DOI: 10.3168/jds.2009-2374

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

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Interpretive summary:

The effect of dry period duration and feeding level on energy balance, metabolic status, and onset of cyclicity was examined in Holstein-Friesian cows. Omitting the dry period reduced milk production in the subsequent lactation, improved postpartum energy balance and metabolic status, and advanced the onset of cyclicity. Feeding a higher energy density diet increased milk production in the subsequent lactation, improved postpartum energy balance and metabolic status, but did not affect the onset of cyclicity. The results indicate that events during late pregnancy and feeding level during early lactation critically affect energy balance, metabolic status, and the reproductive axis.

ABSTRACT

Following parturition, it is typical for dairy cows to enter a period of negative energy balance (NEB) and body condition loss to support mammary milk synthesis, and this is associated with compromised reproductive performance. Alternative management strategies during the prepartum (dry) and early post partum periods may ameliorate this. Forty mature Holstein-Friesian cows were assigned to one of two dry period treatments (standard 8 week dry period (**SDP**) or no planned dry period (**NDP**)) and one of two dietary energy density treatments (standard TMR (**STMR**) or high quality TMR (**HTMR**)). Milk yield during weeks 1 to 12 postpartum was reduced ($P = 0.01$) in cows assigned to the NDP treatment. Energy balance ($P < 0.001$) and body condition score ($P = 0.07$) during weeks 1 to 4 postpartum were increased in cows assigned to the NDP treatment compared to the cows assigned to the SDP, and BCS increased ($P<0.001$) from weeks 5 to 12 postpartum in the NDP cows compared to the SDP cows. During the first 12 weeks postpartum, cows assigned to the HTMR had greater ($P = 0.02$) milk yields and reduced ($P < 0.001$) milk fat concentration compared to the cows assigned the STMR diet. BCS was greater ($P = 0.01$) from weeks 5 to 12 postpartum in HTMR cows compared to STMR cows. During the period from weeks -3 to +3 relative to parturition, circulating concentrations of insulin ($P = 0.001$), glucose ($P < 0.001$) and IGF-I ($P = 0.004$) were greater in cows on the NDP treatment compared to cows on the SDP treatment. Cows assigned to the HTMR had greater circulating insulin ($P = 0.04$) and glucose ($P = 0.001$) concentrations compared to the STMR cows from weeks -3 to +3 relative to parturition. The first postpartum ovulation occurred earlier for cows on the NDP treatment compared to cows on the SDP treatment (16.9 *vs.* 24.8 days postpartum; $P = 0.02$). Cows assigned to the STMR tended to have a higher conception rate to first service (P = 0.07) compared to cows assigned to the HTMR. Energy balance and metabolic status can be improved by either eliminating the dry period or by feeding a higher energy diet, but effects on the reproductive axis appear to be different.

(Keywords: dry period, feeding level, energy balance, resumption of cyclicity)

INTRODUCTION

Reproductive efficiency in high yielding dairy cows has decreased in recent decades (Royal et al., 2000; Butler, 2003; Evans *et al.,* 2006). Intensive genetic selection for increased milk production has led to remarkable improvements in milk yield per cow, but has also been associated with a worldwide decline in dairy cow fertility. It has been demonstrated that a negative correlation between genetic merit for milk yield and reproductive performance exists (Pryce and Veerkamp, 2001; Pryce *et al.,* 1997; Van Arendonk *et al.,* 1989).

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

It is generally accepted that a dry period of 50 to 60 days is required to maximize milk production in the subsequent lactation (for recent reviews, see Bachman and Schairer; 2003; Annen et al., 2004; Grummer and Rastani, 2004). There has been substantial interest recently in decreasing the duration of the dry period. Cows that avoid severe decreases in DMI prepartum have improved energy balance pre- and postpartum (Grummer, 1995). Recently, it has been demonstrated that omitting the dry period results in dramatic alterations in energy balance and metabolic profiles (Rastani et al., 2005). The current study was carried out to examine the effect of dry period duration and dietary energy density on milk production, DMI, energy balance, metabolic status and indicators of reproductive efficiency. Specifically, postpartum follicular dynamics and reproductive hormone profiles were examined to assess the effects of dry period duration and feeding level on resumption of cyclicity.

MATERIALS AND METHODS

Animals and Experimental Design

This experiment was a completely randomized block design with a 2 × 2 factorial arrangement of treatments. Forty mature Holstein-Friesian cows were blocked on the basis of expected calving date, previous lactation yield, bodyweight and body condition score (BCS), and were randomly assigned to one of two dry period treatments (standard 8 week dry period (**SDP**) or no planned dry period (**NDP**)) and one of two dietary energy density treatments (standard TMR (**STMR**) or high quality TMR (**HTMR**)). Cows assigned to SDP were fed ad libitum grass silage prepartum, and either the STMR or HTMR during the first 12 weeks postpartum. Cows assigned to the NDP treatment were fed either the STMR or HTMR diet during the dry period and the first 12 weeks postpartum. If prepartum daily milk yield dropped below 2 kg/day for cows on the NDP treatments, milking was discontinued for the remainder of the prepartum period. Actual dry period lengths (mean ± SEM) were 62.1 ± 1.9 days and 6.3 ± 1.7 days for cows on the SDP and NDP treatments, respectively. Two cows were dropped from the SDP treatment and two cows were dropped from the NDP treatment due to their dry periods being too short (SDP), too long (NDP), or illnesses unrelated to the study.

Animal Measurements

Cows were housed in free stall housing from 4 weeks before expected calving until 12 weeks postpartum. Cows were milked twice daily (prepartum and postpartum) and milk production was recorded at each milking using electronic milk meters (Dairymaster, Causeway, Co. Kerry, Ireland). Milk composition (protein, fat and lactose g/kg) was measured once per week by near-infrared reflectance spectroscopy (Milkoscan 605; Foss Electric, Hillerød, Denmark). Solids corrected milk yield was calculated using the equation of Tyrell and Reid (1965).

Daily measurements of dry matter intake were recorded using the Griffith-Elder MealMaster system (Griffith Elder & Co Ltd, Suffolk, UK). The ingredients for all diets were sampled on a weekly basis. Silage pH was measured on the juice pressed from the silage using a glass electrode and a pH meter (Radiometer pHM2 standard pH meter-radiometer, Copenhagen). The dry matter, crude protein, NDF, ash, starch and oil content of the feed samples were analysed as described by McNamara *et al.* (2003). The ingredient and nutrient composition of the diets is summarized in Table 1.

