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INTERPRETIVE SUMMARY

Lactation responses to fat and protein supplementation in the dry period. *By Jaurena et al.*

Dairy cow nutrition during the dry period (DP) can be critical to dairy enterprise profitability. We hypothesized that supplementing grass silage with extra protein (Pr) or fat (F) during the DP would improve subsequent milk production or composition. Supplementation in the DP enhanced the cow's body condition score and Pr supplementation increased the *Longissimus dorsi* depth, the calf birth weight and subsequent milk Pr concentration. Supplementation with F in the DP reduced milk casein concentration at wk 3 of lactation, but mature cows (parity ≥ 3) fed with F enriched-diets increased their backfat depth, milk volume and protein yields over 20 wk of lactation.

RESPONSES TO FAT AND PROTEIN IN THE DRY PERIOD

Lactation and Body Composition Responses to Fat and Protein Supplies During the Dry Period in Underconditioned Dairy Cows

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26 **ABSTRACT**

27 An experiment was designed to study the effect of precalving supplementation with protein (**Pr**)
28 and rumen-inert fat (**F**) on body composition, and subsequent milk production and composition. Forty
29 Holstein-Friesian dairy cows were allocated to one of four dietary treatments in the dry period (**DP**)
30 based on a first cut ryegrass silage, with 6 Mature (in their third or greater pregnancy) and 4 Young (in
31 their second pregnancy) cows per treatment. These were: low Pr, low F (**LI**): silage alone; low Pr, high
32 F (**Lh**): silage with 10 % rumen-inert fat (mixed on a dry matter (**DM**) basis); high Pr, low F (**HI**): silage
33 with 5 % high protein corn gluten meal (**CGM**); high Pr, high F (**Hh**): silage with 5 % CGM and 10 %
34 rumen-inert fat. All the diets were individually offered ad libitum and DM intake (**DMI**) was recorded
35 daily during the **DP**. After calving all cows received ryegrass silage plus 8 kg/d of a commercial dairy
36 concentrate. During the DP, DMI was higher for Mature than for Young cows. All animals recovered
37 body condition score (**BCS**, 0.13 units/week, 1-5 scale) reaching a maximum BCS of 2.4 some days
38 before calving. Precalving maximum muscle *Longissimus dorsi* (**LD**) depth was greater for Mature (47.5
39 mm) than for Young cows (45.7 mm), and milk fat concentration was also higher for Mature than for
40 Young cows (40.2 and 39.0 g/kg respectively). Supplementation with CGM increased maximum LD
41 depth from 45.9 to 47.6 mm, calf birth weight (low Pr 43.2, high Pr = 46.3 kg), and milk crude protein
42 concentration from 30.8 to 31.6 g/kg. Fat supplementation in the DP of the Mature cows increased
43 maximum backfat depth (from 3.6 to 4.5 mm), milk yield (low fat = 26.3, high fat = 28.7 kg/day) and
44 protein yields (low fat = 837, high fat = 899 g/day). Inclusion of F in the DP diets reduced casein
45 concentration in milk at wk 3 of lactation from 26.3 to 24.5 g/kg. Milk CP yield was also increased by
46 CGM supplementation when compared within cows receiving F supplemented silages (Lh = 832, Hh =
47 877 g/day). It can be concluded that CGM supplementation in the DP increased subsequent milk protein
48 concentration, but milk protein yield increased only in those animals also receiving F supplementation.

49 Dry period diet supplementation with F increased maximum backfat depth, milk and CP yields in the
50 Mature cows, and led to more LD muscle mobilization during early lactation. Second calving cows had
51 a lower DMI and milk fat concentration than Mature cows.

52

53 **(Key words:** dry cow, milk production, milk quality, body composition)

54

55 **Abbreviation key:** **DP**, dry period; **F**, dietary fat; **Hh**, High protein, high fat; **Hl**, High protein, low fat;
56 **LD**, *Longissimus dorsi*; **Lh**, Low protein, high fat; **Ll**, Low protein, low fat; **Pr**, dietary protein; **CGM**,
57 high protein corn gluten meal; **TPr**, milk true protein.

58

INTRODUCTION

59
60 The dry period (**DP**) of the dairy cow occurs during late gestation, when the highest nutrient
61 demands from the conceptus and mammary tissue development occur (Prior and Laster, 1979; Bell et al.,
62 1995). Many authors have suggested the importance of the DP on the subsequent lactation performance
63 of dairy cows (Grummer, 1998, Drackley, 1999), but many dairy producers still tend to think of the dry
64 cow as having relatively low energy and protein requirements. The metabolic, physiological and
65 behavioral changes associated with this relatively short period of the lactation cycle suggest a phase of
66 high metabolic activity and producers should consider the DP as a linking-phase between successive
67 lactations, when management aims to prepare the cow to cope with the next lactation. The aim of DP
68 management should be to avoid subsequent metabolic disorders, to support fetal calf growth and
69 mammary gland development, and optimize subsequent milk production and composition without
70 compromising reproductive performance.

