additional concentrates into the diet of dairy cows at day 14 (pre peak-yield but at the time of maximum lipid mobilisation and milk yield acceleration), at day 42 (peak-yield) and at day 70 (after peak milk yield and lipid mobilisation) of lactation.

MATERIALS AND METHODS

Cows

Eighty winter calving Holstein-Friesian dairy cows (40 primiparous and 40 multiparous (mean parity of 2.4)) were used to examine the effect on cow performance of introducing additional concentrates into the diet at various time points in early lactation. Cows had a mean Predicted Transmitting Ability, expressed on a 2000 year basis (PTA_{2000}) for milk yield and fat plus protein yield of 116 kg and 16.2 kg, respectively, and had a mean calving date of 12 November (s.d, 24 days).

Treatments and diets

Cows were moved to a free stall cow house within 24 hours of calving, and were managed as a single group for the duration of the experiment (28 weeks). Post calving, and until the end of the experiment, all cows were offered a common basal diet comprising proportionally 0.5 concentrate and 0.5 forage on a dry matter (DM) basis. The forage component of the diet comprised proportionally 0.5 grass silage and 0.5 maize silage (DM basis). The ingredient composition of the concentrate component of the basal diet (g/kg, air dry basis) was as follows: barley 155 g, wheat 130 g, citrus pulp 130 g, sugar beet pulp 130 g, soya bean meal (Hi Pro) 310 g, rape meal 40 g, Megalac 20 g, minerals and vitamins 30 g and molasses 30 g. The grass silage component of the diet was produced from primary growth and primary re-growth herbages, harvested from predominantly perennial ryegrass-based swards using a precision chop forage harvester. The maize silage offered (Justina) was harvested between 24 October and 4 November. In addition, all cows were offered 1.0 kg/day of a second concentrate in the milking parlour, with this divided equally between morning and evening milking. The ingredient composition of this concentrate (g/kg, air dry basis) was as follows: barley 200 g, wheat 200 g, citrus pulp 160 g, sugar beet pulp 160 g, soya bean meal (Hi Pro) 150 g, rape meal 50 g, Megalac 20 g, minerals and vitamins 30 g and molasses 30 g.

Cows were allocated to one of four dietary treatments (Con, S2, S6 or S10) at calving (primiparous and multiparous cows allocated separately) with treatment groups balanced for live weight and condition score two weeks pre-calving, genetic merit, and in the case of multiparous cows, for parity. With treatments S2, S6 and S10, an additional 4.0 kg/day of 'parlour concentrate' was introduced into the diet of these cows at 2, 6 or 10 weeks post calving (day 14, 42 or 70) respectively, with these additional concentrates being offered thereafter until the end of the experiment. Cows on Treatment Con continued to be offered the basal diet plus 1.0 kg 'parlour concentrate' throughout the experiment.

The basal diet was offered in the form of a 'mixed ration', and this was prepared and offered daily between 10.00 and 11.00 hours. The concentrate, grass silage and maize silage components of the diet were placed in a complete diet mixer wagon and mixed for approximately ten minutes before being deposited into a series of food boxes. Cows accessed the food in these boxes via a series of Calan gates linked to an automatic cow identification system which

permitted automated recording of individual food intakes (Forbes *et al*., 1986). Cows on each treatment accessed food through gates which were specific to cows on that treatment, with an average of three cows sharing each gate. The basal diet was offered *ad libitum* throughout the study and had a target excess of 10% which was removed twice weekly.

Measurements

Cows were milked twice daily at approximately 07.30 and 18.30, with milk yields recorded automatically at each milking. Milk yields were measured daily and the average daily milk yield calculated for each week of lactation. Milk samples were collected weekly, during two consecutive milkings, and analysed for fat, protein and lactose concentrations and somatic cell count, using an infrared milk analyser (Milkoscan 605, Foss Electric, Hillerød, Denmark), and a weighted composition calculated for each 24-hour sampling period. In addition, milk samples were collected twice weekly for progesterone analysis until pregnancy was confirmed. Milk progesterone concentrations were determined using an enzyme-linked immune-sorbent assay (ELISA) kit (Ridgeway Science Ltd. Gloucestershire) based on the method of Sauer *et al*. (1986), as described in detail by McCoy *et al*. (2006). Interval to commencement of luteal activity (CLA) was defined as the interval from calving to the first of at least two consecutive increases in milk progesterone concentration of >3.0 ng/ml (Darwash *et al*., 1997).

Food intakes for each individual cow were measured daily throughout the experiment using the Calan Gate system described earlier, and a mean weekly intake calculated. Cow live weight (kg) was measured twice daily using an automatic weighbridge at the parlour exit while body condition score was recorded weekly on a scale of 1 (thin) to 5 (fat), as described by (Edmondson *et al*., 1989).

Fortnightly throughout the experiment, blood samples were collected from the coccygeal vein between 0930 and 1130 hours, using heparin-coated or fluoride oxalate (glucose) tubes (Becton Dickinson, Oxford, UK). Plasma was recovered by centrifugation and stored at -20°C prior to being analysed for beta-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA) and glucose (fluoride oxalate tubes) by a clinical analyser (Olympus UK Ltd, Middlesex, UK). Non-esterified fatty acid concentrations were determined using a standard kit (Wako Chemicals

GmbH, Neuss, Germany). The breeding season commenced during the first week of December and finished in early June. Cows were bred by artificial insemination throughout. No cow was bred before day 40 post-calving, while no cow was treated with fertility drugs before day 40 post-calving. The exception to this was cows with uterine discharges/infection, in which case treatment was given as soon as the problem was identified. Cows which had not been seen cycling by day 50 post-calving were examined by a veterinary surgeon, and treated as appropriate.

Silages (grass and maize) were sampled daily and analysed for oven dry matter (ODM) concentrations, with dried samples bulked for each two-week period and analysed for ash, neutral detergent fibre (NDF) and acid detergent fibre (ADF), as described by Cushnahan and Gordon (1995). In addition, fresh samples were taken weekly and analysed using Near Infrared Reflectance Spectroscopy (NIRS) as described by Park *et al*. (1998). Concentrates offered were sampled fortnightly during the experiment and analysed for DM, ash, ADF, NDF and N concentrations.

Three multiparous cows (two due to mastitis and one due to digestive problems) and two primiparous cows (one due to incomplete milk let-down and one due to fertility problems) were removed from the experiment, and their data excluded from the analysis.

Statistical analysis

Data were analysed using Genstat, Version 12.1 (Payne *et al*., 2008), with data for primiparous and multiparous cows analysed separately. Cow performance data (intakes, milk production, condition score, live weight and blood profiles) were analysed by ANOVA. In the case of milk output data for weeks 3 – 28 of lactation (total milk output, daily milk yield, daily fat + protein yield, daily milk energy output), the respective values recorded during week 2 of lactation were used as a covariate within the analysis. Similarly, milk fat, protein and lactose concentrations during week 2 of lactation were used as covariates when analysing milk composition data for weeks 3 – 28 of lactation. Live weight and condition score data during week 2 of lactation were used as a covariate when analysing live weight and condition scores at week 28 post calving and

the mean live weight during the experimental period. Continuous fertility data were analysed by ANOVA, while binomial data were analysed by logistic regression using a generalised linear model analysis.

