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DOI: [10.3168/jds.2009-2374](https://doi.org/10.3168/jds.2009-2374)

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27

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

31

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33

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39

40 Interpretive summary:

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

## ABSTRACT

50

51 Following parturition, it is typical for dairy cows to enter a period of negative  
52 energy balance (NEB) and body condition loss to support mammary milk synthesis,  
53 and this is associated with compromised reproductive performance. Alternative  
54 management strategies during the prepartum (dry) and early post partum periods may  
55 ameliorate this. Forty mature Holstein-Friesian cows were assigned to one of two dry  
56 period treatments (standard 8 week dry period (**SDP**) or no planned dry period  
57 (**NDP**)) and one of two dietary energy density treatments (standard TMR (**STMR**) or  
58 high quality TMR (**HTMR**)). Milk yield during weeks 1 to 12 postpartum was  
59 reduced ( $P = 0.01$ ) in cows assigned to the NDP treatment. Energy balance ( $P <$   
60  $0.001$ ) and body condition score ( $P = 0.07$ ) during weeks 1 to 4 postpartum were  
61 increased in cows assigned to the NDP treatment compared to the cows assigned to  
62 the SDP, and BCS increased ( $P < 0.001$ ) from weeks 5 to 12 postpartum in the NDP  
63 cows compared to the SDP cows. During the first 12 weeks postpartum, cows  
64 assigned to the HTMR had greater ( $P = 0.02$ ) milk yields and reduced ( $P < 0.001$ )  
65 milk fat concentration compared to the cows assigned the STMR diet. BCS was  
66 greater ( $P = 0.01$ ) from weeks 5 to 12 postpartum in HTMR cows compared to STMR  
67 cows. During the period from weeks -3 to +3 relative to parturition, circulating  
68 concentrations of insulin ( $P = 0.001$ ), glucose ( $P < 0.001$ ) and IGF-I ( $P = 0.004$ ) were  
69 greater in cows on the NDP treatment compared to cows on the SDP treatment. Cows  
70 assigned to the HTMR had greater circulating insulin ( $P = 0.04$ ) and glucose ( $P =$   
71  $0.001$ ) concentrations compared to the STMR cows from weeks -3 to +3 relative to  
72 parturition. The first postpartum ovulation occurred earlier for cows on the NDP  
73 treatment compared to cows on the SDP treatment (16.9 vs. 24.8 days postpartum;  $P =$   
74  $0.02$ ). Cows assigned to the STMR tended to have a higher conception rate to first

75 service ( $P = 0.07$ ) compared to cows assigned to the HTMR. Energy balance and  
76 metabolic status can be improved by either eliminating the dry period or by feeding a  
77 higher energy diet, but effects on the reproductive axis appear to be different.

78

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

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81

82

## INTRODUCTION

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

90 The onset of lactation in dairy cattle causes a dramatic increase in mammary  
91 glucose requirements, and marked changes in whole body metabolism are required to  
92 accommodate these needs. Following parturition, high-producing dairy cows typically  
93 experience a variable period of negative energy balance (NEB), as DMI is inadequate  
94 to fully meet the rising energetic requirements of milk production. The severity and  
95 duration of NEB experienced in early lactation affects the postpartum interval to first  
96 ovulation and has a detrimental effect on subsequent likelihood of conception (Butler  
97 and Smith, 1989; Villa Godoy et al 1988). A delay in the onset of ovulatory ovarian  
98 activity limits the number of oestrous cycles prior to breeding, reducing the likelihood  
99 of conception and increasing the number of days open (Butler, 2003). Nutritional

100 approaches to overcome early lactation NEB have been largely unsuccessful. This is  
101 primarily due to the inherent drive to produce additional milk in response to additional  
102 nutrient intake — the hallmark of the modern Holstein-Friesian dairy cow. The  
103 metabolic and endocrine milieu that ensues during NEB is antagonistic to resumption  
104 of ovulatory ovarian activity (Butler et al., 2006), resulting in anestrus and reduced  
105 conception rates.

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

119

120

## MATERIALS AND METHODS

### *Animals and Experimental Design*

122 This experiment was a completely randomized block design with a 2 × 2  
123 factorial arrangement of treatments. Forty mature Holstein-Friesian cows were  
124 blocked on the basis of expected calving date, previous lactation yield, bodyweight

125 and body condition score (BCS), and were randomly assigned to one of two dry  
126 period treatments (standard 8 week dry period (**SDP**) or no planned dry period  
127 (**NDP**)) and one of two dietary energy density treatments (standard TMR (**STMR**) or  
128 high quality TMR (**HTMR**)). Cows assigned to SDP were fed ad libitum grass silage  
129 prepartum, and either the STMR or HTMR during the first 12 weeks postpartum.  
130 Cows assigned to the NDP treatment were fed either the STMR or HTMR diet during  
131 the dry period and the first 12 weeks postpartum. If prepartum daily milk yield  
132 dropped below 2 kg/day for cows on the NDP treatments, milking was discontinued  
133 for the remainder of the prepartum period. Actual dry period lengths (mean  $\pm$  SEM)  
134 were  $62.1 \pm 1.9$  days and  $6.3 \pm 1.7$  days for cows on the SDP and NDP treatments,  
135 respectively. Two cows were dropped from the SDP treatment and two cows were  
136 dropped from the NDP treatment due to their dry periods being too short (SDP), too  
137 long (NDP), or illnesses unrelated to the study.