71 Many cows start the dry period underconditioned, leading to cows calving below the optimum
72 body condition score, and thereby becoming more susceptible to a variety of health problems (NRC,
73 2001). Although the need to improve the body condition of underconditioned cows at drying off has been
74 noted by some authors (Van Saun and Sniffen, 1996), achievement of a moderate amount of body
75 reserves throughout the late pregnancy period is acknowledged as a key factor to maximise dairy cow
76 productive performance in the subsequent lactation (Van Saun and Sniffen, 1996; Studer, 1998; NRC,
77 2001). Cows that begin lactation with a BCS of less than 2.8 (on a 0-5 scale) may not be capable of
78 mobilizing enough energy to support maximal milk production (Otto et al., 1991), and may have sub-
79 optimal reproductive capabilities (Crowe, 2008). Previous experiments have highlighted the effects of
80 body weight gain during the DP, focusing particularly on the consequences of overconditioning (Fronk
81 et al., 1980), but little attention has been paid to recovery of body reserves by thin cows. Grum et al.

82 (1996) indicated that replenishment of the energy reserves of underconditioned cows during the DP could
83 increase milk production and decrease the incidence of metabolic disorders during early lactation, but
84 further research of the same group suggested that recovering BW during the entire DP could bring about
85 peripartum health problems and impaired postpartum performance, even when animals did not become
86 overconditioned (Douglas et al., 2006).

87 Nutrition of dairy cows during the final stages of gestation is further complicated because any
88 nutritional imbalance is exacerbated by a typical DMI reduction (Ingvarsen et al., 2000; NRC, 2001),
89 and the fact that overfeeding can promote fetal overgrowth, which can lead to dystocia and other health
90 problems in the cow (Mee, 2008). At the same time increasing fetal nutrient demands can bring about
91 important maternal body tissue remobilization with undesirable consequences on the cow's postpartum
92 performance (Beever, 2006; Crowe, 2008).

93 In underconditioned cows (BCS typically < 2), supplying large quantities of dietary energy as
94 carbohydrate (grain) during the DP to improve BCS can lead to risk problems such as fatty liver (Grum
95 et al., 1996). However, supplying energy in the form of fat reduces this risk, because the liver is not a
96 lipid depot during positive energy balance (NRC, 2001). In addition to this, feeds with a high
97 concentrations of fat constrain energy supply to the fetus due to low conceptus access to NEFA and
98 ketoacids (Bell, 1993), and it has been speculated that feeding fat to dry cows could lead to increased FA
99 oxidation and reduced FA esterification in liver metabolism (Grum et al., 1996b). According to Grummer
100 (1993), dietary fat could minimise the risk of fatty liver, ketosis or both by: (a) reducing FA mobilisation
101 from adipose tissue, (b) alleviating the shortage of FA precursors for mammary triglyceride synthesis,
102 and (c) by sparing glucose oxidation by reducing the requirement of NADPH for mammary FA synthesis.
103 In other dietary considerations, supplementation with by-pass protein during the DP has shown

104 improvements in milk production and composition (Van Saun et al., 1993, Moorby et al., 1996, Moorby
105 et al., 2002a, b), apparently mediated by replenishment of the labile body protein pool.

106 The leading hypothesis in this study was that increasing the fat and protein supply to the dairy
107 cow during the late DP would improve body fat reserves and labile body protein, hence supporting milk
108 production and composition during the early phase of the subsequent lactation. The objective of this
109 study was to examine the effect of precalving dietary protein and rumen-inert fat supply on body
110 composition, and subsequent milk production and composition of under-conditioned dry dairy cows.

111 **MATERIALS AND METHODS**

112 ***General design and management***

113 In order to investigate the interactive effects of fat and protein in precalving diets, diets based on
114 first cut ryegrass silage supplemented with a rumen-inert fat source and a rumen by-pass protein source
115 were fed. The fat source was Megalac[®] (Volac International Ltd, Royston UK), a calcium soap of long
116 chain fatty acids from palm oil, containing 772 g/kg acid hydrolysis ether extract and, according to
117 manufacturer label specifications, supplied 48% C16:0, 5% C18:0, 36% C18:1 and 9% C18:2. The rumen
118 by-pass protein source was corn gluten meal.

119 Forty Holstein-Friesian dairy cows at the Institute of Grassland and Environmental Research
120 Trawsgoed Research Farm (Wales, UK) were allocated to one of four diets in a factorial treatment
121 arrangement of rumen-inert fat (**F**) and protein (**Pr**). The experimental diets were all based on first cut
122 ryegrass silage and were: low-Pr, low-F (**LI**), the ryegrass silage only; low-Pr, high-F (**Lh**): the same
123 silage with 10 % rumen-inert fat (mixed on a DM basis); high-Pr, low-F (**HI**): the same silage with 5 %
124 high protein corn gluten meal (**CGM**); high Pr, high-Fat (**Hh**): the same silage with 5 % CGM and 10 %
125 rumen-inert fat. Animals were balanced for parity across treatments, with 6 Mature (in their third or
126 greater pregnancy) and 4 Young (in their second pregnancy) cows per treatment. The average age of the

127 16 Young cows at the start of the experiment was $36 (\pm 3.6)$ mo. In the MATURE group, there were 11
128 cows in their third pregnancy (46 ± 0.7 mo old), 8 cows in their fourth pregnancy (58 ± 0.8 mo old) and
129 5 cows in their fifth pregnancy (71 ± 1.5 mo old).