Table 6.1 Chemical composition of the grass silage, maize silage and concentrates offered during the experiment (g/kg DM, unless stated otherwise)

	Forage		Concentrate	
	Grass silage	Maize silage	Concentrate offered in basal diet	Concentrate offered in parlour
Oven dry matter (g/kg)			871	868
Volatile corrected dry matter (g/kg)	183	294		
Crude protein	144	75	232	178
Ammonia-N (% of total N)	8.5	7.8		
pH	4.2	3.7		
Lactic acid	26.6	41.6		
Acetic acid	44.1	31.2		
Acid detergent fibre	350	259	105	100
Neutral detergent fibre	605	485	200	200
Ash	96	40	113	86
Gross energy (MJ/kg DM)	19.0	19.1		
Metabolisable energy (MJ/kg DM)	11.0	11.1	13.2	13.2
Oil			35.5	35.0

In order to examine the response to the additional 4.0 kg concentrate being included in the diet at weeks 2, 6 and 10 post calving, cow performance during the periods after additional concentrates were included in the diet with each of S2, S6 and S10 was compared with the performance of cows offered the Control treatment diet. This was initially undertaken for total concentrate DM

intake, total DM intake, total milk output and total fat + protein yield for weeks 1-7, weeks 8-14 and weeks 1-14 after additional concentrates were included, and also during each two-week period, until week 14, after additional concentrates were included in the diet. In each of these analyses the appropriate cow performance data recorded during the week prior to additional concentrates being offered (weeks 2, 6 and 10 for treatments S2, S6 or S10, respectively) were used as covariates within the analysis, together with the appropriate data from weeks 2, 6 or 10 in the case of the Control. In the case of the data for each two-week period, a repeated measures analysis was not used with data from each time interval analysed separately.

Fortnightly data for DMI, daily milk yield, daily fat + protein yield and live weight were subsequently plotted, with the Control line plotted being that derived from the Control vs S2 comparison. However in reality, due to the introduction of a different covariate at weeks 2, 6 or 10, the Control line differs within each treatment comparison. From a presentational point of view, the response lines for treatments S6 and S10 have been extrapolated back to the Control line at weeks 6 and 10.

RESULTS

The grass silage offered during the experiment had an average DM (volatile corrected), CP and ME content of 183 g/kg, 144 g/kg DM and 11.0 MJ/kg DM, respectively (Table 6.1). Similarly, the maize silage had an average DM (volatile corrected), CP and ME content of 294 g/kg, 75 g/kg DM and 11.1 MJ/kg DM, respectively. Concentrates offered within the basal diet and in parlour had a CP content of 232 and 178 g/kg DM, respectively.

Mean daily intakes of grass silage and maize silage (Table 6.2) during weeks 3 – 28 of lactation were unaffected by treatment ($P<0.05$), while concentrate intakes were higher with treatments S2, S6 and S10, compared to the Control treatment ($P<0.001$ for both primiparous and multiparous cows). Total DM intakes (kg/day) of both primiparous and multiparous cows were significantly lower with the Control treatment compared to treatments S2, S6 or S10 ($P<0.001$ and $P=0.038$, respectively, Table 6.2). Treatment had no effect on daily milk yield (kg/day), daily fat + protein yield or milk composition (fat, protein or milk lactose) during the

experimental period (P>0.05, Table 6.2) for either primiparous or multiparous cows. Across the four treatments, average daily milk yields for primiparous and multiparous cows were 29.2 kg/day and 36.4 kg/day, respectively, while the equivalent values were 2.1 and 2.5 kg/day for fat plus protein yields.

With primiparous cows, none of the live weight or condition score parameters were affected by treatment (P>0.05, Table 6.3). However multiparous cows on treatments S6 and S10 had a higher mean live weight (P=0.038) and live weight at week 28 (P=0.047) of lactation compared to cows on treatments Control and S2. Treatment had no significant effect on mean concentrations of non-esterified fatty acid (NEFA) or beta-hydroxybutyrate (BHB) during the experimental period for either primiparous or multiparous cows.

A number of approaches were used to examine the response to the inclusion of additional concentrates in the diet. Differences in performance (total DM intake, milk yield, fat + protein yield and live weight) of cows offered the Control treatment and each of S2, S6 and S10 was compared during each two-week period following the inclusion of additional concentrates in the diet (up until week 14). For primiparous cows, total DMI with each of S2, S6 and S10 was significantly higher than for the Control treatment during a number of two-week periods during the first 14 weeks after additional concentrates were offered (Table 6.4, Figure 6.1). However, no such effect was observed for milk yield (Figure 2), milk fat + protein yield (Figure 6.3) or live weight (Figure 6.4). In the case of the multiparous cows (Table 6.5), differences in intakes tended to be largely restricted to weeks 7 – 14 after additional concentrates were included with treatments S2, S6 and S10. With milk yield and fat plus protein yield, differences were largely restricted to the S2 vs Control comparison, with significant differences between these two treatments being observed during the 14-week post supplementation period.

Table 6.2 Effect of offering additional concentrates at weeks 2, 6 or 10 post calving on the food intake and milk production performance of primiparous and multiparous dairy cows during weeks 3 - 28 of lactation

	Treatment					
	Control	S2	S6	S10	SEM	P value
Primiparous DMI (kg/day)						
Grass silage	3.4	3.3	3.4	3.5	0.11	0.473
Maize silage	3.4	3.3	3.3	3.5	0.11	0.466
Concentrate	8.0	10.6	10.0	10.0	0.21	<0.001
Total	14.8	17.2	16.6	17.1	0.32	<0.001
Daily milk yield (kg)	28.7	29.7	29.4	29.0	0.86	0.865
Milk fat (g/kg)	39.1	39.3	41.3	42.7	1.56	0.308
Milk protein (g/kg)	33.0	33.1	32.7	33.3	0.59	0.903
Milk lactose (g/kg)	50.4	50.0	50.5	50.1	0.32	0.632
Daily fat plus protein yield (kg)	2.1	2.13	2.14	2.19	0.05	0.328
Daily milk energy output (MJ)	90.0	93.1	94.2	95.0	2.24	0.443
Multiparous DMI (kg/day)						
Grass silage	4.6	4.2	4.3	4.3	0.12	0.216
Maize silage	4.6	4.3	4.3	4.3	0.12	0.252
Concentrate	10.0	12.6	12.2	11.7	0.23	<0.001
Total	19.2	21.0	20.8	20.3	0.46	0.038
Milk yield (kg/day)	34.8	37.1	36.7	36.8	0.98	0.34
Milk fat (g/kg)	37.0	38.3	37.2	36.6	1.17	0.785
Milk protein (g/kg)	32.4	32.6	33.1	32.0	0.57	0.599
Milk lactose (g/kg)	50.0	48.9	49.4	49.0	0.37	0.196
Fat plus protein yield (kg/day)	2.39	2.61	2.56	2.51	0.07	0.176
Milk energy output (MJ/day)	105.8	114.5	112.1	110.7	2.96	0.227

Table 6.3 Effect of offering additional concentrates at weeks 2, 6 or 10 post calving on live weight, condition score and blood metabolites of primiparous and multiparous dairy cows during weeks 3 - 28 of lactation

	Treatment					
	Control	S2	S6	S10	SEM	P value
Primiparous						
Live weight (kg)						
At week-2 post calving	514	514	514	514		
At week-28 post calving	534	555	531	530	10.0	0.282
Mean (weeks 1-28)	512	522	512	509	5.6	0.343
Nadir	482	497	486	485	5.1	0.205
Loss to nadir	40	24	34	35	6.0	0.279
Days to nadir live weight	48	32	43	42	6.6	0.394
Condition score						
At week-2 post calving	2.7	2.7	2.7	2.7		
At week-28 post calving	2.5	2.7	2.5	2.5	0.05	0.403
Mean (weeks 2-28)	2.5	2.6	2.5	2.5	0.03	0.549
Blood metabolites (mean : weeks 2-28)						
Non-esterified fatty acid (meq/l)	0.31	0.25	0.29	0.29	0.02	0.221
Beta-hydroxy butyrate (mmol/l)	0.49	0.48	0.52	0.55	0.03	0.556
Multiparous						
Live weight (kg)						
At week-2 post calving	620	620	620	620		
At week-28 post calving	621	624	656	657	11.5	0.047
Mean (weeks 1-28)	614	613	636	632	6.7	0.038
Nadir	595	596	602	598	5.8	0.821
Loss to nadir	28	32	25	24	6.5	0.809
Days to nadir live weight	33	59	38	40	10.6	0.344
Condition score						
At week-2 post calving	2.5	2.5	2.5	2.5		
At week-28 post calving	2.5	2.6	2.7	2.8	0.09	0.286
Mean (weeks 2-28)	2.4	2.6	2.5	2.5	0.06	0.218
Blood metabolites (mean : weeks 2-28)						
Non-esterified fatty acid (meq/l)	0.26	0.26	0.26	0.24	0.02	0.742
Beta-hydroxy butyrate (mmol/l)	0.45	0.46	0.47	0.39	0.03	0.34