Bodyweight (BW) and body condition score (BCS) were measured weekly by the same technician. Energy Balance (EB) was estimated as the difference between energy intake and the sum of energy requirements for maintenance and milk production, using the French net energy (NE) system (Jarrige, 1989). The French system uses unité fourragère lait (**UFL**) as the unit of net energy, and is equivalent to 1 kg of standard air-dry barley. The following equations were used to determine the energy required for maintenance and output in milk (O'Mara, 1997):

Energy required for maintenance (UFL/day) = $1.4 + 0.6BW/100$;

Energy requirement for milk (UFL/kg of milk) = $0.0054FC + 0.0031PC + 0.0028LC – 0.015$;

Where BW = body weight, FC = fat concentration (%), PC = protein concentration (%) and LC = lactose concentration (%).

Blood Sampling and Analysis

Blood samples were collected 3 times/week for the final 3 weeks prepartum, daily for the first 28 days postpartum and once per fortnight thereafter until day 84 of lactation. All blood samples were collected from the coccygeal blood vessels into 10 ml lithium heparin vacutainers (Becton Dickinson, Plymouth, UK). The blood samples were centrifuged at 2000 × *g* for 15 minutes at 5 °C. The plasma was harvested and decanted into 1.5 ml aliquots, and stored at -20 °C until further analyses.

Plasma samples were analysed for indicators of metabolic status from day -12 to 84 relative to parturition. Glucose, non-esterified fatty acids (NEFA), urea and beta-hydroxybutyrate (BHBA) concentrations were analysed by enzymatic colorimetry using appropriate kits and an ABX Mira autoanalyser (ABX Mira, Cedex 4, France). Plasma insulin concentrations were determined using a solid-phase fluoroimmunoassay (AutoDELFIA, PerkinElmer Life and Analytical Science, Turku, Finland) using appropriate kits (Unitech BD Ltd., Dublin, Ireland). The inter- and intra-assay coefficients of variation were 10.9 and 4.5%, respectively (n = 3).

Circulating IGF-I concentrations were quantified using a validated double-antibody radioimmunoassay following ethanol-acetone-acetic acid extraction (Enright *et al.,* 1989). Recombinant human IGF-I (R&D Systems Europe, UK) was used as a standard and to generate iodinated tracer. The assay was carried out as described by Spicer et al. (1990). Inter- and intra-assay coefficients of variation were 27.5 and 14.9%, respectively (n = 8). Circulating FSH concentrations were analysed in daily plasma samples collected from the day of parturition until 8 days in milk using a validated radioimmunoassay (Crowe *et al.,* 1997). The inter- and intra-assay coefficients of variation were 5.7 and 9.5%, respectively (n = 3).

Circulating estradiol concentrations (E2) were measured in blood samples collected on consecutive days during development of the dominant follicle of the first postpartum follicle wave. If ovulation occurred, plasma E2 concentrations were measured on each of the 7 days immediately prior to ovulation. If the cow had a dominant follicle that underwent atresia or became cystic, E2 concentrations were measured in blood samples collected daily from emergence of the dominant follicle until the point of maximum observed follicle diameter. The concentration of E2 in plasma was determined by radioimmunoassay following extraction (Prendiville, 1995) using E2 MAIA kits (Biostat, UK). Inter- and intra-assay coefficients of variation were 21.5 and 13.1%, (n = 4). For all hormone assays, each treatment was equally represented in each assay, and all samples for a cow on a given treatment were completed in a single assay.

Postpartum Ultrasound Evaluation and Reproductive Management

Ovarian follicular activity was examined by linear array ultrasonography (Aloka 900; 7.5-MHz transrectal transducer, Aloka Ltd., Tokyo, Japan) thrice weekly beginning on day 8-10 postpartum and continuing until first ovulation. Ovulation was deemed to have occurred following the disappearance of a dominant follicle and the subsequent appearance of a corpus luteum (CL). The size of a large follicle was determined by finding the average diameter in two directions at right angles on a single frozen image. The number of small (< 5 mm), medium (5 – 10 mm) and large (> 10 mm) follicles were recorded for each ovary at every ultrasound examination. If ovulation had not occurred by day 60 postpartum, cows were treated to commence cycling with the following hormone programme: Day 0 GnRH (20 μg Buserelin; Receptal, Intervet Ireland, Dublin) and CIDR insertion (InterAg, New Zealand); Day 7 $PGF_{2\alpha}$ (500 μg cloprostenol sodium; Estrumate, BP (Vet) Coopers, Berkhamsted, England); Day 9 CIDR removal. Ovulation was confirmed using transrectal ultrasonography to visualize a CL approximately 7 days after CIDR removal. Initiation of breeding commenced on a calendar mating start date (27th of November 2005). Tail paint was used as a heat detection aid, and all cows were artificially inseminated (AI) following observation of standing estrus and/or removal of tail paint.

Pregnancy status was determined using transrectal ultrasonography on day 30 - 36 and day 60 - 66 post AI. Visualization of a fluid-filled uterine horn with the presence of a viable embryo was used as positive indication of pregnancy.

Data Handling and Statistical Analysis

Daily measurements of milk yield, DMI and EB were collapsed into weekly means. The pre-partum BW and BCS data was lost due to a technical failure in the electronic recording system. The EB, BCS and DMI data for each cow were divided into two time periods; weeks 1 to 4 postpartum and weeks 5 to 12 postpartum. Milk yield, milk composition, SCM, DMI, EB, bodyweight, and plasma FSH data were analysed as repeated measures using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with an autoregressive covariance structure. The fixed effects included in the model were dry period, feeding level, time (day or week), and all possible interactions. Block was included as a random effect. Conception rate data were analysed using Fisher's exact test.

The metabolite and insulin data for each cow were divided into two time points; the transition period lasted from 3 weeks before parturition to 3 weeks postpartum and the post-transition period lasted from 4 weeks to 12 weeks postpartum. The IGF-I data was analysed in a similar manner, with the exception that the post-transition period was weeks 4 to 9 postpartum. The insulin, IGF-I and metabolite data were analysed as repeated measures using the MIXED procedure of SAS with an autoregressive covariance structure with the same fixed and random effects as outlined above.

The peak circulating concentration of FSH during the first 10 days postpartum and the peak E2 during the first postpartum follicular wave were analysed using the

MIXED procedure of SAS. Fixed effects included in the model were dry period, feeding level and the interaction between dry period and feeding level. Block was included as a random effect. The relationship between the total number of small (< 5 mm), medium (5 - 10 mm), and large (> 10 mm) follicles recorded at the first postpartum ultrasound examination, peak circulating FSH concentrations, and the number of days postpartum when peak circulating FSH concentration occurred was analysed using Pearson correlation coefficients.

RESULTS

There was no interaction between dry period duration and feeding level for most variables, so both factors are presented separately; where interactions were observed, these are reported.