130 Animals were adapted to the housing and were trained to use Calan gates over a 2-wk period prior
131 to the start of the experiment. Experimental diets were offered from 6 wk before the expected calving
132 date and cow measurements were collected from then until wk 20 of lactation. Rations were offered ad
133 libitum (to approximately 10 % refusals) as TMR at approximately 9 a.m. each day. Fresh water was
134 available throughout the day and mineral and vitamins were added to all TMR according to manufacturer
135 (Richard Keenan UK Ltd., Kenilworth, UK) specifications.

136 When cows were judged by dairy staff to be about to calve (by changes in behavior and udder
137 volume), they were moved to individual straw pens where they were introduced to the lactation diet. This
138 comprised ad libitum access to ryegrass silage with 4 kg (fresh matter)/d of a dairy concentrate. The
139 composition of the concentrate, per kg freeze DM, was: 13.5 MJ of ME, 225 g CP, 225 g NDF, 111 g
140 ADF, 237 g starch, 54 g acid hydrolysis ether extract. After calving all cows received ad libitum access
141 to the same ryegrass silage together with a daily allocation of concentrate feed. Immediately after calving
142 the fresh matter quantity of concentrate offered to the cows was increased in steps (4, 5, 6, 7 and 8 kg/d
143 respectively for days 0 to 1, 2 to 3, 4 to 5, 6 to 7 and 8 d of lactation), and after the first 8 d of lactation,
144 all cows were offered 8 kg/d for the remainder of the experiment.

145 ***Feed sampling and analysis***

146 The silage was prepared from a first cut ryegrass-dominated sward ensiled using a silage
147 inoculant (Ecosyl Bio-products Ltd., UK) in two adjacent bunkers. Representative samples of all feeds
148 (silage, CGM, Megalac, TMR and concentrates) were collected weekly, and pooled to provide 2 samples
149 per month and stored frozen until analyzed. Fresh (thawed) samples of silage were analyzed for DM (by

150 freeze drying to constant weight), pH, ammonia, lactic, acetic, propionic and butyric acids. All other
151 analyses of silage or concentrate samples were conducted on freeze-dried material.

152 Aqueous extracts of silage samples were prepared by mixing 20 g of thawed silage with 100 mL
153 of distilled water, and kept at 4°C overnight; pH was measured in the solution after allowing it to
154 equilibrate with room temperature for 30 min. Samples were then filtered through fast flow filter paper
155 and aliquots of the filtrate were pipetted into microcentrifuge tubes and frozen for later analysis. Volatile
156 fatty acid concentrations were determined by gas chromatography (Zhu et al., 1996). Lactic acid
157 concentration was determined by a spectrophotometric technique using a kit specific for L-lactic acid
158 (procedure 826-UV; Sigma-Aldrich Co. Ltd., Dorset, UK), followed by a second determination on the
159 same sample using the specific D-lactic acid dehydrogenase (Sigma-Aldrich product L-9636). Ammonia-
160 N concentration of silage was determined by the reaction of ammonia with salicylate and
161 dichloroisocyanurate in alkaline solution to produce a substituted indophenol blue. The color was read
162 in a ChemLab system 4 colorimeter (ChemLab Instruments Ltd., Great Dunmow, Essex, UK) linked to
163 a continuous flow analysis system. Analysis of feed concentrations of organic matter, CP, NDF, ADF,
164 water soluble carbohydrates, ether extract, and acid hydrolysis ether extract were completed as described
165 by Dewhurst et al. (2000). Feed starch concentrations were determined as described by Moorby et al.
166 (2016).

167 ***Measurements and sample collection on animals***

168 Cows were individually offered their allocated diets on a daily basis throughout the experiment
169 using Calan gates. Feed refusals were removed and weighed on Mondays, Wednesdays and Fridays to
170 estimate DMI on a daily basis. Dry matter intake was initially calculated on an oven DM basis (drying
171 at 100°C overnight) and later corrected to a freeze DM basis (freeze drying to a constant weight).

172 Animal BW, BCS, and depths of *Longissimus dorsi* (**LD**) and backfat were measured after morning
173 milking from 6 wk before anticipated calving (*i.e.*, wk -6) until wk 20 of lactation. Body condition score,
174 LD and backfat were assessed weekly from wk -6 to wk 8 of lactation, and once every 4 wk from wk 9
175 of lactation until the end of the experiment at wk 20 of lactation. Around calving (-10 to +10 days of
176 parturition) all these measurements were performed more frequently, on each Monday, Wednesday and
177 Friday. After calving, BW was automatically recorded daily after each morning milking and averaged
178 on a weekly basis. Body condition score was assessed by the same operator throughout the experiment
179 using a 0 to 5 scale (0-5 scale, Mulvany, 1977). *Longissimus dorsi* and backfat depths were measured
180 perpendicular to the skin using real-time ultrasound imaging at the fifth lumbar process (Concept\MCV
181 Ultrasound scanner, Dynamic Imaging Ltd., Livingstone, UK). Udder volume was estimated assuming
182 the udder to be spherical (volume, $\text{cm}^3 = 4/3 \times \pi \times r^3$) as described in Jaurena (2003).