138

### 139 *Animal Measurements*

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

147 Daily measurements of dry matter intake were recorded using the Griffith-Elder  
148 MealMaster system (Griffith Elder & Co Ltd, Suffolk, UK). The ingredients for all  
149 diets were sampled on a weekly basis. Silage pH was measured on the juice pressed

150 from the silage using a glass electrode and a pH meter (Radiometer pHM2 standard  
151 pH meter-radiometer, Copenhagen). The dry matter, crude protein, NDF, ash, starch  
152 and oil content of the feed samples were analysed as described by McNamara *et al.*  
153 (2003). The ingredient and nutrient composition of the diets is summarized in Table  
154 1.

155 Bodyweight (BW) and body condition score (BCS) were measured weekly by the  
156 same technician. Energy Balance (EB) was estimated as the difference between  
157 energy intake and the sum of energy requirements for maintenance and milk  
158 production, using the French net energy (NE) system (Jarrige, 1989). The French  
159 system uses unité fourragère lait (UFL) as the unit of net energy, and is equivalent to  
160 1 kg of standard air-dry barley. The following equations were used to determine the  
161 energy required for maintenance and output in milk (O'Mara, 1997):

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

163 Energy requirement for milk (UFL/kg of milk) =  $0.0054FC + 0.0031PC + 0.0028LC$   
164  $- 0.015$ ;

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

167

### 168 ***Blood Sampling and Analysis***

169 Blood samples were collected 3 times/week for the final 3 weeks prepartum,  
170 daily for the first 28 days postpartum and once per fortnight thereafter until day 84 of  
171 lactation. All blood samples were collected from the coccygeal blood vessels into 10  
172 ml lithium heparin vacutainers (Becton Dickinson, Plymouth, UK). The blood  
173 samples were centrifuged at  $2000 \times g$  for 15 minutes at 5 °C. The plasma was

174 harvested and decanted into 1.5 ml aliquots, and stored at -20 °C until further  
175 analyses.

176 Plasma samples were analysed for indicators of metabolic status from day -12 to  
177 84 relative to parturition. Glucose, non-esterified fatty acids (NEFA), urea and beta-  
178 hydroxybutyrate (BHBA) concentrations were analysed by enzymatic colorimetry  
179 using appropriate kits and an ABX Mira autoanalyser (ABX Mira, Cedex 4, France).

180 Plasma insulin concentrations were determined using a solid-phase  
181 fluoroimmunoassay (AutoDELFIA, PerkinElmer Life and Analytical Science, Turku,  
182 Finland) using appropriate kits (Unitech BD Ltd., Dublin, Ireland). The inter- and  
183 intra-assay coefficients of variation were 10.9 and 4.5%, respectively (n = 3).

184 Circulating IGF-I concentrations were quantified using a validated double-  
185 antibody radioimmunoassay following ethanol-acetone-acetic acid extraction (Enright  
186 *et al.*, 1989). Recombinant human IGF-I (R&D Systems Europe, UK) was used as a  
187 standard and to generate iodinated tracer. The assay was carried out as described by  
188 Spicer *et al.* (1990). Inter- and intra-assay coefficients of variation were 27.5 and  
189 14.9%, respectively (n = 8). Circulating FSH concentrations were analysed in daily  
190 plasma samples collected from the day of parturition until 8 days in milk using a  
191 validated radioimmunoassay (Crowe *et al.*, 1997). The inter- and intra-assay  
192 coefficients of variation were 5.7 and 9.5%, respectively (n = 3).

193 Circulating estradiol concentrations (E2) were measured in blood samples  
194 collected on consecutive days during development of the dominant follicle of the first  
195 postpartum follicle wave. If ovulation occurred, plasma E2 concentrations were  
196 measured on each of the 7 days immediately prior to ovulation. If the cow had a  
197 dominant follicle that underwent atresia or became cystic, E2 concentrations were  
198 measured in blood samples collected daily from emergence of the dominant follicle

199 until the point of maximum observed follicle diameter. The concentration of E2 in  
200 plasma was determined by radioimmunoassay following extraction (Prendiville, 1995)  
201 using E2 MAIA kits (Biostat, UK). Inter- and intra-assay coefficients of variation  
202 were 21.5 and 13.1%, (n = 4). For all hormone assays, each treatment was equally  
203 represented in each assay, and all samples for a cow on a given treatment were  
204 completed in a single assay.

205

### 206 *Postpartum Ultrasound Evaluation and Reproductive Management*

207 Ovarian follicular activity was examined by linear array ultrasonography (Aloka  
208 900; 7.5-MHz transrectal transducer, Aloka Ltd., Tokyo, Japan) thrice weekly  
209 beginning on day 8-10 postpartum and continuing until first ovulation. Ovulation was  
210 deemed to have occurred following the disappearance of a dominant follicle and the  
211 subsequent appearance of a corpus luteum (CL). The size of a large follicle was  
212 determined by finding the average diameter in two directions at right angles on a  
213 single frozen image. The number of small (< 5 mm), medium (5 – 10 mm) and large  
214 (> 10 mm) follicles were recorded for each ovary at every ultrasound examination. If  
215 ovulation had not occurred by day 60 postpartum, cows were treated to commence  
216 cycling with the following hormone programme: Day 0 GnRH (20 µg Buserelin;  
217 Receptal, Intervet Ireland, Dublin) and CIDR insertion (InterAg, New Zealand); Day  
218 7 PGF<sub>2α</sub> (500 µg cloprostenol sodium; Estrumate, BP (Vet) Coopers, Berkhamsted,  
219 England); Day 9 CIDR removal. Ovulation was confirmed using transrectal  
220 ultrasonography to visualize a CL approximately 7 days after CIDR removal.  
221 Initiation of breeding commenced on a calendar mating start date (27<sup>th</sup> of November  
222 2005). Tail paint was used as a heat detection aid, and all cows were artificially  
223 inseminated (AI) following observation of standing estrus and/or removal of tail paint.