Milk Production and Composition

Milk production data are summarized in Table 2. Solids corrected milk yield was reduced by 19.5% in cows assigned to the NDP treatment during the first 12 weeks of lactation compared to cows assigned to the SDP ($P = 0.004$). The yield of fat and protein was reduced by 11.3% in the NDP cows compared with the SDP cows ($P = 0.02$). Cows assigned to the NDP treatment had greater ($P = 0.001$) milk protein concentration compared to cows assigned to the SDP.

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

STMR diet. The HTMR treatment tended ($P = 0.08$) to increase milk protein concentration.

Dry Matter Intake, Energy Balance and Body Condition Score

The DMI, EB and BCS data are summarized in Table 3. Cows assigned to the NDP treatment had greater ($P < 0.001$) pre-partum DMI compared to cows assigned to the SDP treatment, but there was no difference in postpartum DMI. Mean daily energy balance was greater in cows assigned to the NDP treatment compared to cows on the SDP treatment for weeks 1 to 4 postpartum ($P < 0.001$) and weeks 5 to 12 postpartum ($P = 0.02$). The energy balance nadir was lower ($P = 0.004$) and the mean duration from parturition to return to zero energy balance was longer ($P = 0.003$) for cows on the SDP treatment compared to the NDP treatment. Cows assigned to the NDP treatment had greater BCS compared to SDP treatment cows from weeks 1 to 4 postpartum ($P = 0.07$) and weeks 5 to 12 postpartum ($P < 0.001$).

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

Plasma Insulin, IGF-I and Metabolites

The cows on the NDP treatment had greater circulating concentrations of glucose ($P < 0.0001$), insulin ($P = 0.001$) and IGF-I ($P = 0.004$) concentrations during the transition period compared to cows on the SDP treatment (Figure 1), whereas cows assigned to the SDP treatment had greater ($P = 0.009$) NEFA concentrations compared to cows on the NDP treatment (Table 4). During the post-transition period, cows assigned to the NDP treatment had increased circulating IGF-I concentrations compared to cows assigned to the SDP treatment ($P = 0.02$), whereas cows on the SDP treatment had greater circulating NEFA concentrations ($P = 0.02$). There were no differences between the dry period treatments from week 4 to 12 postpartum in plasma glucose or BHBA concentrations, but circulating insulin was greater ($P= 0.02$) in cows assigned to the NDP treatment compared to the SDP treatment (Table 4).

During the transition period, cows fed the HTMR diet had increased concentrations of glucose compared to cows fed the STMR diet ($P < 0.001$) whereas cows on the STMR had significantly greater concentrations of BHBA ($P < 0.001$). There was no effect of dietary energy density on insulin or IGF-I during the transition period. During the post-transition period cows fed the HTMR diet had greater circulating concentrations of insulin ($P = 0.015$), glucose ($P < 0.001$) and urea ($P < 0.001$) compared to cows on the STMR diet. Cows on the STMR diet had increased circulating concentrations of NEFA ($P = 0.04$) and BHBA ($P < 0.001$) compared to cows fed the HTMR diet during the post-transition period. Cows on the HTMR diet had greater circulating IGF-I concentrations ($P = 0.006$) compared to cows fed the STMR diet for weeks 4 to 9 relative to parturition (Figure 2 and Table 4).

Reproductive hormones and follicular dynamics

Cows assigned to the SDP treatment had greater mean FSH concentrations during days 1 to 10 postpartum (P = 0.006) and greater peak FSH concentrations (P=0.008) compared to those assigned to the NDP treatment (Figure 3). Dry period duration did not affect the interval from calving until peak circulating FSH concentrations (5.1 ± 0.5 *vs.* 4.6 ± 0.4 DIM; P = 0.4, NDP *vs.* SDP, respectively). There was a weak, but statistically significant, negative correlation between the diameter of the dominant follicle at first postpartum ultrasound examination and the days in milk when peak FSH occurred (r = -0.34; P = 0.04). The follicle data recorded at the first postpartum ovarian ultrasound examination (9.4 ± 0.5 *vs.* 8.6 ± 0.6 DIM, NDP *vs.* SDP, respectively; P = 0.2) is summarized in Table 5. There was no effect of dry period treatment on the number of small follicles observed (P = 0.8). At the same ultrasound examination, cows assigned to the SDP treatment had a greater number of medium size follicles (P = 0.04), whereas cows assigned to the NDP treatment had a greater number of large follicles (P = 0.04).

Ovulation occurred later in cows assigned to the SDP treatment compared to cows assigned to the NDP treatment (16.9 ± 2.5 vs. 24.8 ± 2.6 days in milk; NDP *vs.* SDP, respectively; P = 0.02). This corresponded to 83.3%, 11.1% and 5.6%, of cows assigned to the NDP treatment having their first ovulation during the 1st, 2nd, and 3rd or later postpartum follicular waves, respectively, whereas for cows assigned to the SDP treatment these values were 64.7%, 23.5% and 11.8% respectively.

For cows that had an ovulation during the first postpartum follicle wave, there was no effect of dry period duration on peak E2 concentrations, day postpartum when peak E2 occurred, maximum follicle diameter, or day postpartum when maximum follicle diameter was observed (results not shown). For cows that failed to ovulate the first postpartum follicle wave, follicles underwent atresia (n = 3 and 3) or developed into follicular cysts (n = 3 and 0) in SDP and NDP treatment cows, respectively. There was no significant effect of dry period duration on calving to service interval, conception rate to first service, calving to conception interval or overall pregnancy rate (Table 6).

There were no differences in FSH concentrations during days 1 to 10 postpartum (0.27 *vs.* 0.27 ng/ml; P = 0.9) nor was there a difference in peak FSH concentration (0.46 *vs.* 0.45 ng/ml; P = 0.7) between cows assigned to the HTMR and STMR diets. Dietary energy density did not affect the number of days from calving until peak circulating FSH concentration (4.9 ± 0.4 *vs.*4.8 ± 0.49; HTMR *vs.* STMR, respectively). The follicle data recorded at the first postpartum ovarian ultrasound examination (9.1 ± 0.5 *vs.* 8.8 ± 0.6 DIM, HTMR *vs.* STMR, respectively; P = 0.6) is summarized in Table 5. Cows on the STMR diet tended to have a greater number of small follicles (P = 0.07), but there were no differences between feeding level treatments in the number of medium follicles (P = 0.47) or large follicles (P = 0.87).

Dietary energy density did not affect the timing of the first postpartum ovulation (20.8 ± 2.7 *vs.* 20.9 ± 2.5 DIM, HTMR *vs.* STMR, respectively; P = 0.9). This corresponded to 73.7%,10.5% and 15.8% of cows assigned to the HTMR having their first ovulation during the 1st, 2nd, and 3rd or later postpartum follicular waves, respectively, whereas the values for cows assigned to the STMR were 70.6%, 23.5% and 5.9%, respectively.