183 Milk yields were measured and recorded automatically at each milking and samples were taken
184 until wk 20 of lactation: milk samples were collected from each cow at two consecutive milkings weekly
185 and analyzed for fat, protein and lactose by infrared milk analysis (National Milk Records Central
186 Laboratory, Somerset, UK). Gross energy of the milk samples was estimated by the formulae of Tyrrell
187 and Reid (1965; quoted by AFRC, 1993) using milk fat, protein and lactose contents for the current
188 lactation data, and the formulae based on milk fat and protein contents for the previous lactation data.
189 At wk 3 and 8 of lactation, an extra sample of milk was taken and analyzed for milk CP fractions. Milk
190 CP (total N \times 6.38) was estimated in duplicate by Kjeldahl analysis, and milk protein fractions were
191 separated according to the International Dairy Federation Standard (FIL-IDF, 1964) into true protein
192 (**TPr**), casein N (**CN**), non-protein N (**NPN**), and whey proteins by difference. Milk urea concentration
193 was estimated by a Sigma kit for urea-N determination (No. 640), and read spectrophotometrically at
194 570 nm.

195 **Statistical analysis**

196 Preliminary analysis of results showed an important interaction of the dietary factors under study
197 with animal maturity (Young versus Mature cows). Consequently, data was analyzed in a factorial
198 arrangement ($2 \times 2 \times 2$) of maturity, Pr and F supplementation in a complete randomized design.
199 Statistical analyses were carried out using GenStat (5th edition; Genstat Committee, 2000), and unless
200 otherwise stated fitted to the following model:

$$Y_{ijk} = \mu + Cov + M + Pr + F + M \times Pr + M \times F + Pr \times F + M \times Pr \times F + \varepsilon$$

201 Where: μ is the grand mean; *Cov*, covariable; *M*, Maturity; *Pr*, Protein; *F*, Fat; and ε is the random error
202 estimated by the residual of the model.

203 The DMI data from the DP were fitted to an exponential model ($y = a + b \times [1 - b^{k \times \text{days}}]$) using the
204 Genstat Standard Curves procedure (GenStat Committee, 2000). Starting BW measurements and BW
205 measured at the first wk of lactation were used as covariates for intake data for pre- and post-calving
206 periods were respectively to allow for differences in cow body size. Pre- and post-calving weekly DMI
207 means were studied by linear correlation analysis.

208 Maximum values before calving, and minimum values after calving for BW, BCS, backfat and
209 LD were analyzed by analysis of covariance using the first measurement of each variable in the DP as
210 the covariate. Analysis of the time (in wk) between maximum precalving BCS, BW, LD and backfat
211 records and calving was carried out using a complete randomised block design (blocking by cow). A
212 similar analysis was completed for the interval between calving and minimum record postpartum.

213 Calf birth weights were analyzed by using calf sex as an additional factor in the model. Milk
214 composition and yield (volume and components) were analyzed using each animal's previous lactation
215 records as covariates. Milk protein fraction concentrations and yields at wk 3 and 8 of lactation were
216 analyzed by a model including previous lactation CP concentrations or yields as covariates.

217 Health events were analyzed by logistic regression (GenStat Committee, 2000). Health events
218 including retained placenta, reproductive tract infections, cystic ovary, postpartum anestrus and hormone
219 treatment to resume reproductive cycling were grouped together and analyzed as “reproduction
220 problems”. Incidents of general lameness, sole ulcers and interdigital dermatitis were grouped and
221 analyzed as “feet problems”.

222 RESULTS

223 All cows were dried off 8 wk before their expected calving date, and calved between 1 September
224 and 19 October 1999. The average time on precalving treatment was 39 to 44 d.

225 Five cows were diagnosed and successfully treated for mastitis within the first wk of calving, one
226 cow was treated for milk fever and successfully recovered after calving. Crude protein fraction data
227 collected from the mastitic cows (which were on treatments Ll: 1, Lh: 2, Hl: 1 and Hh: 1) were excluded
228 from subsequent statistical analyses. One cow in the Mature-Hl treatment group calved twins. One cow
229 (treatment Lh) did not adapt to using the Calan gates, and could not be replaced with a suitable animal,
230 therefore only the remaining 39 animals were used for the final analyses.

231 Although the study of health problems was beyond the scope of this work due to the limited
232 number of animals, a higher incidence of reproductive problems was detected in those cows receiving F
233 supplementation ($P \leq 0.05$; Ll = 1, Hl = 1; Lh = 6; Hh = 3). Furthermore, an F \times Pr interaction ($P \leq 0.05$)
234 occurred for total health incidents (Ll = 2; Hl = 4; Lh = 8; Hh = 7). No differences were detected for the
235 incidence of calving problems, mastitis or milk fever.