Table 6.4 Difference in total dry matter intake, daily milk yield, daily fat + protein yield and live weight between the Control and each of S2, S6 and S10 during each 2-week period (until week 14) after additional concentrates were offered (P value in brackets) to primiparous cows

			Weeks post additional concentrate inclusion in diet						
			1-2	3-4	5-6	7-8	9-10	11-12	13-14
Total DMI (kg/day)	Con vs	S2	1.5 (0.61)	2.9 (0.001)	2.3 (0.038)	2.4 (0.039)	1.5 (0.090)	1.4 (0.078)	2.7 (0.006)
		S6	0.8 (0.447)	1.7 (0.105)	1.6 (0.071)	1.4 (0.138)	2.1 (0.027)	3.3 (0.001)	3.0 (<0.01)
		S10	1.6 (0.044)	2.2 (0.007)	3.2 (<0.001)	3.7 <0.001)	3.8 (<0.001)	3.2 (<0.001)	3.0 (0.001)
Daily milk yield (kg)	Con vs	S2	-0.1 (0.905)	1.0 (0.380)	1.7 (0.135)	2.3 (0.060)	1.7 (0.128)	1.1 (0.440)	1.2 (0.407)
		S6	0.2 (0.830)	1.2 (0.320)	2.2 (0.090)	1.0 (0.405)	1.2 (0.453)	1.5 (0.402)	1.1 (0.524)
		S10	0.1 (0.896)	0.5 (0.639)	-0.2 (0.878)	-0.2 (0.873)	-1.2 (0.465)	-0.5 (0.719)	-0.9 (0.600)
Daily fat and protein yield (kg)	Con vs	S2	-0.04 (0.677)	-0.03 (0.721)	0.04 (0.670)	0.14 (0.149)	0.11 (0.254)	0.05 (0.651)	0.09 (0.508)
		S6	-0.04 (0.676)	0.14 (0.176)	0.22 (0.043)	0.10 (0.347)	0.06 (0.535)	0.16 (0.114)	0.08 (0.394)
		S10	0.15 (0.183)	0.13 (0.245)	0.12 (0.245)	0.18 (0.086)	0.18 (0.216)	0.18 (0.105)	0.18 (0.224)
Live weight (kg)	Con vs	S2	0 (0.934)	5 (0.480)	7 (0.400)	9 (0.245)	5 (0.571)	2 (0.845)	6 (0.636)
		S6	-4 (0.374)	-2 (0.823)	-3 (0.677)	-7 (0.380)	-5 (0.581)	-1 (0.883)	3 (0.728)
		S10	1 (0.613)	1 (0.827)	6 (0.279)	13 (0.018)	15 (0.010)	14 (0.020)	13 (0.041)

Table 6.5 Difference in total dry matter intake, daily milk yield, daily fat + protein yield and live weight between the Control and each of S2, S6 and S10 during each 2-week period (until week 14) after additional concentrates were offered (P value in brackets) to multiparous cows

			Weeks post additional concentrate inclusion in diet						
			1-2	3-4	5-6	7-8	9-10	11-12	13-14
Total DMI (kg/day)	Con vs	S2	0.4 (0.714)	1.2 (0.314)	2.0 (0.130)	2.1 (0.057)	2.4 (0.015)	2.7 (0.020)	1.6 (0.053)
		S6	1.7 (0.150)	1.6 (0.142)	1.8 (0.110)	1.7 (0.137)	1.8 (0.020)	2.6 (0.005)	2.6 (0.015)
		S10	1.3 (0.237)	1.8 (0.122)	1.2 (0.201)	2.0 (0.035)	2.0 (0.620)	1.4 (0.143)	2.2 (0.025)
Daily milk yield (kg)	Con vs	S2	2.7 (0.014)	3.8 (0.009)	2.9 (0.085)	3.9 (0.018)	3.7 (0.026)	3.4 (0.038)	1.9 (0.364)
		S6	1.4 (0.240)	1.8 (0.196)	1.7 (0.238)	0.6 (0.702)	1.8 (0.169)	0.8 (0.522)	0.8 (0.544)
		S10	0.5 (0.565)	1.4 (0.060)	0.2 (0.819)	2.3 (0.072)	0.9 (0.517)	-0.8 (0.589)	-0.5 (0.788)
Daily fat and protein yield (kg)	Con vs	S2	0.12 (0.239)	0.30 (0.016)	0.23 (0.081)	0.25 (0.100)	0.27 (0.044)	0.41 (0.019)	0.18 (0.119)
		S6	0.11 (0.285)	0.14 (0.247)	0.10 (0.458)	0.05 (0.644)	0.10 (0.247)	0.06 (0.448)	0.11 (0.184)
		S10	0.03 (0.828)	0.03 (0.731)	-0.07 (0.316)	0.11 (0.249)	0.01 (0.897)	-0.079 (0.430)	-0.10 (0.454)
Live weight (kg)	Con vs	S2	12 (0.191)	4 (0.604)	5 (0.563)	-3 (0.759)	-3 (0.746)	-5 (0.613)	-2 (0.868)
		S6	4 (0.507)	-4 (0.590)	-1 (0.911)	-2 (0.848)	6 (0.591)	10 (0.338)	7 (0.564)
		S10	-1 (0.877)	5 (0.363)	7 (0.408)	17 (0.059)	10 (0.335)	18 (0.103)	17 (0.157)

The second approach involved undertaking a similar comparison during weeks 1-7, 8 –14 and weeks 1 – 14 after additional concentrates were included in the diet. With the primiparous cows, total concentrate DMI and total DMI were different between the Control treatments and S2, S6 and S10 on each of these occasions ($P<0.01$) (Table 6.6). However there were no significant differences in any of the comparisons of either milk yield or fat + protein yield ($P>0.05$). A similar trend was observed with the intake data for the multiparous cows (Table 6.7; $P<0.05$). However there were also significant differences in milk yield and fat plus protein yield in comparison of the Control treatment and S2 at weeks 1 – 7, 7 – 14 and 1 - 14 ($P<0.05$). No such significant effects were observed for the comparisons of the Control treatment with S6 or S10 ($P>0.05$).