For cows that had an ovulation during the first postpartum follicle wave, there was no effect of feeding level on peak E2 concentration, day postpartum when peak E2 occurred, maximum follicle diameter, or day postpartum when maximum follicle diameter was observed (results not shown). For cows that failed to ovulate the first postpartum follicle wave, follicles underwent atresia (n = 3 and 2) or developed into follicular cysts (n = 2 and 2) in the HTMR and STMR treatments, respectively. Cows assigned to the STMR diet tended to have a greater conception rate to first service compared to cows on the HTMR diet (50.0 vs. 21.1%; STMR *vs.* HTMR, respectively; P = 0.07) but there was no effect of feeding level on the overall pregnancy rate at the end of the breeding period (Table 6).

Interactions between dry period duration and feeding level

Time of EB nadir, plasma NEFA and BHBA. An interaction between dry period duration and feeding level was observed for the interval from parturition to the EB nadir. For cows on the NDP treatment, the HTMR diet resulted in a shorter interval from parturition to the EB nadir compared to the STMR diet (2.86 ± 0.30 vs. 1.71 ± 0.28 weeks; P = 0.03), but there was no effect of dietary feeding level for cows on the SDP treatment (2.25 ± 0.34 vs. 2.88 ± 0.27 weeks; P = 0.4). Similarly, cows on the SDP treatment had a longer interval to EB nadir compared to cows on the NDP treatment when fed the HTMR diet (2.88 ± 0.27 vs. 1.71 ± 0.28 weeks; P = 0.02), but there was no effect of dry period treatment when cows were fed the STMR diet (2.25 ± 0.34 vs. 2.86 ± 0.30 weeks; P = 0.5).

An interaction between dry period duration and feeding level was also observed for circulating NEFA concentrations (Figure 4). HTMR decreased circulating NEFA concentrations for SDP cows (0.32 ± 0.05 vs. 0.18 ± 0.04 mmol/L; P = 0.03), but had no effect for NDP cows (0.12 ± 0.04 vs. 0.19 ± 0.04 mmol/L; P > 0.3) during the transition period (interaction between dry period length and feeding level: P = 0.003). Similarly during the post-transition period, HTMR decreased circulating NEFA concentrations for SDP cows (0.19 ± 0.03 vs. 0.09 ± 0.03 mmol/L;

P = 0.04), but had no effect for NDP cows (0.08 ± 0.03 vs. 0.09 ± 0.03 mmol/L; P > 0.9), resulting in a significant interaction (P = 0.045).

A similar interaction (P = 0.018) was observed for circulating BHBA concentrations during the transition period. HTMR decreased circulating BHBA for cows on the SDP treatment (0.64 ± 0.05 vs. 0.34 ± 0.04 mmol/L; P < 0.001), but had no effect for cows on the NDP treatment (0.52 ± 0.04 vs. 0.42 ± 0.04 mmol/L; P = 0.3).

Pre-ovulatory circulating estradiol-17β. For cows that had an ovulatory first postpartum follicle wave, there were no significant effects of either dry period duration or feeding level on peak circulating E2 concentrations prior to ovulation. However, an interaction (P = 0.03) between dry period duration and feeding level was observed whereby cows assigned to the SDP treatment had a significantly higher preovulatory peak E2 concentrations when fed the HTMR diet compared to cows fed the STMR diet. No effect of feeding level was observed for the cows assigned to the NDP treatment (Figure 5).

DISCUSSION

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

Short dry periods reduce milk production in the subsequent lactation in a number of species including cattle, rats, and humans (Annen et al., 2004); in cattle this occurs due to reduced mammary epithelial cell turnover and secretory capacity (Annen et al., 2007). In the current study, average daily milk production during the first 12 weeks of lactation was decreased by 16%. Remond et al. (1992) reported that continuously milked cows had a 17% reduction in average daily milk yield, and Rastani et al. (2005) observed a 20% and 16% decrease in mean daily milk yield and solids-corrected milk yield, respectively, during the first 70 days postpartum in continuously milked cows. It should be noted that the milk production potential of the cows in the current study was similar to those in the report of Remond et al., (1992) but lower than the cows in the study reported by Rastani et al. (2005).

The cows assigned to the NDP treatment experienced only mild NEB for a short duration, and accordingly did not lose BCS postpartum. In contrast, the cows on the SDP treatment were in NEB for an average duration of 7.1 weeks following parturition, and on average lost 0.5 units of body condition. The improved EB status of the NDP cows was achieved via a reduction in milk energy output during the first 12 weeks of lactation (2.4 UFL/day) while maintaining similar energy intake and maintenance requirements to cows on the SDP treatment. Similar to the cows on the NDP treatment, cows assigned to the HTMR treatment did not lose BCS during the postpartum period. However, in contrast to the NDP treatment, the HTMR diet resulted in a non-significant increase in total milk energy output, a significant increase in energy intake, with an overall effect of a non-significant improvement in calculated energy balance. Hence, reducing the duration of the dry period decreased the inherent drive to produce milk in the subsequent lactation, whereas increasing dietary energy density allowed dietary energy intake to more closely meet energy requirements, albeit at a higher daily milk yield. During the first 12 weeks postpartum, cows assigned to the NDP treatment had increased milk protein concentrations compared to SDP treatment cows. This is in agreement with previous reports that continuous milking results in higher milk protein concentrations (Remond *et al.*, 1997; Rastani *et al.*, 2005), and likely reflects their superior energy balance status. In contrast, the HTMR diet tended to increase milk protein concentration, and resulted in a significant reduction in milk fat concentration. Reduced milk fat concentration is commonly observed with high energy diets, and is thought to be due to rumen biohydrogenation intermediates exerting direct inhibitory effects on mammary milk fat synthesis (Bauman and Griinari, 2003).

Following expulsion of the foetal-placental unit and clearance of gestational steroids, plasma FSH concentrations increase within 3-5 days postpartum, and this plays a pivotal role in orchestrating the emergence of a new follicular wave (Beam and Butler, 1997). In the current study, dietary energy density had no effect on postpartum FSH concentrations, but cows on the SDP treatment had greater concentrations of FSH compared to the NDP treatment group. Gümen et al. (2005) reported that cows assigned to a continuous milking treatment had lower postpartum circulating FSH concentrations compared to cows on a traditional dry period treatment on Day 6 postpartum. Those authors speculated that the cows on the NDP treatment had their postpartum FSH surge earlier than Day 6 postpartum, but this could not be detected because blood samples were not collected from Day 1 to 6 postpartum in their experimental protocol. Our results do not support their hypothesis as we observed no differences in the timing of the postpartum FSH surge between the NDP and SDP treatment groups. Prepartum circulating E2 concentrations are reduced by omitting the dry period (Gümen et al., 2005), and could potentially impact postpartum pituitary release of FSH.