236 ***Feed characteristics and intake***

237 Feed characteristics were homogeneous throughout the experiment (Table 1 and 2). Although the
238 CP concentration of Hh ration was significantly (Tukey test, $P < 0.05$) higher than that of diet Lh,
239 inclusion of CGM with ryegrass silage did not lead to a statistically significant difference (Tukey test, P

240 > 0.05; HI vs. LI ration). Ether extract concentrations were similar for LI and HI rations, and were
241 considerably lower than concentrations in Lh and Hh rations.

242 Analysis of intake data showed that Mature cows ate more than their younger counterparts (means
243 of 13.2 and 11.1 kg/d respectively, SEM = 0.27, $P < 0.001$) in correspondence with their BW, but when
244 adjusted for initial BW, DMI increased from 11.8 to 13.3 kg/d within the high F treatments in association
245 with CGM supplementation ($P_{Ft \times Pr} < 0.05$; Table 3), this increase was particularly large for the Mature
246 cows offered the high F rations (Mature-Hh; $P_{M \times F \times Pr} < 0.05$), as they ate 26% more than those offered
247 the Lh treatment ($P \leq 0.05$). During the DP, DMI tended to decrease between 3 and 17 d before calving
248 (Figure 1). In addition to this reduction, analysis of DMI measurements made before the animals were
249 dried off (data not shown) indicated that the DMI of 60 % (23) of the animals had started to decrease
250 before the dry period started, and this is seen in the overall intake patterns of groups Young-Lh, Mature-
251 HI and Mature-Hh in Figure 1.

252 After calving, Mature cows consumed more DM (mean of 18.2 kg/d) than the Young cows (16.7
253 kg/d; $P < 0.001$, model without covariate), but there were no differences in covariate-adjusted DMI, with
254 grand means for the first 20 wk of lactation for total DMI, silage DMI and proportion of concentrate of
255 17.6 kg/d, 10.6 kg/d and 40 % respectively.

256 **Body composition characteristics**

257 Cows started the experiment with mean actual BCS of 1.6 (SEM = 0.10) and 2.0 (SEM = 0.08),
258 and BW of 593 kg (SEM = 10.2) and 686 kg (SEM = 12.5), for Young and Mature groups respectively
259 ($P < 0.01$; model without covariable; Table 3). Mean maximum LD depth was greater for the Mature
260 group (46.4 mm) than for the Young group (45.6 mm; $P = 0.047$), and increased with CGM
261 supplementation from 45.6 to 47.4 mm (SEM = 0.64; $P = 0.06$). Supplementation with F increased
262 maximum backfat thickness only in the Mature animals (Mature-low F = 3.6, Mature-high F = 4.5 mm;

263 SEM = 0.20, $P_F < 0.05$), and reduced the time from maximum BCS ($P_F = 0.039$) and backfat ($P_F =$
264 0.024) to calving. Body tissue mobilization started before calving as shown by Figures 2 and 3, and time
265 between the maximum BCS, BW, LD and backfat and calving were presented in Table 3. The maxima
266 of the various variables differed in time: backfat (3.45^a wk) > LD (2.87^a wk) > BCS (1.63^b wk) > BW
267 (1.29^b wk; numbers with differing superscript differed significantly, $P < 0.05$).

268 The minimum LD was recorded at 5.5 wk postcalving (grand mean for all treatments; Table 4),
269 despite differences due to maturity (Mature > Young cows; $P_M < 0.05$), protein inclusion (high Pr > low
270 Pr; $P = 0.045$), and F supplementation (low F > high F; $P_F = 0.007$). Postpartum LD loss was greater for
271 those cows receiving rations with F during the DP ($P = 0.042$). There were also significant differences
272 in the time between calving and minima of the different variables studied (*i.e.* BW, 4.2^c; LD, 5.6^b; BCS,
273 6.1^b; and backfat, 11.1^a wk; $P < 0.001$). No differences among treatments were observed in estimated
274 udder volume at calving (grand mean = 38.5 L, SEM = 1.53; $P > 0.10$).

275 ***Calf birth weights, milk composition and yield***

276 Male calf birth weights were higher than those of females calves (47.0 and 42.6 kg respectively;
277 SEM = 1.25 kg, $P = 0.014$), and cows receiving Pr supplementation delivered heavier calves than their
278 non-supplemented counterparts (low Pr = 43.2, high Pr = 46.3, SEM = 1.10 kg, $P = 0.048$). There was
279 no difference in birth weights of calves from Young and Mature cows. Colostrum CP concentration
280 averaged 141 g/kg and did not show any differences among treatments ($P > 0.05$).

281 Cow maturity was associated with higher milk fat concentrations (Mature cows = 40.2; Young
282 cows = 39.0, SEM = 0.49 g/kg; $P_M = 0.09$; Table 5), and milk ($P_{M \times F} < 0.10$) and protein yields ($P_{M \times F} =$
283 0.01) of F supplemented cows. Inclusion of CGM in the DP diet tended to increase milk protein
284 concentration (between 1 and 1.5 g CP/kg, $P_P = 0.086$), particularly during the first month postpartum,

285 but milk protein yield only increased when CGM was included with the F-supplemented silages ($P <$
286 0.07); within the low F DP treatments the average milk protein yield was 839 g/d ($P > 0.10$).