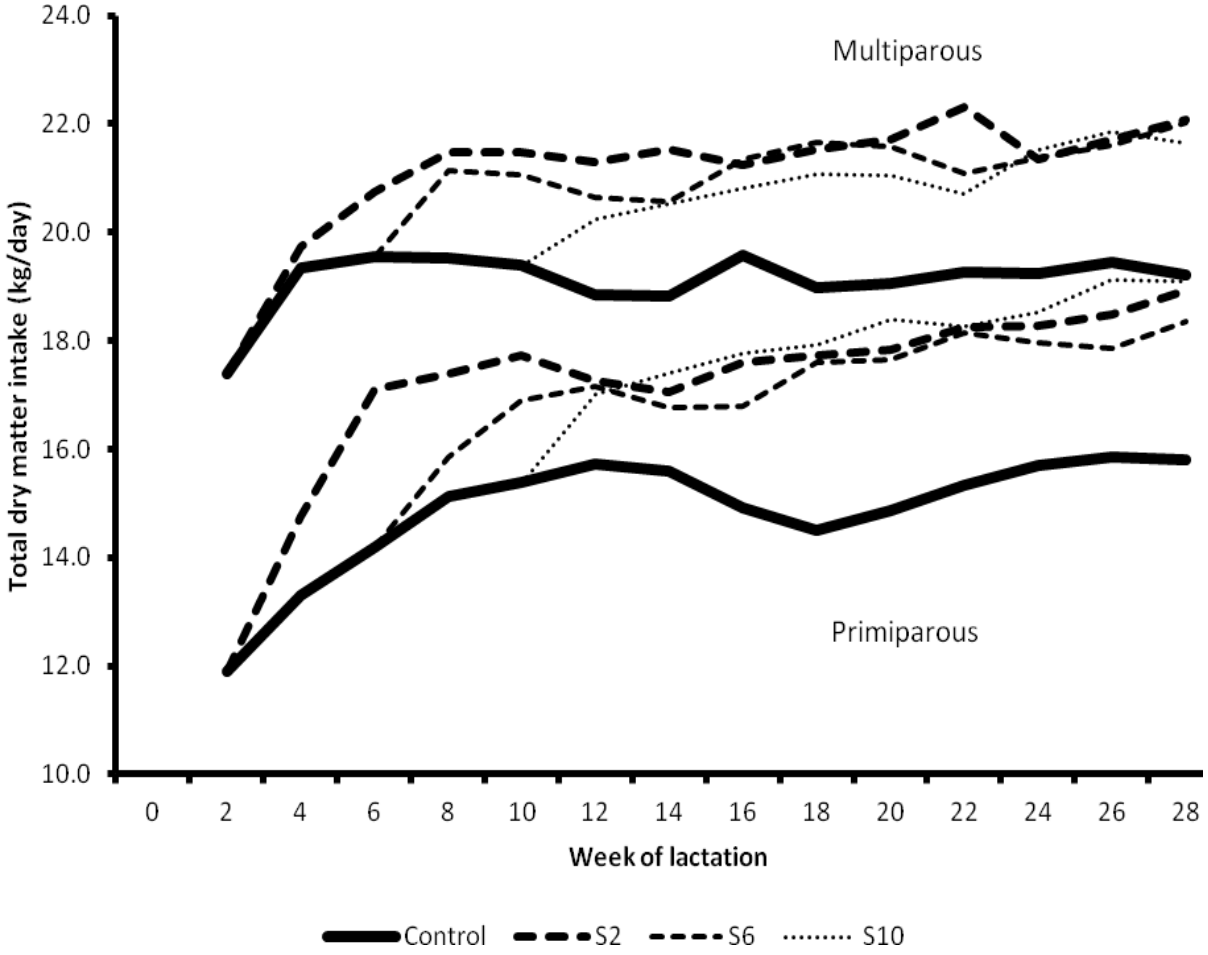


Figure 6.1 Effect of offering additional concentrate at 2 (S2), 6 (S6) and 10 (S10) weeks post calving on the total dry matter intake (kg/day) of primiparous and multiparous cows

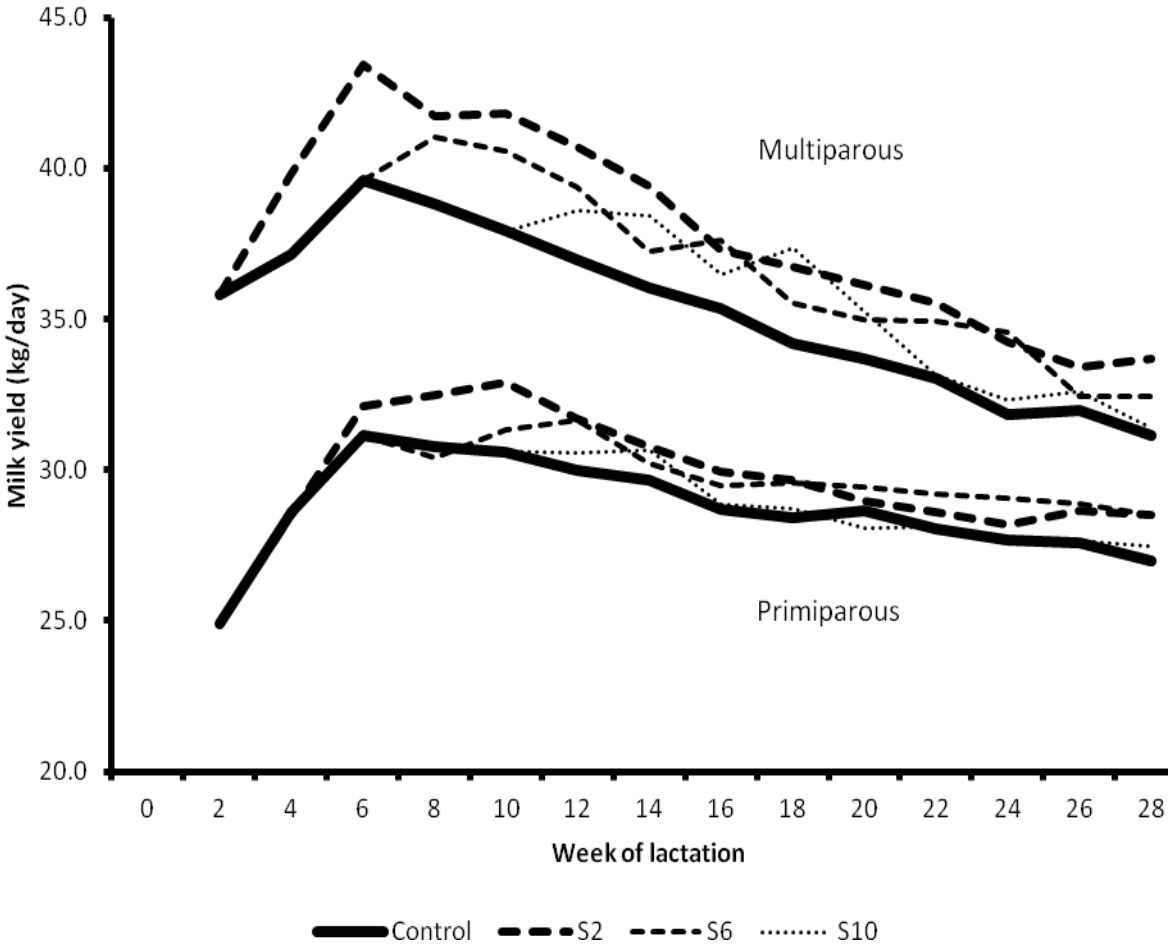


Figure 6.2 Effect of offering additional concentrate at 2 (S2), 6 (S6) and 10 (S10) weeks post calving on the milk yield (kg/day) of primiparous and multiparous cows