The greater circulating FSH concentrations observed in the SDP treatment group did not affect the number of small follicles at the first postpartum ultrasound examination. Interestingly, at the same ultrasound examination, the SDP treatment had a greater number of medium follicles (5 – 10 mm), whereas the NDP treatment had a greater number of large follicles (> 10 mm). It is likely that this simply reflects a more advanced stage of follicle development, in that the cows on the NDP treatment had already developed a dominant follicle, whereas the SDP treatment cows had a number of follicles at the selection stage of development. Our observations on follicle development are consistent with those of Gümen et al., (2005), who carried out the first postpartum scan on day 6 postpartum, and reported a significantly larger mean follicle diameter for continuously milked cows compared to cows with a normal dry period. These data clearly indicate that increased circulating concentrations of FSH do not increase the rate of follicular development. A negative correlation was observed between the diameter of the dominant follicle at the first postpartum ultrasound examination and the day postpartum when peak FSH concentration occurred. Hence, the earlier the postpartum increase in FSH concentrations occurred, the greater the size of the DF on day 8 to 10 postpartum. This is consistent with previous reports indicating that emergence of the first follicle wave postpartum is related to the timing of the postpartum FSH surge (Beam and Butler, 1997). Despite not observing differences in the timing of peak FSH concentrations between either the dry period duration or dietary energy density treatments, the first postpartum ovulation occurred earlier for cows on the NDP treatment compared to cows on the SDP treatment, but dietary energy density had no significant effect.

Negative energy balance is associated with a decrease in circulating concentrations of insulin, glucose and IGF-I, and increased circulating concentrations of NEFA and BHBA (Grummer, 1995). In the current study, cows on the NDP treatment had greater circulating insulin, glucose and IGF-I concentrations, and lower circulating NEFA and BHBA concentrations compared to SDP cows, consistent with their superior energy balance status. Beam and Butler (1997) reported that circulating estradiol concentrations during the first postpartum follicle wave were greater and interval to first ovulation was shorter in cows with increased circulating IGF-I concentrations. In the current study, we observed greater circulating IGF-I concentrations in cows on the NDP compared to the SDP treatment, and also in cows on the HTMR compared to the STMR treatment. The cows assigned to the NDP treatment ovulated earlier compared to cows on the SDP treatment, but there was no difference in interval to first ovulation between cows on the two dietary energy density treatments, despite the differences in plasma IGF-I concentrations. Butler et al. (2004) reported that a 2.6 fold increase in circulating insulin during the first postpartum follicle wave resulted in increased circulating IGF-I and E2 concentrations without any apparent change in LH pulse release. Despite observing differences in circulating insulin and IGF-I concentrations in the current study due to either omitting the dry period or increasing dietary energy density, circulating E2 concentrations during the first postpartum follicular wave were not affected. It should be noted that average daily milk yield in the current study was moderate; consequently NEB was also moderate, and a high proportion of cows on all treatments had an ovulatory first postpartum follicle wave.

Lucy et al. (1991) reported that as predicted energy balance increased during the first 25 days postpartum, there was a decrease in the average number of small follicles (6 to 9 mm) and an increase in the average number of large follicles (10 to 15+ mm), indicating that small follicles mature to larger follicles earlier in cows in superior energy balance status. It is likely that the superior EB status and increased concentrations of insulin and IGF-I for cows on the NDP treatment resulted in greater LH pulse frequency (Canfield et al., 1990), and accordingly the NDP treatment had an earlier onset of cyclicity compared to the SDP treatment. Interestingly, the HTMR treatment also resulted in greater circulating concentrations of insulin, IGF-I, improved BCS, and circulating metabolite concentrations indicative of superior EB. Despite this, feeding the HTMR diet did not advance the onset of cyclicity, and tended to have a negative effect on subsequent conception rate to first service. It is plausible to hypothesise that the greater metabolic burden of increased milk output and greater liver blood flow due to increased DMI increased steroid hormone clearance, but neither the milk production nor the DMI in the current study were high by the standards of the modern Holstein-Friesian dairy cow. Nevertheless, the observation of poorer reproductive performance with increased concentrate supplementation is not consistent with other reports from this research centre and elsewhere (Horan et al., 2004; Pollott et al. 2008), and may be an artefact of the small number of animals enrolled in the study.

CONCLUSION

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

ACKNOWLEDGEMENTS

The authors would like to thank Mr. J.P. Murphy, Mr. J. Kenneally and the Moorepark farm staff for management and care of the animals. The technical assistance of Ms. N. Galvin and Ms. N. Hynes is also appreciated. National Development Plan funding is gratefully acknowledged.

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Table 1: Ingredient and nutrient composition of STMR and HTMR diets.

Diet ingredients	STMR	HTMR
Grass Silage	0.50	0.20
Barley	0.35	0.24
Brewers grains and beet pulp mix	-	0.30
Soya bean meal	0.13	0.10
Soya hulls	-	0.15
Vitamins and minerals[1]	0.02	0.01
Nutrient composition (DM basis)		
DM (g/kg)	892	904
Net energy (UFL/kg DM)	0.96	1.02
Ash (g/kg DM)	71	65
Crude protein (g/kg DM)	164	178
NDF (g/kg DM)	385	415
Oil (Acid Hydrolysis) %	2.9	3.3

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

Table 2. The effect of dry period duration and dietary energy density on milk production and composition for weeks 1 to 12 of lactation.

	Dry Period		Feeding Level		SEM	P - value		
	SDP	NDP	STMR	HTMR		DP[1]	FL[2]	DP×FL
	n = 18	n = 18	n = 18	n = 18				
SCM[3] (kg/d)	28.7	23.1	25.5	26.3	1.3	0.004	0.67	0.62
Milk Yield(kg/d)	29.4	24.6	24.9	29.1	1.50	0.01	0.02	0.4
Fat (g/kg)	40.0	41.4	42.8	38.6	1.10	0.2	<0.001	0.8
Protein (g/kg)	33.7	37.0	34.5	36.2	0.67	0.001	0.081	0.06
Lactose (g/kg)	46.8	47.0	47.0	46.8	0.33	0.5	0.5	0.3
F&P[4] (kg/d)	2.13	1.89	1.89	2.13	0.10	0.02	0.02	0.4
Energy[5](UFL/d)	12.8	10.4	11.2	12.0	0.57	<0.001	0.15	0.9

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

Table 3. The effect of dry period duration and dietary energy density on dry matter intake, body condition score and energy balance for weeks 1 to 4 and 5 to 12 of lactation.