287 Analysis of milk protein fractions showed lower CN at wk 3 of lactation after inclusion of F in
288 the precalving diet (low F = 26.3, high F = 24.5 g/kg; $P_F = 0.002$; Table 6); and a maturity \times F interaction
289 for milk NPN and urea (which was deemed meaningless due to the lack of difference when tested by
290 least significant difference $P = 0.05$). At wk 8 of lactation, the only experimental effect on milk
291 composition was on urea concentration ($P_{M \times Pr \times F} = 0.001$) associated with CGM inclusion (0.33 g/kg for
292 the Young-Hh group versus 0.27 g/kg for the Young-Lh group; $P < 0.05$) and within the Mature group
293 (Mature-Ll = 0.25 g/kg, Mature-Hl = 0.30 g/kg; $P < 0.05$). Otherwise, the overall mean milk urea
294 concentration for Young cows in low F diets was 0.30 g/kg, and for Mature cows in high F diets was
295 0.24 g/kg. The other protein fraction grand means were: CP = 32.9 g/kg; TPr = 30.1 g/kg; casein = 23.4
296 g/kg and WP = 6.7 g/kg.

297 Milk yield, CP, TPr, CN and NPN yields all increased in Mature cows with inclusion of dietary
298 F in the DP ($P_{M \times F} < 0.05$; Table 7). The $F \times Pr$ interaction effect was significant for CP, TPr and CN
299 yields. Within the low F treatments this difference was probably brought about by a depression in CN
300 yields with Pr supplementation (Ll = 828, Hl = 717 g/d, $P < 0.05$). At wk 8 of lactation the inclusion of
301 F in the DP diet led to increased milk yields only in the Mature animals ($P_{M \times F} \leq 0.01$). Milk urea yields
302 within the young animals ($P_{M \times Pr \times F} = 0.029$) showed significant differences between Lh (6.9 g/d) and Hh
303 (9.1 g/d; $P < 0.05$) DP groups.

304 DISCUSSION

305 The lowest incidence of health problems was associated with cows offered ryegrass silage alone
306 in the DP (*i.e.* Ll). Although the number of animals was too small to draw definite conclusions, the higher
307 incidence of reproductive and health problems among animals that received additional dietary fat during

308 the DP did not agree with the beneficial effects hypothesized by some authors (Kronfeld, 1982,
309 Grummer, 1993, Grum et al., 1996), and would support the concerns expressed by Douglas et al. (2006)
310 about the potential detrimental effects of allowing ad libitum access to diets containing moderate to high
311 energy densities throughout the entire DP.

312 ***Feed characteristics and intake***

313 Both silages used pre- and postcalving showed acceptable fermentation characteristics and were
314 within the range of values commonly found in the UK (Haigh, 1996a, b). The ash content of Megalac
315 was higher than manufacturer specifications because the ashing procedure used (combustion at 550°C)
316 could have retained Ca as CaCO₃ instead of CaO (Dedman and Owen, 1962).

317 The greatest differences in DMI in the DP were between the two age groups as a reflection of
318 BW differences, so that as a proportion of BW, DMI recorded at the beginning of the experiment agreed
319 with other reports (Van Saun and Sniffen, 1996, Dewhurst et al., 2000). These results agree with studies
320 that have shown that primigravid and even second-calving cows, as in this experiment, have lower DMI
321 than multiparous cows (Grummer, 1998, Ingvarsten and Andersen, 2000), and thus should be considered
322 separately from mature cows for diet formulation, as recognized by the NRC standard for dairy cattle
323 (2001).

324 Inclusion of fat in dairy cow rations, despite the potential improvements in energy intake, has
325 often been found to induce a reduction in feed intake in lactating dairy cows (Choi and Palmquist, 1996,
326 Staples et al., 1998). In our study, a noticeable response in DMI during the DP was observed to CGM
327 supplementation (which induced an increase in diet CP concentration from 143 to 170 g/kg DM) in the
328 cows fed with high fat concentrations. This was particularly significant for the Mature cow group. In this
329 experiment the Hh ration was 19% higher in CP concentration than the Lh ration, which could have
330 brought about a positive response in microbial activity due to the release of dietary AA in the rumen

331 (Orskov, 1982, Dawson et al., 1988). Furthermore, voluntary intake increases could have been promoted
332 by the increase in metabolizable protein supply, something that is not frequently reported but has
333 previously been observed in lactating dairy cows (Allen, 2000, Faverdin et al., 2003). Precalving DMI
334 (average of wk -5 to -1) of Young and Mature cows offered the low F rations was 1.9% of their BW, but
335 within the Mature group offered the high F rations DMI increased from 1.8% (Mature-Lh) to 2.2% BW
336 (Mature-Hh) in association to PM supplementation. The typical DMI reduction during the DP as calving
337 approaches constitutes a restriction in energy and nutrient inputs during a period of particularly high
338 nutrient demands. The pattern of intake reduction showed, as in other reports, the DMI decline before
339 the DP (Ingvarlsen and Andersen, 2000) and the final drop during the last 3 wk of gestation (Van Saun
340 et al., 1993, NRC, 2001).