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

227

### 228 *Data Handling and Statistical Analysis*

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

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

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

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

256

257

## RESULTS

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

261

### 262 *Milk Production and Composition*

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

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

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

275

### 276 *Dry Matter Intake, Energy Balance and Body Condition Score*

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

287 There was no difference in DMI between cows assigned to STMR compared  
288 to those assigned to HTMR during weeks 1 to 4 ( $P = 0.8$ ), but during weeks 5 to 12  
289 cows assigned to the HTMR had increased DMI compared to those on the STMR diet  
290 ( $P = 0.01$ ). There was no difference in calculated EB between cows on the HTMR and  
291 STMR diets during either weeks 1 – 4 or weeks 5 – 12. The EB nadir tended to be  
292 lower ( $P = 0.07$ ) and the duration from parturition to return to zero EB tended to be  
293 longer ( $P = 0.08$ ) for cows on the STMR diet compared to cows on the HTMR diet.  
294 There was no effect of diet on BCS during weeks 1 – 4 postpartum, but cows fed the  
295 HTMR had greater BCS during weeks 5 – 12 compared to cows fed the STMR ( $P =$   
296  $0.01$ ).

297

298 ***Plasma Insulin, IGF-I and Metabolites***

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

310           During the transition period, cows fed the HTMR diet had increased  
311 concentrations of glucose compared to cows fed the STMR diet ( $P < 0.001$ ) whereas  
312 cows on the STMR had significantly greater concentrations of BHBA ( $P < 0.001$ ).  
313 There was no effect of dietary energy density on insulin or IGF-I during the transition  
314 period. During the post-transition period cows fed the HTMR diet had greater  
315 circulating concentrations of insulin ( $P = 0.015$ ), glucose ( $P < 0.001$ ) and urea ( $P <$   
316  $0.001$ ) compared to cows on the STMR diet. Cows on the STMR diet had increased  
317 circulating concentrations of NEFA ( $P = 0.04$ ) and BHBA ( $P < 0.001$ ) compared to  
318 cows fed the HTMR diet during the post-transition period. Cows on the HTMR diet  
319 had greater circulating IGF-I concentrations ( $P = 0.006$ ) compared to cows fed the  
320 STMR diet for weeks 4 to 9 relative to parturition (Figure 2 and Table 4).

321

322 ***Reproductive hormones and follicular dynamics***

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

337 Ovulation occurred later in cows assigned to the SDP treatment compared to  
338 cows assigned to the NDP treatment ( $16.9 \pm 2.5$  vs.  $24.8 \pm 2.6$  days in milk; NDP vs.  
339 SDP, respectively;  $P = 0.02$ ). This corresponded to 83.3%, 11.1% and 5.6%, of cows  
340 assigned to the NDP treatment having their first ovulation during the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup>  
341 or later postpartum follicular waves, respectively, whereas for cows assigned to the  
342 SDP treatment these values were 64.7%, 23.5% and 11.8% respectively.

343 For cows that had an ovulation during the first postpartum follicle wave, there  
344 was no effect of dry period duration on peak E2 concentrations, day postpartum when  
345 peak E2 occurred, maximum follicle diameter, or day postpartum when maximum  
346 follicle diameter was observed (results not shown). For cows that failed to ovulate the  
347 first postpartum follicle wave, follicles underwent atresia ( $n = 3$  and 3) or developed

348 into follicular cysts (n = 3 and 0) in SDP and NDP treatment cows, respectively.  
349 There was no significant effect of dry period duration on calving to service interval,  
350 conception rate to first service, calving to conception interval or overall pregnancy  
351 rate (Table 6).

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

362 Dietary energy density did not affect the timing of the first postpartum  
363 ovulation ( $20.8 \pm 2.7$  vs.  $20.9 \pm 2.5$  DIM, HTMR vs. STMR, respectively; P = 0.9).  
364 This corresponded to 73.7%, 10.5% and 15.8% of cows assigned to the HTMR having  
365 their first ovulation during the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> or later postpartum follicular waves,  
366 respectively, whereas the values for cows assigned to the STMR were 70.6%, 23.5%  
367 and 5.9%, respectively.

368 For cows that had an ovulation during the first postpartum follicle wave, there  
369 was no effect of feeding level on peak E2 concentration, day postpartum when peak  
370 E2 occurred, maximum follicle diameter, or day postpartum when maximum follicle  
371 diameter was observed (results not shown). For cows that failed to ovulate the first  
372 postpartum follicle wave, follicles underwent atresia (n = 3 and 2) or developed into

373 follicular cysts (n = 2 and 2) in the HTMR and STMR treatments, respectively. Cows  
374 assigned to the STMR diet tended to have a greater conception rate to first service  
375 compared to cows on the HTMR diet (50.0 vs. 21.1%; STMR vs. HTMR,  
376 respectively; P = 0.07) but there was no effect of feeding level on the overall  
377 pregnancy rate at the end of the breeding period (Table 6).

378

### 379 *Interactions between dry period duration and feeding level*

380 *Time of EB nadir, plasma NEFA and BHBA.* An interaction between dry period  
381 duration and feeding level was observed for the interval from parturition to the EB  
382 nadir. For cows on the NDP treatment, the HTMR diet resulted in a shorter interval  
383 from parturition to the EB nadir compared to the STMR diet ( $2.86 \pm 0.30$  vs.  $1.71 \pm$   
384  $0.28$  weeks; P = 0.03), but there was no effect of dietary feeding level for cows on the  
385 SDP treatment ( $2.25 \pm 0.34$  vs.  $2.88 \pm 0.27$  weeks; P = 0.4). Similarly, cows on the  
386 SDP treatment had a longer interval to EB nadir compared to cows on the NDP  
387 treatment when fed the HTMR diet ( $2.88 \pm 0.27$  vs.  $1.71 \pm 0.28$  weeks; P = 0.02), but  
388 there was no effect of dry period treatment when cows were fed the STMR diet ( $2.25$   
389  $\pm 0.34$  vs.  $2.86 \pm 0.30$  weeks; P = 0.5).