	Dry Period		Feeding Level		SEM	P - value		
	SDP	NDP	STMR	HTMR		DP	FL	DP×FL
	n = 18	n = 18	n = 18	n = 18				
DMI wk -6 to 0 (kg/d)	9.9	15.5	13.0	12.4	0.59	<0.001	0.5	0.4
DMI wk 1 to 4 (kg/d)	16.0	16.9	16.3	16.6	0.63	0.3	0.8	0.9
DMI wk 5 to 12 (kg/d)	19.6	18.6	18.6	19.8	0.43	0.1	0.01	0.6
BCS wk 1 to 4	2.96	3.25	3.06	3.16	0.12	0.07	0.5	0.3
BCS wk 5 to 12	2.74	3.34	2.83	3.25	0.11	<0.001	0.01	0.6
EB wks 1 to 4 (UFL/d)	-1.92	1.61	-0.83	0.53	0.80	<0.001	0.15	0.3
EB wks 5 to12 (UFL/d)	0.74	2.41	1.23	1.91	0.53	0.02	0.3	0.9
EB nadir (UFL/d)	-5.9	-2.1	-5.2	-2.8	1.05	0.004	0.07	0.9
Week of EB nadir	2.6	2.3	2.5	2.3	0.23	0.3	0.4	0.004
Weeks to zero EB	7.1	4.2	6.5	4.7	0.76	0.003	0.08	0.26

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

	Dry Period		Feeding Level		SEM	P - value		
Transition period	SDP	NDP	STMR	HTMR		DP	FL	DP×FL
	n = 18	n = 18	n = 18	n = 18				
Glucose (mmol/L)	3.35	3.69	3.43	3.61	0.04	<0.001	0.001	0.4
Insulin (µIU/mL)	4.37	7.94	5.35	6.97	0.62	<0.001	0.04	0.14
IGF-I[1] (ng/ml)	110	165	131	144	13.2	0.004	0.5	0.6
NEFA (mmol/L)	0.25	0.16	0.22	0.19	0.03	0.009	0.3	0.003
BHBA (mmol/L)	0.49	0.47	0.58	0.38	0.03	0.5	<0.001	0.018
Urea (mmol/L)	4.80	5.36	4.88	5.28	0.19	0.02	0.09	0.5
Post transition period								
Glucose (mmol/L)	3.52	3.60	3.42	3.70	0.04	0.2	<0.001	0.6
Insulin (µIU/mL)	5.44	7.21	4.69	7.97	0.58	0.02	<0.001	0.7
IGF-I[2] (ng/ml)	114	150	109	155	13.2	0.02	0.006	0.7
NEFA (mmol/L)	0.14	0.09	0.14	0.09	0.02	0.02	0.05	0.046
BHBA (mmol/L)	0.48	0.41	0.54	0.35	0.04	0.2	<0.001	0.12
Urea (mmol/L)	6.09	5.89	5.38	6.59	0.18	0.4	<0.001	0.7

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

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

Table 5. The effect of dry period duration and dietary energy density on the number of small, medium, and large follicles observed at the first postpartum ultrasound examination.

	Dry Period		Feeding level		SEM	P-value		
	SDP n = 18	NDP n = 18	STMR n = 18	HTMR n = 18		DP	FL	DP×FL
Small follicles	11.1	10.8	12.6	9.4	1.55	0.9	0.07	0.9
Medium follicles	5.0	3.3	4.4	3.8	0.86	0.05	0.5	0.9
Large follicles	0.4	0.9	0.6	0.7	0.19	0.004	0.9	0.3
DF diameter[1]	10.0	11.3	10.3	10.9	0.78	0.16	0.5	0.7

[1] DF diameter = dominant follicle diameter

[2] DIM = days in milk at first ultrasound scan

Table 6. The effect of dry period duration and dietary energy density on the reproductive performance of Holstein-Friesian cows during the breeding season

	Dry Period		Feeding Level		SEM	P value		
	SDP	NDP	STMR	HTMR		DP	FL	DP×FL
	n = 18	n = 18	n = 18	n = 18				
CSI (days)	78	83	77	85	7.1	0.5	0.3	0.8
CR1 (%)	29.4	38.9	50	21.1		0.6	0.07	
CCI (days)	126	112	117	120	11.1	0.3	0.8	0.9
Overall PR (%)	64.7	83.3	81.3	68.4		0.2	0.4	

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

Figure 1. The effect of dry period duration on circulating glucose and insulin concentrations from weeks -3 to 12 relative to parturition and IGF-I concentrations from weeks -2 to 9 relative to parturition (n = 18 cows per treatment). *Upper panel*: Cows assigned to the NDP had greater glucose concentrations compared to cows assigned to the SDP during week -3 to 3 relative to parturition (P < 0.001; pooled SEM was 0.04 mmol/L) but not during weeks 4 to 12 postpartum (P = 0.2; pooled SEM was 0.07 mmol/L). *Middle panel*: Cows assigned to the NDP had greater insulin concentrations compared to cows assigned to the SDP during week -3 to 3 relative to parturition (P < 0.001; pooled SEM was 0.62 μIU/ml) and during weeks 4 to 12 (P = 0.02; pooled SEM was 0.83 μIU/ml). *Lower panel*: Cow assigned to the NDP had greater IGF-I concentrations during weeks -2 to 3 relative to parturition (P = 0.004; pooled SEM was 13.1 ng/ml) and during weeks 4 to 9 (P = 0.02; pooled SEM was 13.2 ng/ml) postpartum compared to the SDP treatment.

Figure 2. The effect of dietary energy density on circulating glucose and insulin concentrations from weeks -3 to 12 relative to parturition and IGF-I concentrations from weeks -2 to 9 relative to parturition (n = 18 per treatment). *Upper panel*: cows assigned to the HTMR had greater glucose concentrations during weeks -3 to 3 relative to parturition (P = 0.001; pooled SEM was 0.04 Mmol/L) and during weeks 4 to 12 postpartum (P <0.001; pooled SEM was 0.068 Mmol/L). *Middle panel*: Cows assigned to the HTMR had greater plasma insulin concentrations during weeks -3 to 3 relative to parturition (P = 0.04; pooled SEM was 0.62 μIU/ml) and from weeks 4 to 12 (P, 0.001; pooled SEM was 0.43 μIU/ml). *Lower panel*: There was no effect of diet on circulating IGF-I during weeks -2 to 3 relative to parturition (P = 0.49; pooled SEM was 13.15pg/ml), but cows on the HTMR had greater plasma IGF-I concentrations compared to the STMR cows during weeks 4 to 9 postpartum (P =0.006; pooled SEM was 13.24 ng/ml).

Figure 3. Effect of dry period duration on circulating FSH concentrations during the first 8 days postpartum (n = 18/treatment). Cows assigned to the SDP treatment had greater FSH concentrations than cows assigned to the NDP treatment (P = 0.007; pooled SEM = 0.034 ng/ml).