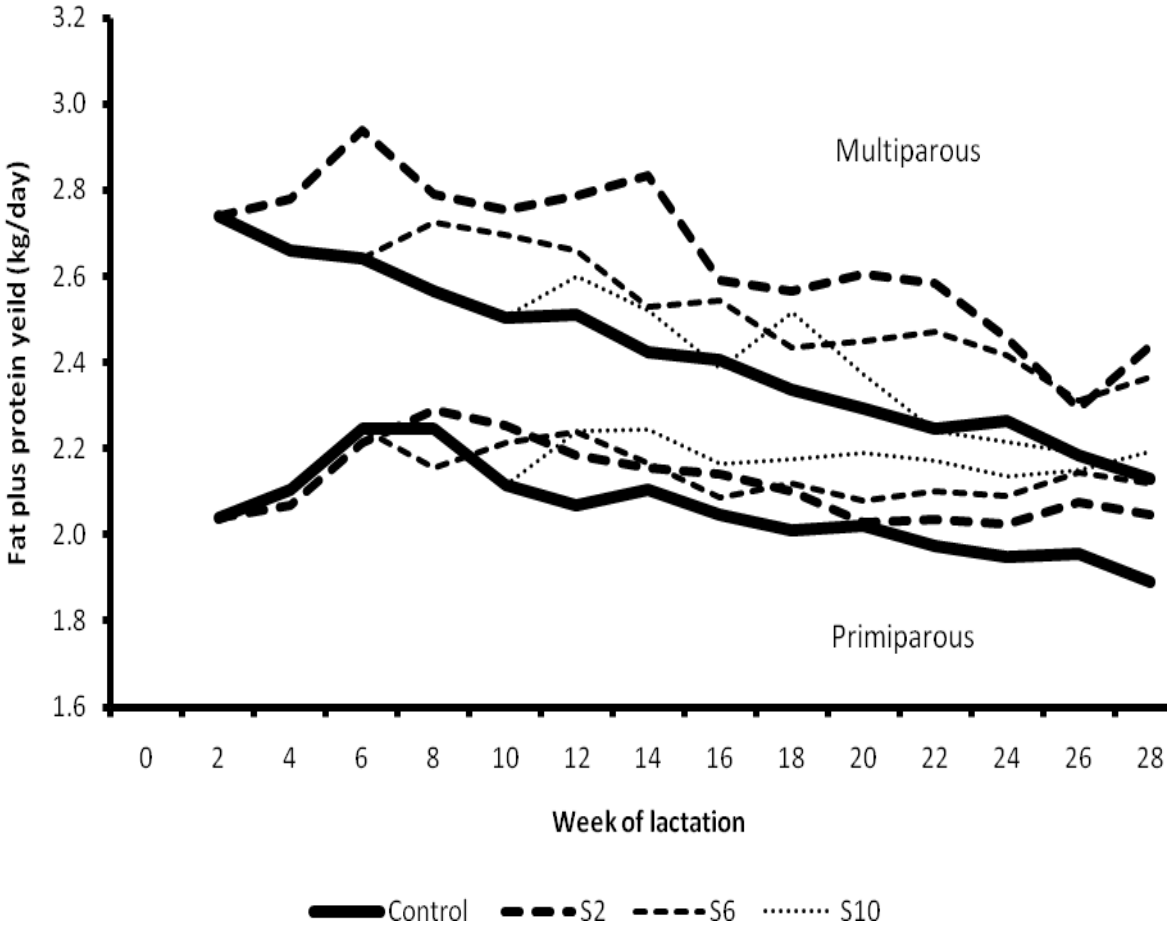


Figure 6.3 Effect of offering additional concentrate at 2 (S2), 6 (S6) and 10 (S10) weeks post calving on the fat plus protein yield (kg/day) of primiparous and multiparous cows

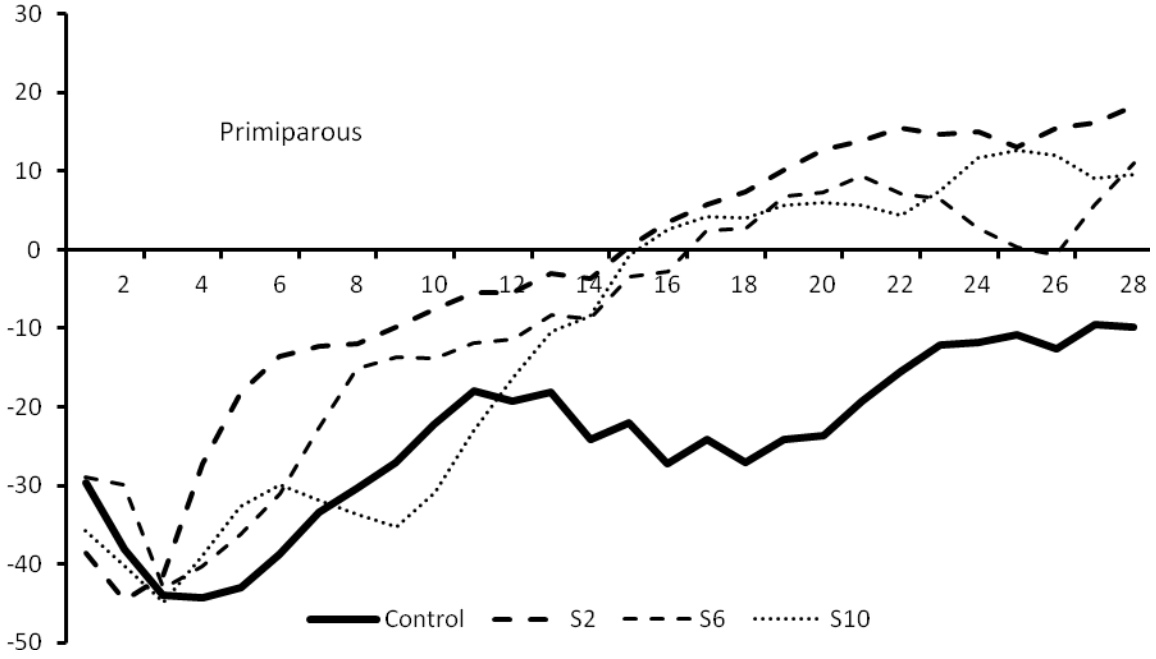


Figure 6.4: Effect of offering additional concentrate at 2 (S2), 6 (S6) and 10 (S10) weeks post calving on the daily energy balance (MJ) of primiparous cows.

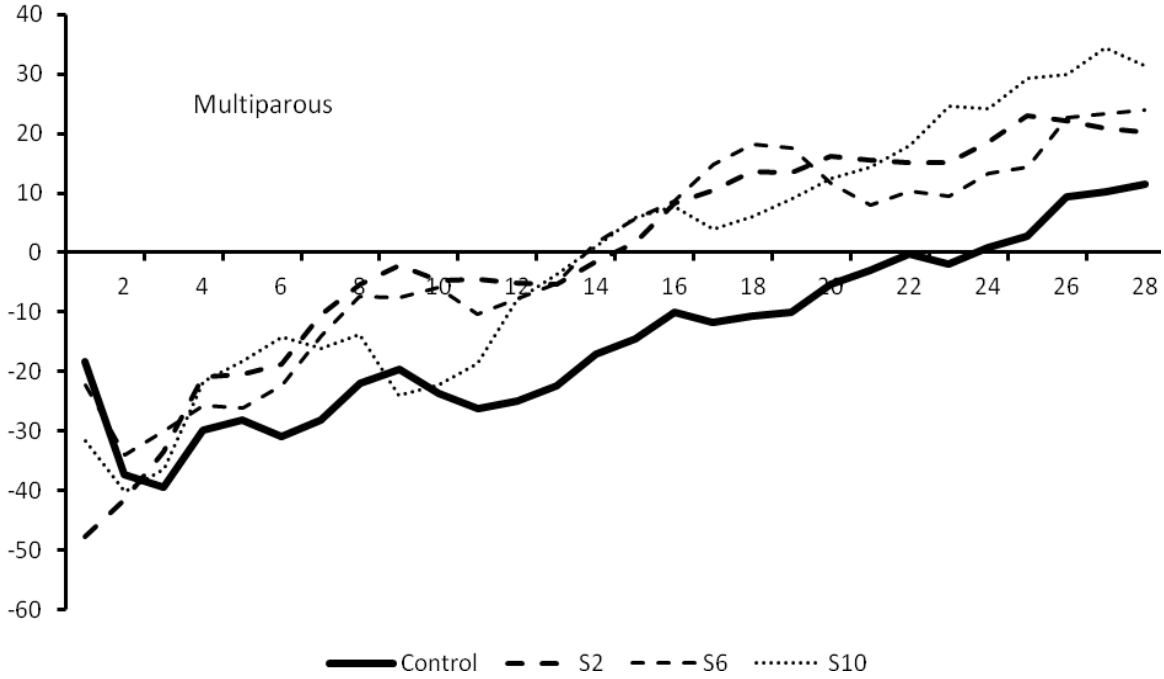


Figure 6.5: Effect of offering additional concentrate at 2 (S2), 6 (S6) and 10 (S10) weeks post calving on the daily energy balance (MJ) of multiparous cows.