341 ***Body composition characteristics***

342 All cows were in relatively poor condition at dry-off (about 1.8 on a 0-5 scale) in relation to
343 targets reported in the literature, e.g., 2 to 3, Garnsworthy (1988) and Palmquist et al. (1993); 2.5 to 3
344 (Van Saun and Sniffen, 1996); 3 (Mulvany, 1977, Moorby et al., 2002a). However, the cows in the
345 current study recovered significant quantities of BCS after being dried off and achieved a maximum
346 precalving BCS of 2.4 a few days before calving. The inclusion of fat in the precalving diet increased the
347 maximum backfat thickness by a mean of about 1 mm in the Mature cows (a mean of 4.54 mm versus
348 3.53 for Young cows, SEM = 0.247), and reduced the time interval between maximum BCS and backfat
349 thickness, and calving. These results suggest that fat supplementation during the DP improved the energy
350 balance of Mature cows and delayed the initiation of tissue mobilization before calving.

351 The depth of LD at the loin was measured as an estimate of labile body protein (Moorby et al.,
352 2002a). All animals gained LD depth during the DP, and started mobilization of LD before calving, in
353 agreement with Moorby et al. (2002b). Maximum LD muscle depth increased significantly with cow

354 maturity and Pr supplementation, with both diet and maturity producing different patterns of LD
355 mobilization over the course of the experiment. This indicates that labile body protein can be increased
356 by the provision of a protein supplement during the DP, and this would increase BCS as well, particularly
357 for cows in poor body condition (Jaurena et al., 2005).

358 Cow maturity had widespread effects, as it was the significant main factor found to influence
359 initial BCS and BW, maximum BCS, and maximum LD depth. Response differences have been noted
360 due to age at first calving (2 versus 3 years old, Dewhurst et al., 2002) and parity on lactation performance
361 (Waltner et al., 1992, Dewhurst et al., 2002) and pattern of change of BCS (Waltner et al., 1992). Animals
362 that calve for the first time at about two years old are still growing during the first and second lactations,
363 which was indicated in this study by differences in plasma somatotropin concentrations observed in this
364 herd (data not shown), which could affect the partitioning of nutrients between fetal and maternal tissues.
365 There can also be differences in the response to DP nutrition between first calving heifers and older cows
366 (Robinson et al., 2004), which is probably a consequence of the same effect, and highlights the
367 importance of managing young and older dry cows separately under commercial conditions.

368 Postpartum mobilization of body tissues was apparent through losses in BW and BCS. Changes
369 in backfat thickness and in plasma concentrations of NEFA and BOHB (data not shown) indicate the
370 mobilization of body fat, and losses of LD depth indicate concomitant body protein mobilization.
371 Supplementation of the DP diet with CGM reduced the amount of LD lost, and increased the minimum
372 depth of LD measured during early lactation, agreeing with the results of Hutjens (1996) and Moorby et
373 al. (2002b) respectively. Inclusion of fat in the DP ration did not affect the maximum LD depth before
374 calving, but led to greater LD losses postpartum, particularly in the Mature animals. This likely due to
375 the higher milk and protein yields observed from the Mature cows offered high fat DP rations, and agrees
376 with the hypothesis of Moorby et al. (2002b) that the availability of body nutrients to support milk

377 production does not drive increased rate of milk synthesis. Further support for this is provided by changes
378 in plasma prolactin concentrations among the animals in this study (data not shown).

379 The developing udder and conceptus together constitute an increasing proportion of body weight
380 gain as the animal approaches calving. No differences in udder volume were detected due to the
381 experimental treatments, but a positive correlation was detected between udder volume and milk yield at
382 wk 3 of lactation. The amount of secretory tissue is an important determinant of milk yield, and selection
383 for output has resulted in a positive relationship between mammary gland size and milk yield (Tomar,
384 1973). Although external non-invasive measurements associated with udder volume have proven to be
385 effective in estimating udder weight (Dewhurst et al., 1993), it must be recognized that udder volume is
386 a crude measurement of total tissue mass, and does not provide information on the relative proportion of
387 secretory tissue or cisternal volume (Dewhurst and Knight, 1993).

388 ***Calf birth weight, milk composition and milk yield***

389 Colostrum protein concentration was within the normal range (approximately 70 to 230 g/kg;
390 Kehoe et al., 2007) expected for Holstein dairy cows, and as in previous reports (Tesfa et al., 1999, Santos
391 et al., 2001), no association was found between colostrum composition and precalving diet, or cow
392 maturity, as would suggest differences in density reported by Robinson et al. (2009).