390 An interaction between dry period duration and feeding level was also  
391 observed for circulating NEFA concentrations (Figure 4). HTMR decreased  
392 circulating NEFA concentrations for SDP cows ( $0.32 \pm 0.05$  vs.  $0.18 \pm 0.04$  mmol/L;  
393 P = 0.03), but had no effect for NDP cows ( $0.12 \pm 0.04$  vs.  $0.19 \pm 0.04$  mmol/L; P >  
394 0.3) during the transition period (interaction between dry period length and feeding  
395 level: P = 0.003). Similarly during the post-transition period, HTMR decreased  
396 circulating NEFA concentrations for SDP cows ( $0.19 \pm 0.03$  vs.  $0.09 \pm 0.03$  mmol/L;

397 P = 0.04), but had no effect for NDP cows ( $0.08 \pm 0.03$  vs.  $0.09 \pm 0.03$  mmol/L; P >  
398 0.9), resulting in a significant interaction (P = 0.045).

399 A similar interaction (P = 0.018) was observed for circulating BHBA  
400 concentrations during the transition period. HTMR decreased circulating BHBA for  
401 cows on the SDP treatment ( $0.64 \pm 0.05$  vs.  $0.34 \pm 0.04$  mmol/L; P < 0.001), but had  
402 no effect for cows on the NDP treatment ( $0.52 \pm 0.04$  vs.  $0.42 \pm 0.04$  mmol/L; P =  
403 0.3).

404

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

413

414

## DISCUSSION

415 The main findings from this study are that (i) omitting the dry period or  
416 feeding a higher energy density TMR resulted in improved energy balance and  
417 metabolic status, but the improvements were achieved via different mechanisms; (ii)  
418 postpartum plasma FSH concentrations and ovarian follicular development were  
419 affected by dry period duration; (iii) interval to first ovulation was reduced by  
420 omitting the dry period, but feeding a higher energy TMR had no effect. The results  
421 indicate that periparturient energy balance can be improved by altering management

422 practices (dry period duration, feeding level). The results also suggest that improving  
423 energy balance/metabolic status *per se* will not necessarily result in an earlier onset of  
424 cyclicity.

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

436         The cows assigned to the NDP treatment experienced only mild NEB for a  
437 short duration, and accordingly did not lose BCS postpartum. In contrast, the cows on  
438 the SDP treatment were in NEB for an average duration of 7.1 weeks following  
439 parturition, and on average lost 0.5 units of body condition. The improved EB status  
440 of the NDP cows was achieved via a reduction in milk energy output during the first  
441 12 weeks of lactation (2.4 UFL/day) while maintaining similar energy intake and  
442 maintenance requirements to cows on the SDP treatment. Similar to the cows on the  
443 NDP treatment, cows assigned to the HTMR treatment did not lose BCS during the  
444 postpartum period. However, in contrast to the NDP treatment, the HTMR diet  
445 resulted in a non-significant increase in total milk energy output, a significant increase  
446 in energy intake, with an overall effect of a non-significant improvement in calculated

447 energy balance. Hence, reducing the duration of the dry period decreased the inherent  
448 drive to produce milk in the subsequent lactation, whereas increasing dietary energy  
449 density allowed dietary energy intake to more closely meet energy requirements,  
450 albeit at a higher daily milk yield. During the first 12 weeks postpartum, cows  
451 assigned to the NDP treatment had increased milk protein concentrations compared to  
452 SDP treatment cows. This is in agreement with previous reports that continuous  
453 milking results in higher milk protein concentrations (Remond *et al.*, 1997; Rastani *et*  
454 *al.*, 2005), and likely reflects their superior energy balance status. In contrast, the  
455 HTMR diet tended to increase milk protein concentration, and resulted in a significant  
456 reduction in milk fat concentration. Reduced milk fat concentration is commonly  
457 observed with high energy diets, and is thought to be due to rumen biohydrogenation  
458 intermediates exerting direct inhibitory effects on mammary milk fat synthesis  
459 (Bauman and Griinari, 2003).

460       Following expulsion of the foetal-placental unit and clearance of gestational  
461 steroids, plasma FSH concentrations increase within 3-5 days postpartum, and this  
462 plays a pivotal role in orchestrating the emergence of a new follicular wave (Beam  
463 and Butler, 1997). In the current study, dietary energy density had no effect on  
464 postpartum FSH concentrations, but cows on the SDP treatment had greater  
465 concentrations of FSH compared to the NDP treatment group. Gümen *et al.* (2005)  
466 reported that cows assigned to a continuous milking treatment had lower postpartum  
467 circulating FSH concentrations compared to cows on a traditional dry period  
468 treatment on Day 6 postpartum. Those authors speculated that the cows on the NDP  
469 treatment had their postpartum FSH surge earlier than Day 6 postpartum, but this  
470 could not be detected because blood samples were not collected from Day 1 to 6  
471 postpartum in their experimental protocol. Our results do not support their hypothesis

472 as we observed no differences in the timing of the postpartum FSH surge between the  
473 NDP and SDP treatment groups. Prepartum circulating E2 concentrations are reduced  
474 by omitting the dry period (Gümen et al., 2005), and could potentially impact  
475 postpartum pituitary release of FSH.