Figure 4. Effect of dry period duration and dietary energy density on NEFA concentrations. Upper panel: Circulating NEFA concentrations during weeks -3 to =3 relative to parturition. Significant effects of dry period duration (P = 0.01), and the interaction between dry period duration and feeding level were observed (P value = 0.003). The effect of feeding level was not significant (P = 0.3). The pooled SEM was 0.03 mmol/L. Lower panel: Circulating NEFA concentrations during weeks 4 to 12 postpartum. Significant effects of dry period duration (P = 0.03), feeding level (P = 0.049) and the interaction between dry period duration and feeding level were observed (P value = 0.046). The pooled SEM was 0.02 Mmol/L. The number of animals per treatment was 8, 10, 9 and 9 for SDP/STMR, SDP/HTMR, NDP/STMR, and NDP/HTMR, respectively.

Figure 5. Effect of dry period duration and feeding level on peak circulating E2 concentrations during the first postpartum follicular wave. A significant interaction between dry period duration and feeding level was observed (P = 0.03), but the effects of dry period duration (P = 0.2) and feeding level were not significant (P = 0.4). The

pooled SEM was 0.46 pg/ml. The number of animals per treatment was 8, 10, 9 and 9 for SDP/STMR, SDP/HTMR, NDP/STMR, and NDP/HTMR, respectively.