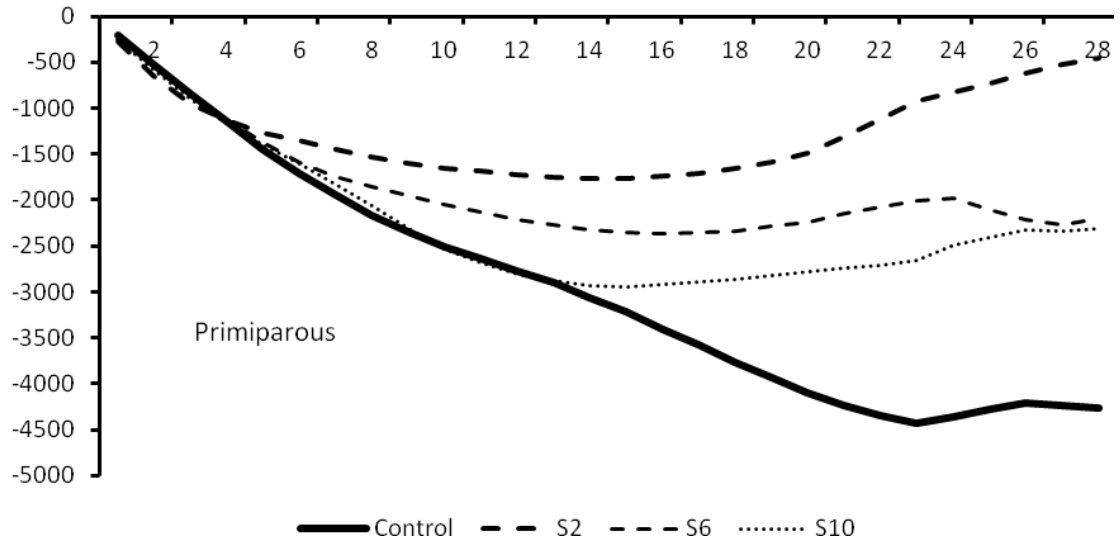


Figure 6.6: Effect of offering additional concentrate at 2 (S2), 6 (S6) and 10 (S10) weeks post calving on the cumulative daily energy balance (MJ) of primiparous cows.

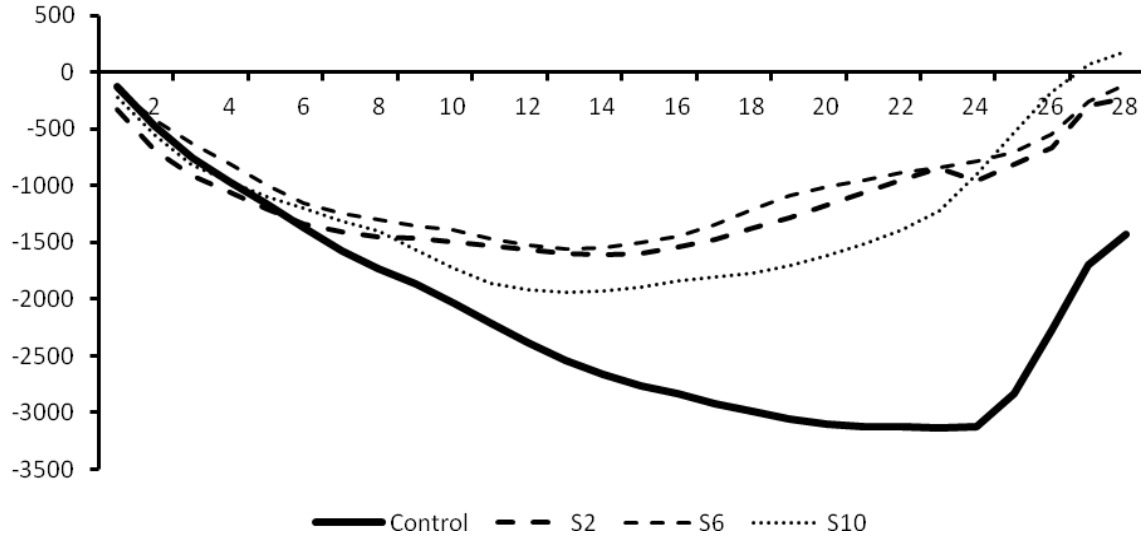


Figure 6.7: Effect of offering additional concentrate at 2 (S2), 6 (S6) and 10 (S10) weeks post calving on the cumulative daily energy balance (MJ) of multiparous cows.

Trends in daily energy balance associated with primiparous and multiparous cows on each of the three treatments are presented in Figures 6.4 and 6.5, respectively, while cumulative energy balance for primiparous and multiparous cows are presented in Figures 6.6 and 6.7 respectively. Cows on the Control treatment remained in negative energy balance for longer than those on S2,

S6 and S10. Primiparous cows on the three supplementation treatments returning to positive energy balance between weeks 14 – 16 of lactation, while multiparous cows returned to positive energy balance between weeks 13 – 15 of lactation.

Treatment had no significant effect on any of the fertility parameters examined within the current experiment ($P>0.05$, Table 6.8).

Table 6.6 Total concentrate DMI, total DMI, total milk output and total fat + protein yield for the Control and each of treatments S2, S6 or S10 during weeks 1-7, 8-14 and 1-14 post additional concentrate being offered in the diet of primiparous cows

	Total concentrate DMI (kg)			Total DMI (kg)			Total milk output (kg)			Total fat + protein yield (kg)		
Weeks	1-7	8-14	1-14	1-7	8-14	1-14	1-7	8-14	1-14	1-7	8-14	1-14
Control	382	402	786	698	742	1440	1482	1450	2932	107.1	102.0	209.0
S2	509	538	1046	818	865	1684	1532	1526	3057	107.6	106.6	214.2
SEM	14.8	15.8	26.7	24.0	30.4	51.1	31.3	41.9	72.4	1.73	3.58	4.89
Significance	<0.001	<0.001	<0.001	0.003	0.011	0.004	0.276	0.222	0.239	0.827	0.376	0.466
Control	414	395	809	746	717	1463	1466	1386	2853	103.0	97.8	200.8
S6	491	515	1006	815	850	1666	1518	1351	2969	107.8	103.1	210.9
SEM	16.8	16.8	30.7	27.7	24.4	47.9	33.4	112.2	82.9	2.81	3.07	5.65
Significance	0.005	<0.001	<0.001	0.096	0.001	0.008	0.295	0.825	0.335	0.244	0.238	0.223
Control	406	400	807	734	735	1469	1461	1410	2871	89.6	85.9	175.0
S10	513	541	1054	855	895	1750	1461	1375	2836	99.4	96.8	196.0
SEM	21	20.4	40.9	28.3	25.6	52.6	33.4	50.3	80.6	10.98	10.71	21.6
Significance	0.003	<0.001	<0.001	0.008	<0.001	0.002	0.990	0.913	0.759	0.535	0.479	0.506

Table 6.7 Total concentrate DMI, total DMI, total milk output and total fat + protein yield for the Control and each of treatments S2, S6 or S10 during weeks 1-7, 8-14 and 1-14 post additional concentrate being offered in the diet of multiparous cows