476         The greater circulating FSH concentrations observed in the SDP treatment  
477 group did not affect the number of small follicles at the first postpartum ultrasound  
478 examination. Interestingly, at the same ultrasound examination, the SDP treatment  
479 had a greater number of medium follicles (5 – 10 mm), whereas the NDP treatment  
480 had a greater number of large follicles (> 10 mm). It is likely that this simply reflects  
481 a more advanced stage of follicle development, in that the cows on the NDP treatment  
482 had already developed a dominant follicle, whereas the SDP treatment cows had a  
483 number of follicles at the selection stage of development. Our observations on follicle  
484 development are consistent with those of Gümen et al., (2005), who carried out the  
485 first postpartum scan on day 6 postpartum, and reported a significantly larger mean  
486 follicle diameter for continuously milked cows compared to cows with a normal dry  
487 period. These data clearly indicate that increased circulating concentrations of FSH  
488 do not increase the rate of follicular development. A negative correlation was  
489 observed between the diameter of the dominant follicle at the first postpartum  
490 ultrasound examination and the day postpartum when peak FSH concentration  
491 occurred. Hence, the earlier the postpartum increase in FSH concentrations occurred,  
492 the greater the size of the DF on day 8 to 10 postpartum. This is consistent with  
493 previous reports indicating that emergence of the first follicle wave postpartum is  
494 related to the timing of the postpartum FSH surge (Beam and Butler, 1997). Despite  
495 not observing differences in the timing of peak FSH concentrations between either the  
496 dry period duration or dietary energy density treatments, the first postpartum

497 ovulation occurred earlier for cows on the NDP treatment compared to cows on the  
498 SDP treatment, but dietary energy density had no significant effect.

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

522 Lucy et al. (1991) reported that as predicted energy balance increased during  
523 the first 25 days postpartum, there was a decrease in the average number of small  
524 follicles (6 to 9 mm) and an increase in the average number of large follicles (10 to  
525 15+ mm), indicating that small follicles mature to larger follicles earlier in cows in  
526 superior energy balance status. It is likely that the superior EB status and increased  
527 concentrations of insulin and IGF-I for cows on the NDP treatment resulted in greater  
528 LH pulse frequency (Canfield et al., 1990), and accordingly the NDP treatment had an  
529 earlier onset of cyclicity compared to the SDP treatment. Interestingly, the HTMR  
530 treatment also resulted in greater circulating concentrations of insulin, IGF-I,  
531 improved BCS, and circulating metabolite concentrations indicative of superior EB.  
532 Despite this, feeding the HTMR diet did not advance the onset of cyclicity, and  
533 tended to have a negative effect on subsequent conception rate to first service. It is  
534 plausible to hypothesise that the greater metabolic burden of increased milk output  
535 and greater liver blood flow due to increased DMI increased steroid hormone  
536 clearance, but neither the milk production nor the DMI in the current study were high  
537 by the standards of the modern Holstein-Friesian dairy cow. Nevertheless, the  
538 observation of poorer reproductive performance with increased concentrate  
539 supplementation is not consistent with other reports from this research centre and  
540 elsewhere (Horan et al., 2004; Pollott et al. 2008), and may be an artefact of the small  
541 number of animals enrolled in the study.

542

543

## CONCLUSION

544 The results indicate that omitting the dry period and feeding a higher energy density  
545 diet results in superior metabolic status. The improved bioenergetic status was  
546 achieved via contrasting mechanisms. Omitting the dry period reduced the drive to

547 produce milk, whereas increasing dietary energy density allowed the feed consumed  
548 to more closely meet energy requirements, despite increased milk output. Omitting  
549 the dry period advanced the interval to first postpartum ovulation, whereas feeding a  
550 high energy TMR had no effect on onset of cyclicity. This study clearly shows that  
551 events during the dry period and early lactation critically affect nutrient partitioning,  
552 metabolism, milk production, and the reproductive axis.

553

554

### **ACKNOWLEDGEMENTS**

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

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

<b>Diet ingredients</b>	<b>STMR</b>	<b>HTMR</b>
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 <sup>1</sup>	0.02	0.01
<b>Nutrient composition (DM basis)</b>		
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

636 <sup>1</sup>Vitamin and mineral mix: 15g/kg DiCa P, 8g/kg Limestone Flour, 5g/kg Salt, 2.5 g/kg Cal-Mag, 80gm  
637 Manganous Oxide, 200gm Copper Sulphate, 125gm Zinc Oxide, 18gm Potassium Iodate, 20gm  
638 Sodium Selenite (4.6%), 10gm Cobalt Sulphate, 8MIU/t vitamin A, 2MIU/t vitamin D3, 15,000iu/t  
639 vitamin E.  
640

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

	Dry Period		Feeding Level		SEM	P - value		
	SDP n = 18	NDP n = 18	STMR n = 18	HTMR n = 18		DP <sup>1</sup>	FL <sup>2</sup>	DP×FL
SCM <sup>3</sup> (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 <sup>4</sup> (kg/d)	2.13	1.89	1.89	2.13	0.10	0.02	0.02	0.4
Energy <sup>5</sup> (UFL/d)	12.8	10.4	11.2	12.0	0.57	<0.001	0.15	0.9

643 <sup>1</sup>DP = dry period duration

644 <sup>2</sup>FL = feeding level

645 <sup>3</sup>SCM = solids corrected milk yield

646 <sup>4</sup>F&P = fat and protein yield

647 <sup>5</sup>Milk energy output

648

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

	Dry Period		Feeding Level		SEM	P - value		
	SDP n = 18	NDP n = 18	STMR n = 18	HTMR n = 18		DP	FL	DP×FL
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 to 12 (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