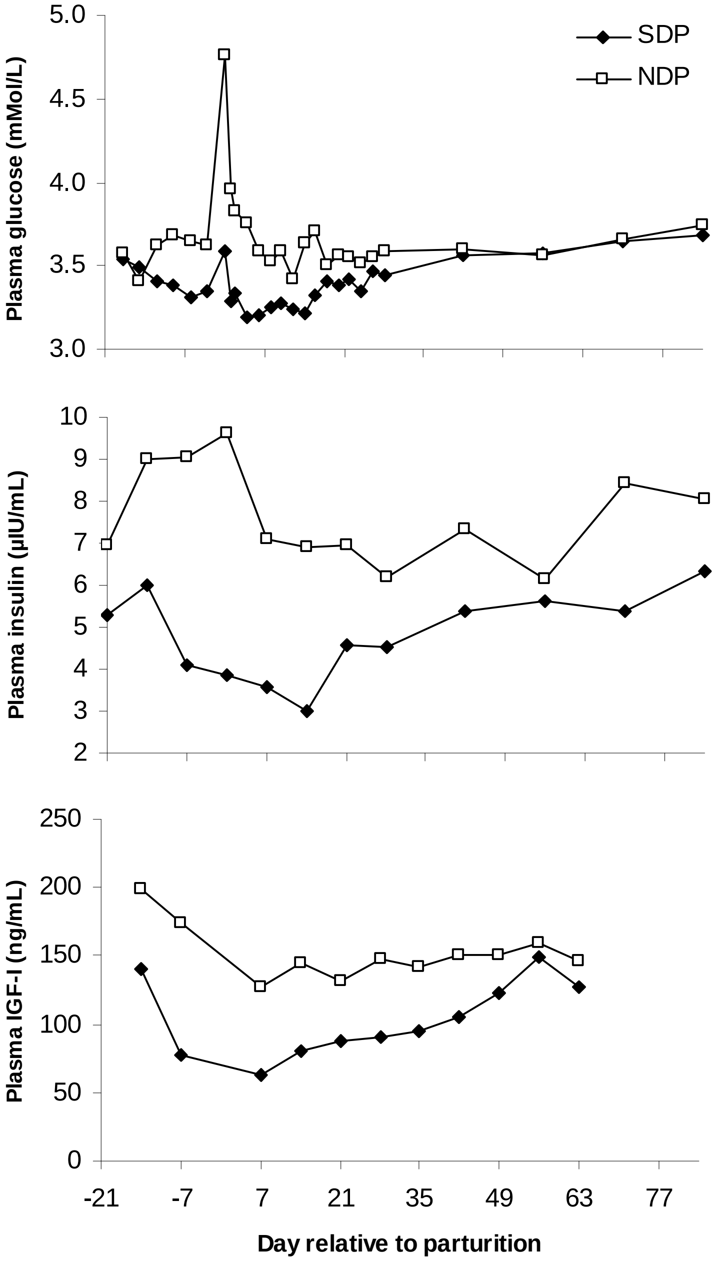

Figure 1 - de Feu

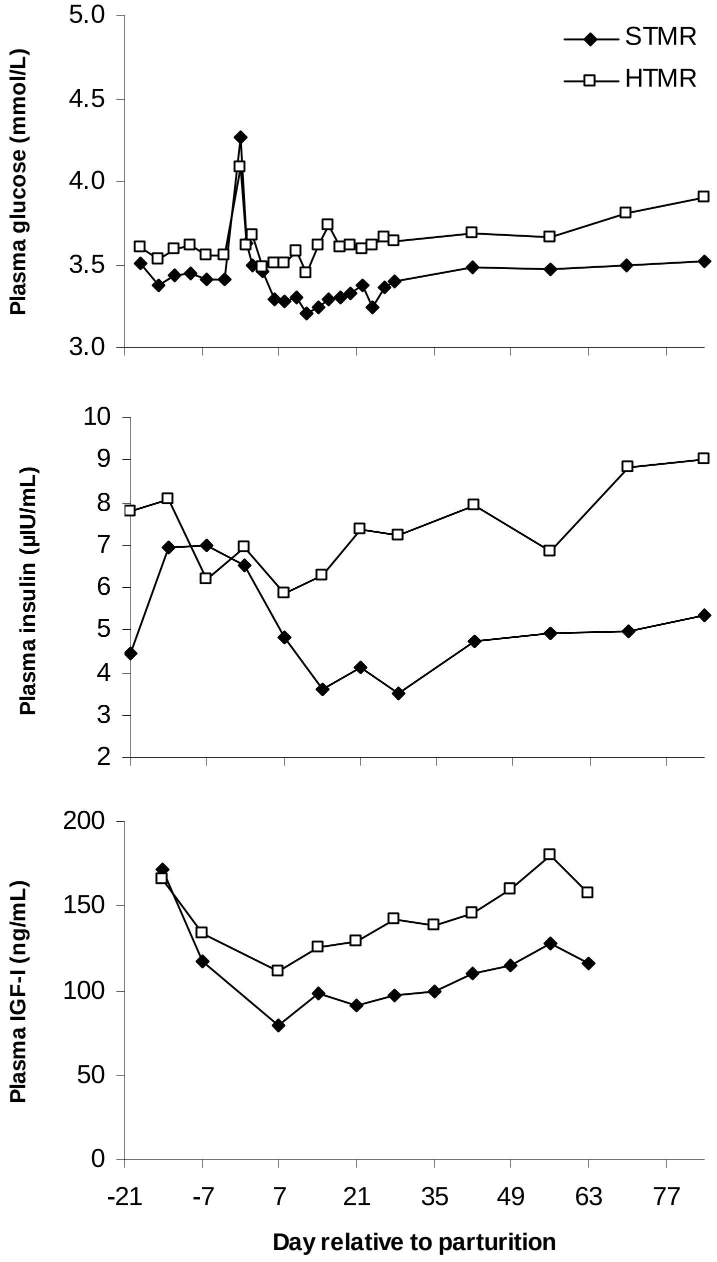

Figure 2 - de Feu

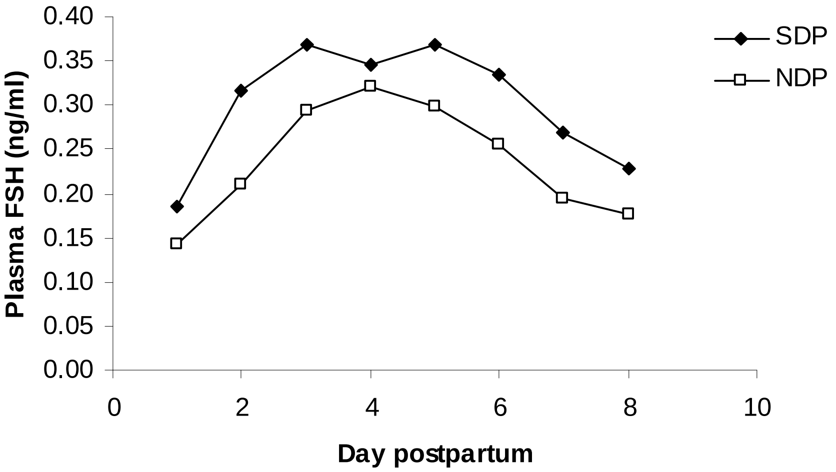

Figure 3 - de Feu

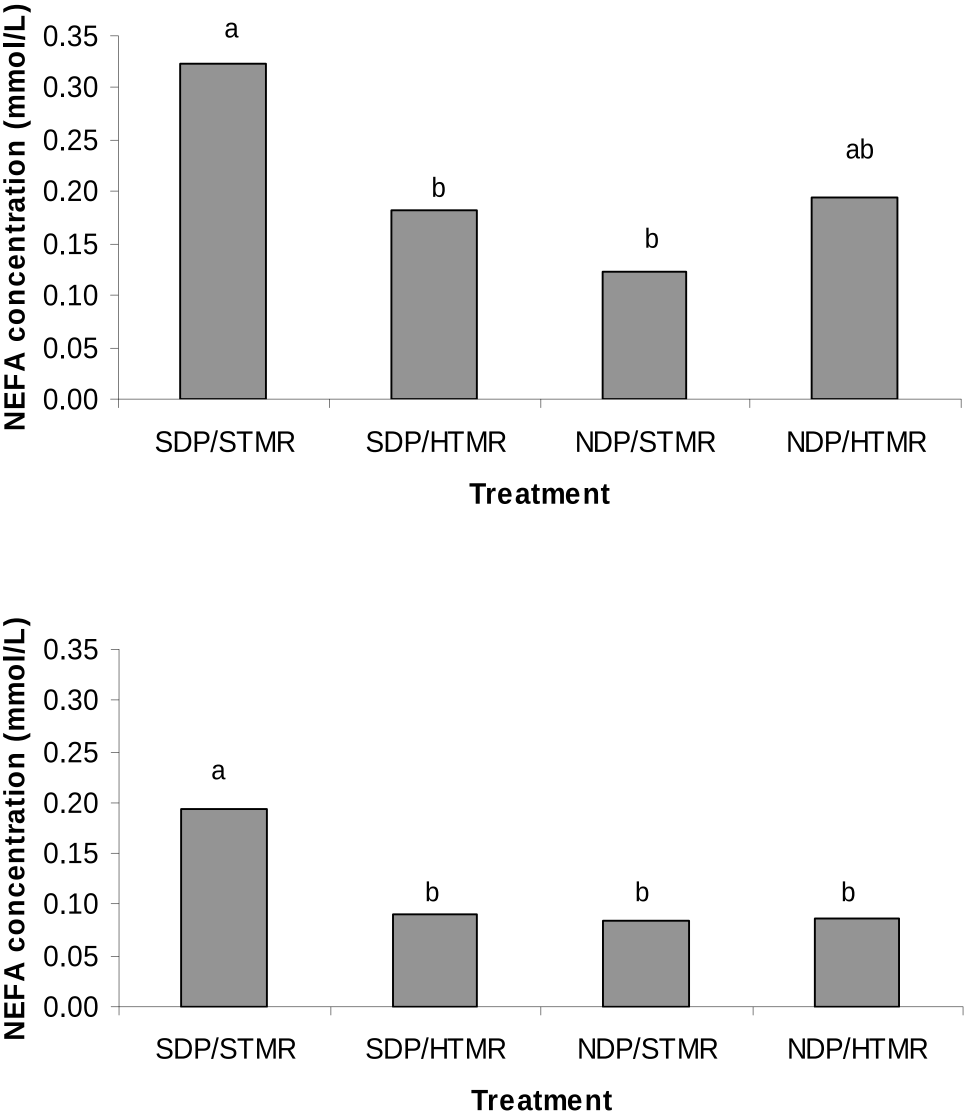

Figure 4 - de Feu

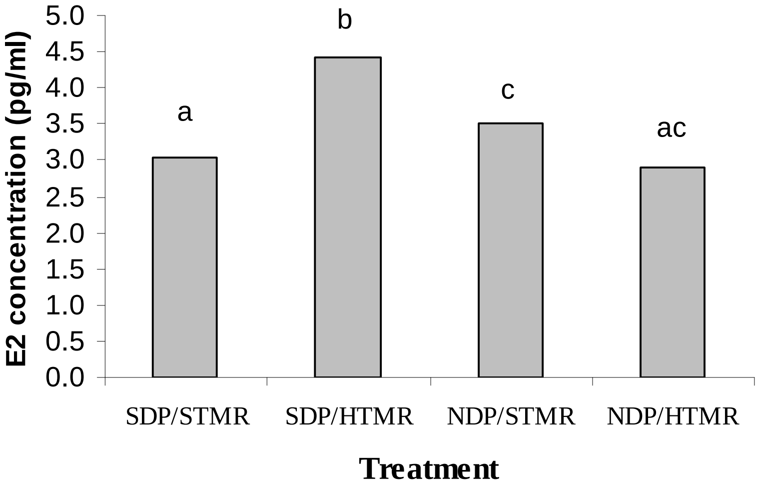

Figure 5 - de Feu