	Total concentrate DMI (kg)			Total DMI (kg)			Total milk output (kg)			Total fat + protein yield (kg)		
Weeks	1-7	8-14	1-14	1-7	8-14	1-14	1-7	8-14	1-14	1-7	8-14	1-14
Control	487	482	965	946	926	1872	1887	1780	3667	127.9	120.1	248.0
S2	630	633	1267	1029	1055	2084	2047	1933	3980	138.8	133.9	272.7
SEM	15.2	14.6	26.6	36.9	29.3	62.8	39.4	52.4	84.5	3.45	4.38	7.44
Significance	<0.001	<0.001	<0.001	0.134	0.006	0.029	0.011	0.056	0.018	0.039	0.040	0.032
Control	486	488	974	930	935	1865	1876	1729	3605	124.4	117.3	241.7
S6	619	631	1250	1031	1054	2085	1950	1779	3729	131.9	122.1	253.9
SEM	15.3	10.9	24.6	32.7	25.9	56.0	39.3	42.1	76.4	4.83	3.65	8.21
Significance	<0.001	<0.001	<0.001	0.043	0.005	0.012	0.203	0.409	0.267	0.289	0.367	0.305
Control	475	484	958	928	936	1864	1806	1660	3466	120.6	112.9	233.5
S10	623	629	1252	1019	1036	2055	1849	1673	3522	124.1	115.2	239.3
SEM	11.7	12.9	23.8	28.4	25.5	51.9	26.8	47.5	69.6	3.76	4.27	7.73
Significance	<0.001	<0.001	<0.001	0.039	0.013	0.020	0.278	0.848	0.579	0.516	0.715	0.604

Table 6.8 Effects of offering additional concentrate supplementation at week 2, 6 or 10 post calving on dairy cow fertility (mean of primiparous and multiparous cows)

	Treatment					
	Control	S2	S6	S10	SEM	Significance
Days to 1st progesterone rise	41	36	38	37	3.6	0.803
Peak progesterone concentration during 1st cycle (ng/ml)	16.6	19.6	20.0	23.7	3.7	0.589
Cows with luteal activity before day-42 post calving (proportion)	0.53	0.67	0.65	0.59	0.11	0.810
Days to 1st observed heat	60	57	55	65	4.5	0.415
Conception to 1st and 2nd AI (proportion)	0.63	0.61	0.65	0.59	0.11	0.983
Proportion of cows in calf at end of breeding season	0.79	0.89	0.90	0.82	0.08	0.741
Calving interval (days)	375	386	386	398	11.7	0.581

DISCUSSION

Effects of additional concentrates on food intake

While many studies have examined the response of dairy cows to concentrates supplementation at a fixed point in time during the lactation, few studies have compared the response to concentrates offered at different stages of the lactation. Offering additional concentrates will normally promote total dry matter intake, although the extent of this increase is normally related to the quality of silage being offered as the basal diet. Within the current experiment total DM intake across the experimental period increased with the inclusion of additional concentrates in the diet, although with the multiparous cows, this increase was only significant when intakes with treatment S2 was compared with the Control treatment. Across the duration of the study the inclusion of an additional 3.5 kg concentrate DM in the diet with treatment S2 resulted in a 2.6 kg increase in total concentrate DM intake with both primiparous and multiparous cows, and a corresponding increase in total intake of 2.4 and 1.8 kg DM/day with the primiparous and multiparous cows, respectively. The latter suggests a greater substitution rate with multiparous cows, compared to primiparous cows. Data presented in Tables 6.6 and 6.7 highlights the impact of offering additional concentrates at weeks 2, 6 and 10 post calving, on total concentrate and total DM intake during weeks 1 – 7, 8 - 14 and 1 – 14 after additional concentrates were offered, in comparison to the Control treatment. Irrespective of stage of offering additional concentrates, total DM intake was increased by a relatively similar amount, compared to the Control treatment. For example, total DM intake during the 14-week period after additional concentrates were offered was 244, 203 and 280 kg higher with treatments S2, S6 and S10, respectively, compared to the Control treatment (primiparous cows) while respective values were 212, 220 and 191 kg with the primiparous cows. Indeed Figure 6.1 highlights that irrespective of when the additional concentrates were introduced into the diet, cows showed a rapid response in terms of total DM intake, with the total intake response lines for S6 and S10 achieved a similar trajectory as for S2 within 2 – 4 weeks of the additional concentrates being offered. In addition to increasing total DM intake, the inclusion of additional concentrates will also have resulted in an increase in the energy density of the total diet, the extra concentrate offered having a ME content of 13.2 MJ/kg DM, compared to an ME content of approximately 11.0 MJ/kg DM with the forages.

Effect of additional concentrate on milk yield and composition

When examined over the entire experimental period, the inclusion of additional concentrates in the diet at 2, 6 or 10 weeks post calving had no effect on milk yield, milk fat + protein yield or milk composition with cows of either parity. This apparent lack of an overall response to additional concentrates, especially with treatment S2 which involved additional concentrates being offered during the entire experimental period, is perhaps surprising in view of the increase in total DM intake described above. However the absence of a response may reflect the high concentrate inclusion level in the Control diet (proportionally 0.53, DM basis) and the relatively high quality silage offered within the study. For example, previous research has clearly demonstrated that the response to each kilogramme of additional concentrate declines with increasing concentrate feed level, and is lower with high compared to medium quality silages.

However, examining milk yield responses over the entire period may mask the immediate milk yield responses to additional concentrates offered at different stage of lactation. Indeed, in an earlier study by Broster *et al*. (1963) involving primiparous cows, the response to the inclusion of additional concentrates decreased with increasing stage of lactation. In that study, offering additional concentrates during weeks 1-9 of lactation resulted in a total milk yield response of 532 kg, a 10% increase in the lactation yield, whereas when additional feeding was offered during weeks 10-18 of lactation, the total milk yield response was only 69 kg. Similarly, data from the primiparous cows presented in Figures 6.2 and 6.3 suggest a numerical milk yield and fat plus protein yield response to additional concentrate inclusion, especially when additional concentrates were offered at two weeks post calving (S2). However, when the responses were examined during weeks 1 – 7 and weeks 8 – 14 after additional concentrates were offered, none of these responses were found to be significant. While previous research has shown that primiparous cows tend to show a lower response to concentrate supplementation compared to multiparous cows, the fact that basal concentrate feed levels in the study by Broster *et al*. were lower than in the current study may also have been a contributing factor to the different responses between the two studies.

In contrast, multiparous cows on treatment S2 showed a significant milk yield and fat + protein yield response during the 14-week period after additional concentrates were offered, in comparison to the Control treatment. However, when additional concentrates were offered at 6 and 10 weeks post calving (treatments S6 and S10), both the milk yield and fat + protein yield responses achieved were not significant. Nevertheless, there was a numerical trend for both the milk yield and fat plus protein yield response to decline when concentrates were offered in later lactation, with the actual difference in milk yield between the Control and treatments S2, S6 and S10 being 313, 124 and 56 kg, respectively (during the 14-week period after additional concentrates were offered), while the respective values for fat plus protein yield were 24.7, 12.2 and 5.8 kg, respectively. Thus these findings with multiparous cows support the earlier observations by Broster *et al*. with primiparous cows, namely that the maximum response to concentrate supplementation is achieved in early lactation. While there does not appear to be any other similar work undertaken in recent years with high yielding dairy cows, the expectation might have been that these higher yielding cows would have retained the genetic potential to respond to additional concentrates in mid/late lactation. Indeed, in a study in which the response to concentrate supplementation was examined with grazing dairy cows (Ferris *et al*., 2003), late lactation cows with a high genetic potential showed almost an linear response to concentrate supplementation up to a concentrate feed level of 9.0 kg/day (0.73 kg milk/kg concentrate). However the basal diet in that study (autumn grass as the sole diet) was very different from the high quality diet offered in the current study. Thus the results of the current study suggest that when high yielding cows are offered a quality diet in mid lactation, their potential to exhibit a milk yield response to additional concentrates is limited.