652

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

<u>Transition period</u>	Dry Period		Feeding Level		SEM	P - value		
	SDP n = 18	NDP n = 18	STM n = 18	HTM n = 18		DP	FL	DP×FL
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 <sup>1</sup> (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
<u>Post transition period</u>								
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 <sup>2</sup> (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

656 <sup>1</sup> IGF-I was measured from weeks -2 to 3 relative to parturition

657 <sup>2</sup> IGF-I was measured from weeks 4 to 9 relative to parturition

658 **Table 5.** The effect of dry period duration and dietary energy density on the number  
 659 of small, medium, and large follicles observed at the first postpartum ultrasound  
 660 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 <sup>1</sup>	10.0	11.3	10.3	10.9	0.78	0.16	0.5	0.7

661 <sup>1</sup>DF diameter = dominant follicle diameter  
 662 <sup>2</sup>DIM = days in milk at first ultrasound scan

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

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

665 CSI = calving to service interval  
 666 CR1 = conception rate to first service  
 667 CCI = calving to conception interval  
 668 PR = pregnancy rate.

669

670

671 **Figure 1.** The effect of dry period duration on circulating glucose and insulin  
672 concentrations from weeks -3 to 12 relative to parturition and IGF-I concentrations  
673 from weeks -2 to 9 relative to parturition (n = 18 cows per treatment). *Upper panel:*  
674 Cows assigned to the NDP had greater glucose concentrations compared to cows  
675 assigned to the SDP during week -3 to 3 relative to parturition (P < 0.001; pooled  
676 SEM was 0.04 mmol/L) but not during weeks 4 to 12 postpartum (P = 0.2; pooled  
677 SEM was 0.07 mmol/L). *Middle panel:* Cows assigned to the NDP had greater insulin  
678 concentrations compared to cows assigned to the SDP during week -3 to 3 relative to  
679 parturition (P < 0.001; pooled SEM was 0.62  $\mu$ IU/ml) and during weeks 4 to 12 (P =  
680 0.02; pooled SEM was 0.83  $\mu$ IU/ml). *Lower panel:* Cow assigned to the NDP had  
681 greater IGF-I concentrations during weeks -2 to 3 relative to parturition (P = 0.004;  
682 pooled SEM was 13.1 ng/ml) and during weeks 4 to 9 (P = 0.02; pooled SEM was  
683 13.2 ng/ml) postpartum compared to the SDP treatment.  
684

685 **Figure 2.** The effect of dietary energy density on circulating glucose and insulin  
686 concentrations from weeks -3 to 12 relative to parturition and IGF-I concentrations  
687 from weeks -2 to 9 relative to parturition (n = 18 per treatment). *Upper panel:* cows  
688 assigned to the HTMR had greater glucose concentrations during weeks -3 to 3  
689 relative to parturition (P = 0.001; pooled SEM was 0.04 Mmol/L) and during weeks 4  
690 to 12 postpartum (P < 0.001; pooled SEM was 0.068 Mmol/L). *Middle panel:* Cows  
691 assigned to the HTMR had greater plasma insulin concentrations during weeks -3 to 3  
692 relative to parturition (P = 0.04; pooled SEM was 0.62  $\mu$ IU/ml) and from weeks 4 to  
693 12 (P, 0.001; pooled SEM was 0.43  $\mu$ IU/ml). *Lower panel:* There was no effect of  
694 diet on circulating IGF-I during weeks -2 to 3 relative to parturition (P = 0.49; pooled  
695 SEM was 13.15pg/ml), but cows on the HTMR had greater plasma IGF-I  
696 concentrations compared to the STMR cows during weeks 4 to 9 postpartum (P  
697 = 0.006; pooled SEM was 13.24 ng/ml).  
698

699 **Figure 3.** Effect of dry period duration on circulating FSH concentrations during the  
700 first 8 days postpartum (n = 18/treatment). Cows assigned to the SDP treatment had  
701 greater FSH concentrations than cows assigned to the NDP treatment (P = 0.007;  
702 pooled SEM = 0.034 ng/ml).  
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704 **Figure 4.** Effect of dry period duration and dietary energy density on NEFA  
705 concentrations. Upper panel: Circulating NEFA concentrations during weeks -3 to =3  
706 relative to parturition. Significant effects of dry period duration (P = 0.01), and the  
707 interaction between dry period duration and feeding level were observed (P value =  
708 0.003). The effect of feeding level was not significant (P = 0.3). The pooled SEM was  
709 0.03 mmol/L. Lower panel: Circulating NEFA concentrations during weeks 4 to 12  
710 postpartum. Significant effects of dry period duration (P = 0.03), feeding level (P =  
711 0.049) and the interaction between dry period duration and feeding level were  
712 observed (P value = 0.046). The pooled SEM was 0.02 Mmol/L. The number of  
713 animals per treatment was 8, 10, 9 and 9 for SDP/STMR, SDP/HTMR, NDP/STMR,  
714 and NDP/HTMR, respectively.  
715

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

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

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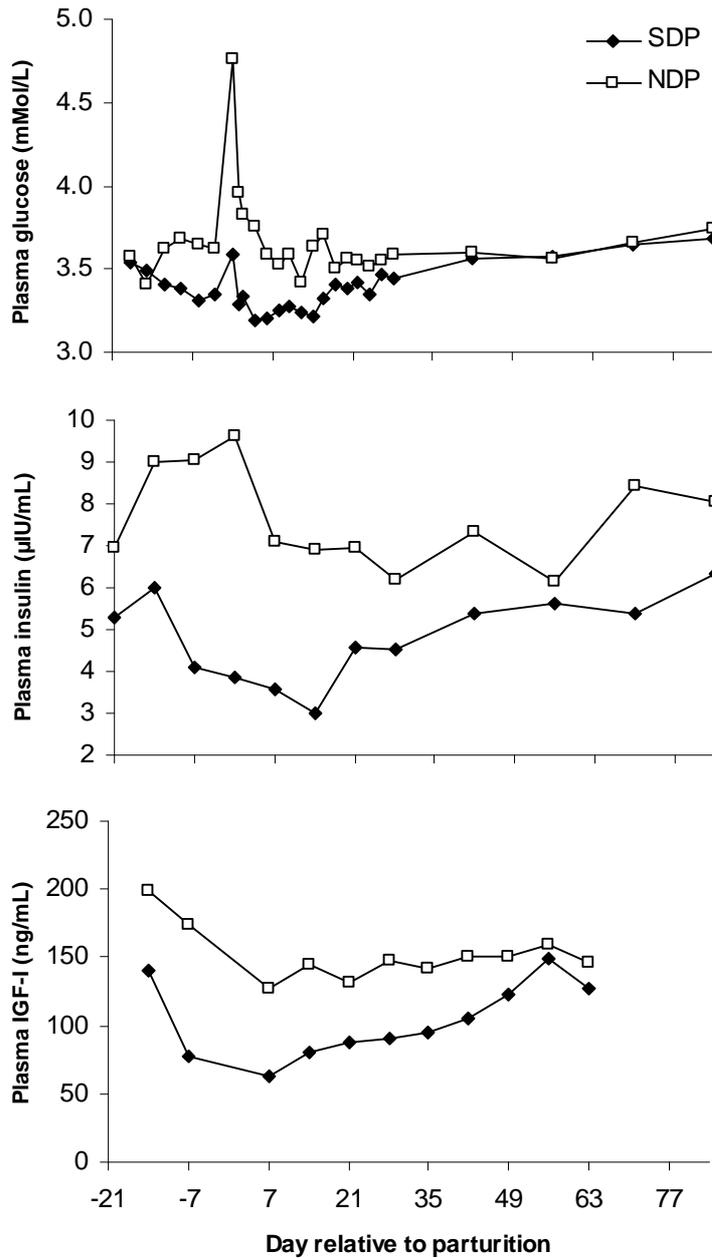


Figure 1 - de Feu

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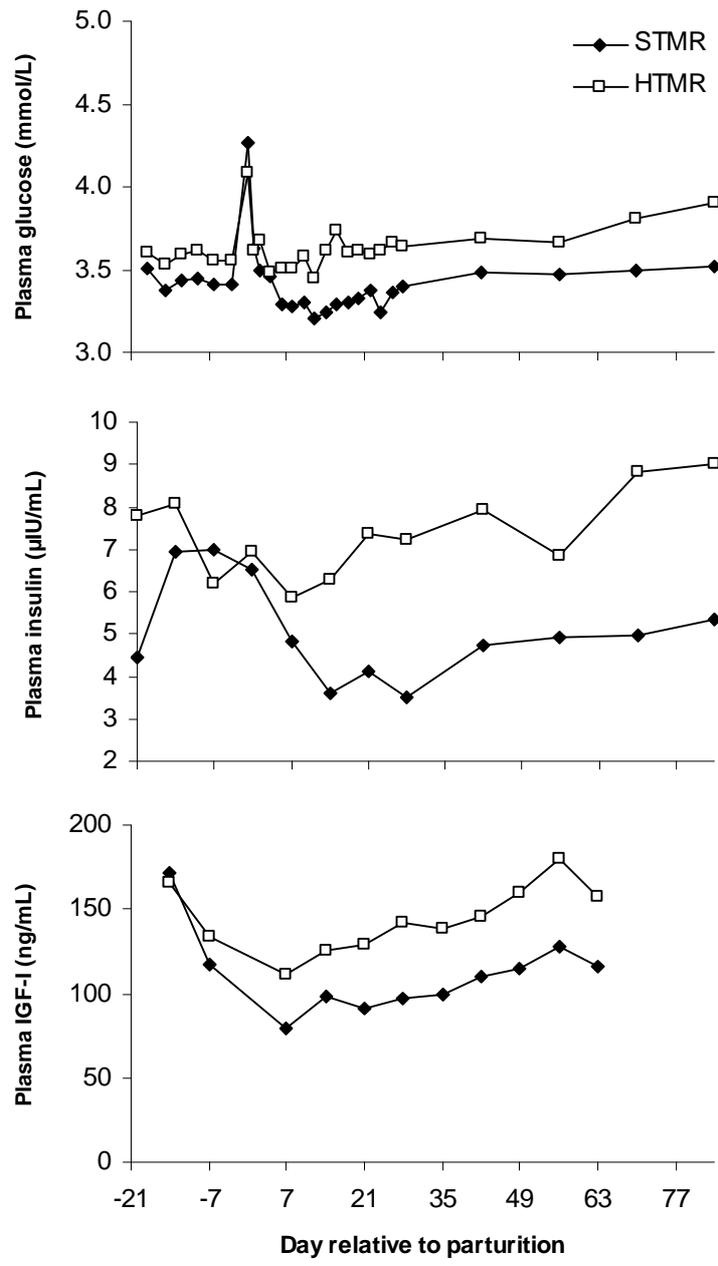
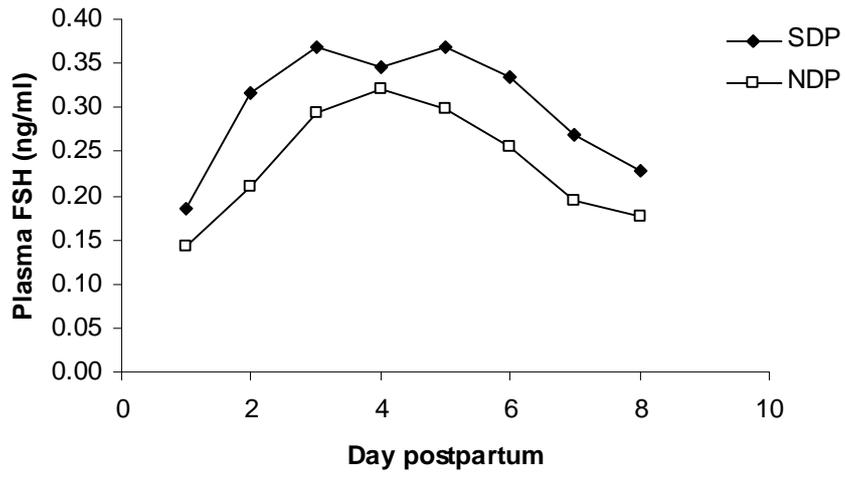