Effect of offering additional concentrates on body tissue reserves and energy balance

The lack of a treatment effect on either plasma NEFA or BHB concentrations (higher concentrations of which are indicative of tissue mobilisation), suggest similar levels of tissue mobilisation across treatments. Indeed, treatment had remarkably few effects on any of the indices of body tissue reserves observed within the current study, the exception being mean live weight and live weight at the end of the study, which was higher with multiparous cows on

treatments S6 and S10, compared to the Control treatment. While a similar, although non-significant trend was observed for body condition score, these relatively minor effects contrast with the trends observed in daily energy balance, whereby both primiparous and multiparous cows on the Control treatment remained in negative energy balance for considerably longer than cows on any other treatment. For example, primiparous cows on the Control treatment were still in negative energy balance when the experiment finished at week-28 of lactation, while multiparous cows returned to positive energy balance at approximately week-21 of lactation. In contrast, with each of S2, S6 and S10, return to positive energy balance took place within a relatively narrow time window (approximately weeks 14 – 16 and weeks 13 – 14 with primiparous and multiparous cows, respectively). This more prolonged period of negative energy balance with the Control treatment reflects the fact that intakes were significantly lower with this treatment, and yet milk yield was not significantly affected by treatment over the entire lactation. The increased levels of tissue deposition observed with the multiparous cows offered concentrates at 6 and 10-weeks post calving reflects the numerically higher intakes of these cows, the higher ME content of the diets offered, and the absence of a significant milk yield response to additional concentrates as lactation proceeds. That no such increase in tissue deposition was observed with the primiparous cows is perhaps surprising given the more dramatic lift in food intake following concentrate supplementation, and the much reduced trend for a milk yield response. However, the degree of positive energy balance observed with the primiparous cows once they returned to positive energy balance, was considerably less than for the multiparous cows. The need for primiparous cows to partition energy toward growth, especially during their first lactation, may provide an explanation for where part of the additional energy was utilised.

Effect of concentrate supplementation on fertility

The dramatic change in the trajectory of the energy balance curve with each of S2, S6 and S10 following immediately after the additional concentrates were incorporated into the diet, reflects the similar trends observed in the food intake responses. These energy balance curves clearly demonstrate that relatively small increases in additional concentrate supplementation during early lactation can be used to manipulate energy balance, and that this approach could perhaps be

used to achieve a predefined energy balance trajectory, similar to that achieved by Law et al. (2011) through modifying the protein content of the diet. Indeed, given the clear relationship that has been established between dairy cow fertility and the extent of negative energy balance in a number of studies, fertility benefits with treatments S2, S6 and S10 might have been expected in the current study. This is especially true in view of the fact that cows on the Control treatment remained in negative energy balance for considerably longer than cows on any other treatment. Nevertheless, in common with other studies involving relatively small numbers of cows, there was no evidence that any aspect of dairy cow fertility performance was affected by treatment.

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TECHNOLOGY TRANSFER ASSOCIATED WITH PROJECT

PhD

Law, R. A. (2007) The effects of dietary energy and protein on behaviour and fertility in the modern high yielding dairy cow. Ph.D. Dissertation, Queen's University, Belfast.

Gilmore, H. S. (2010) An investigation of factors affecting reproductive function in the high genetic merit Holstein-Friesian dairy cow. Ph.D. Dissertation, Queen's University, Belfast.

Refereed Publications

Law, R. A., Young, F. J., Patterson, D. C., Kilpatrick, D. J., Wylie, A. R. G., M. A. McCoy, Mayne, C. S. and Ferris, C. P. (2010) Effect of pre- and post calving dietary energy content on animal production and blood metabolites of dairy cows during lactation. *Journal of Dairy Science*, 94(2):808-823.

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Law, R. A, Young, F. J., Patterson, D. C., Kilpatrick, D. J., Wylie, A. R. G., and Mayne, C. S. (2009) Effect of dietary energy content pre- and post-calving on production and blood metabolites of dairy cows during early lactation. In: *Proceedings of the British Society of Animal Science*, page 11.

Gilmore, H. S., Young, F. J., Law, R. A, Patterson, D. C., Wylie, A. R. G., and Mayne, C. S. (2009) An evaluation of the effect of nutritional strategy in early lactation on reproductive performance of Holstein-Friesian dairy cows. In: *Proceedings of the British Society of Animal Science*, page 12

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Farmers/Industry Meetings and Press Releases

Law, R.A., and Young, F.J., Recent developments in supplementation strategies for high-yielding dairy cows during the winter. (2010) AgriSearch Dairy Seminar 'Improving the sustainability of Dairy Farming within Northern Ireland'.

Reducing protein in diets is an environmentally friendly option (October 2009)

Lower Nitrogen, higher performance (October 2009)

The effect of reducing the protein content of the diet on the performance of dairy cows, United News / Cow management (Experimental results 2010)

Visitors to Hillsborough

1) **Presentations to industry**

- Association of Veterinary Surgeons Practitioning in NI — 6th June 2007
- McLarnon Feeds — 7th June 2007
- CAFRE Dairy Advisors — 13th June 2007
- Chris Granger, Australia — 14th June 2007
- Frank Wright Ltd — 26th June 2007
- Kite Consulting — 26th June 2007
- Agritech Group — 27th June 2007
- Dairy Hygiene Inspectorate — 17th July 2007
- Kingshay — 5th September 2007

- BOCM PAULS — 26th October 2007
- United Feeds Technical staff — 6th December 2007
- Adrian Cane, Dairy Consultant — 11th December 2007
- UFU milk committee — 18th December 2007
- BOCM PAULS — 5th February 2008
- John Comerford (Pennstate University) — 4th April 2008
- Alltech/Harbro — 16th April 2008
- French Feed Merchants — 19th May 2008
- Gary Waghorn, New Zealand — 28th May 2008
- John Thompson and Sons technical team — 12th June 2008
- Volac — 19th August 2008
- Corby Rock Technical Team — 1st October 2008
- Kemin UK — 10th December 2008
- Presentation to Trouw nutrition representatives — 23rd April 2009
- Dairy Co visit — 30th September 2009
- Dairy Science Forum — 17th November 2009
- Mole Valley technical team — 3rd December 2009
- United Feeds — 20th January 2010
- John Thompson and Sons technical team — 26th January 2010
- Presentation on fertility at UCD — 15th February 2010
- AgriSearch Dairy Committee — 23rd February 2010
- Grass Check Visit — 26th March 2010
- Frank Buckley et al. — 14th April 2010
- Karen Wonacott, MDC — 15th April 2010
- Tom Phillips, Pasture to Profit — 28th May 2010
- Dairy Co Extension Officers — 29th June 2010
- AgriSearch Dairy Committee — 27th August 2010
- John Roche, DairyNZ — 31st August 2010
- Julie Lee, DairyNZ — 8th October 2010
- United Feeds Technical Team — 19th January 2011

2) Presentations to farmer groups at Hillsborough

- Devon Dairy Farmers — 24th July 2007
- CAFRE Dairy Students — 6th December 2007
- Chilean Dairy Farmers — 6th March 2008
- Chilean Dairy Farmers — 23rd May 2008
- Enniskillen Dairy Farmers — 26th August 2008
- Hugh Black and Dairy Farmers — 10th September 2008
- Navan Dairy Farmers — 3rd October 2008
- Blacklion discussion group — 6th May 2009
- Greenmount and farmer group — 18th November 2009
- Navan Farmers — 3rd February 2010
- Chilean Dairy Farmers — 14th May 2010
- Scottish Dairy Farm Managers — 9th June 2010
- Vision Farmers Group — 22nd July 2010
- DairyCo Farmers Group — 30th September 2010
- Antrim Dairy Farmers — 26th October 2010