Figure 2 - de Feu



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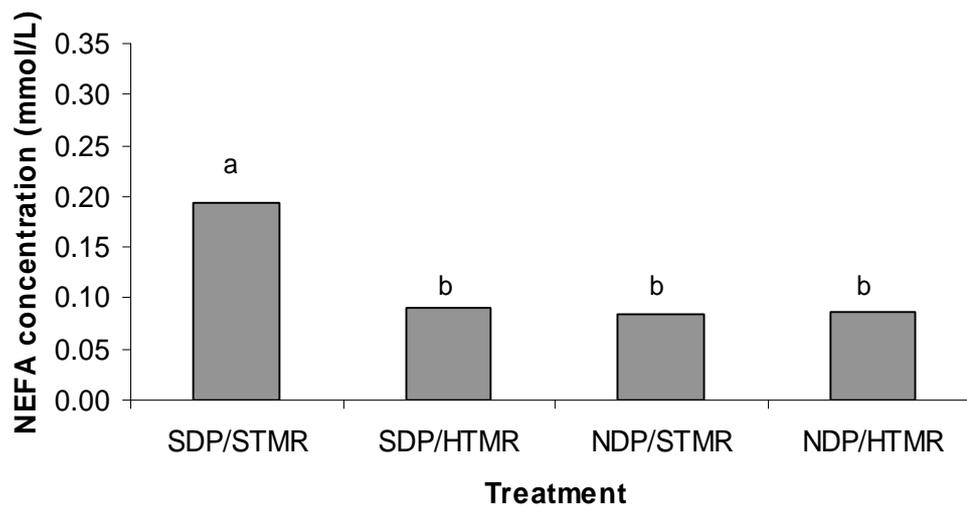
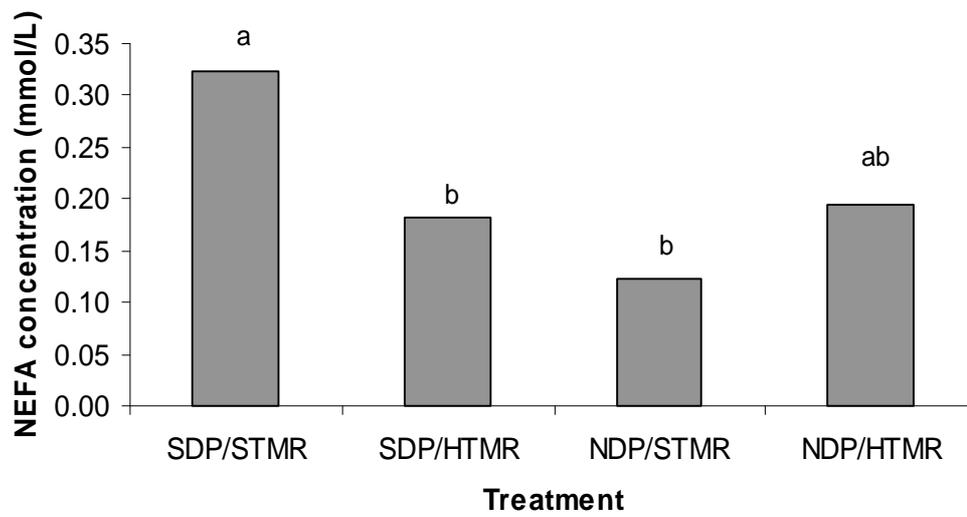
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Figure 3 - de Feu

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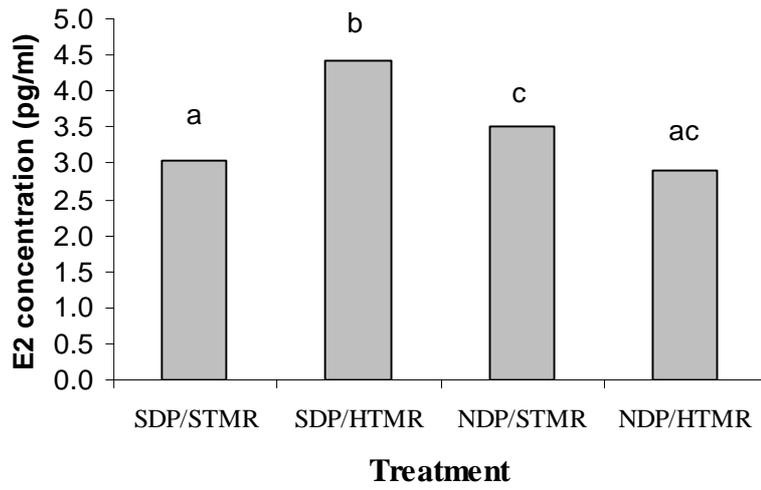
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Figure 4 - de Feu



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Figure 5 - de Feu