393 There were limited effects of experimental treatment on milk composition over the first 20 wk of
394 lactation. Milk protein concentration increased with the inclusion of the protein supplement in the DP
395 diet, as previously noted for primiparous (Van Saun et al., 1993, Santos et al., 1999) and multiparous
396 (Moorby et al., 1996) Holstein dairy cows. However, other reports have failed to find any relationship
397 between precalving CP intake and subsequent milk production or composition (Wu et al., 1997, Putnam
398 and Varga, 1998, Huyler et al., 1999, Murphy, 1999), which could be associated with the protein
399 concentration or quality of the control diets. Analysis of milk protein fraction concentrations at wk 3 and

400 8 of lactation found typical values for CN and whey proteins concentrations, but NPN was slightly above
401 the range 250 to 300 mg/L of milk assumed as normal (DePeters and Ferguson, 1992). Milk urea
402 concentrations were within the normal range (DePeters and Ferguson, 1992), and did not exceed the
403 threshold set for Holstein dairy cows fed according to requirements (Jonker et al., 1998) and rumen-
404 degraded protein balance (Schepers and Meijer, 1998).

405 Several small but significant effects of treatments were observed among milk and milk
406 component yields. The Mature cows had higher concentrations of milk fat than Young cows, and DP Pr
407 supplementation increased milk protein concentrations. However, fat supplementation of the DP diet of
408 the Mature cows increased milk yield and protein yields, whereas no differences were detected for Young
409 cows. Perhaps the most notable result was the effect of CGM supplementation on milk protein yield in
410 fat-supplemented animals; this was associated with differences in DP diet CP contents and intake.

411 The results obtained from the sets of milk samples studied at wk 3 and 8 of lactation indicated
412 that the positive effect of precalving dietary fat diminished as lactation progressed, as is expected if
413 mobilization of body tissues is playing an important role in lactation (Garnsworthy, 1988, Holter et al.,
414 1990).

415 **CONCLUSIONS**

416 Precalving supplementation of underconditioned dry dairy cows with both fat and protein
417 apparently improved body fat reserves and labile body protein, and delayed body tissue mobilization,
418 although differences were found between primiparous and multiparous cows. Precalving intakes of cows
419 receiving the high fat diets were increased by CGM supplementation, particularly for Mature cows.
420 Supplementation of the DP diet with protein also led to a significant increase in calf birth weight, and a
421 small increase in milk protein concentration over the first 20 wk of the subsequent lactation, however,
422 milk protein yield was only increased when the DP diet was also enriched with fat.

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TABLES

Table 1. Mean chemical composition of the diets used during the precalving period and the ryegrass silage offered after calving. Values in g/kg DM unless stated otherwise.

	Precalving experimental TMR ¹								Postcalving	
	Ll		Hl		Lh		Hh		Silage	
	Mean	SEM ²	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
n	3		3		3		3		5	
DM, ³ g/kg fresh matter	246	6.5	255	5.8	263	5.6	272	5.5	235	1.4
Ash	95	4.0	85	3.2	106	0.6	99	1.3	80	3.0
Crude protein	156	6.8	162	3.8	143	5.2	170	4.1	170	2.1
Ether extract ⁴	37	---	41	---	67	---	85	---	---	---
Acid detergent fiber	287	2.0	275	4.9	266	6.3	244	5.6	284	4.6
Neutral detergent fiber	461	2.8	452	7.6	434	6.2	414	5.2	471	8.1
ADIN ⁵	0.7	0.02	0.8	0.01	0.6	0.02	0.8	0.03	0.5	0.02
WSC ⁶	9.0	2.3	8.7	1.5	7.5	0.8	8.8	1.5	9.0	0.5
Gross energy, MJ/kg DM	17.6	0.08	17.6	0.13	18.8	0.13	18.8	0.04	18.0	0.04
E _{ADig} , MJ/MJ ⁷	0.70	---	0.72	---	0.67	---	0.71	---	---	---
ME, ⁸ MJ/kg DM ⁴	10.5	---	10.9	---	10.5	---	11.3	---	---	---
Fermentation characteristics										
pH	4.1	0.19	---	---	---	---	---	---	4.0	0.06
Ammonia-N, g/kg total N	100	30	---	---	---	---	---	---	100	7
Lactic acid	133.4	17.10	---	---	---	---	---	---	114.6	6.44
Acetic acid	19.8	4.04	---	---	---	---	---	---	9.5	1.32
Propionic acid	3.1	0.82	---	---	---	---	---	---	0.6	0.03
Butyric acid	7.1	4.23	---	---	---	---	---	---	0.1	0.06

¹ Ll, Low protein, low fat, ryegrass silage; Lh, Low protein, high fat; Hl, High protein, low fat; Hh, High protein, high fat.

² Standard error of the mean.

³ By lyophilization.

⁴ From samples collected for in vivo estimates of whole tract digestibility.

⁵ Acid detergent insoluble N.

⁶ Water soluble carbohydrates.

⁷ Apparent digestibility of energy.

⁸ Metabolizable energy.

3

Table 2. Mean chemical composition of concentrates used before and after calving. Values in g/kg FDM unless stated otherwise.

	Rumen-inert fat ¹		Corn gluten meal		Dairy concentrate	
	Mean	SEM ²	Mean	SEM	Mean	SEM
N	3		3		7	
Dry matter, g/kg fresh matter	980 ³	---	905	0.7	897	4.9
Ash	242	6.8	24	9.4	82	1.8
Crude protein	0.7	0.51	624	2.9	225	4.0
NDF	---	---	---	---	225	7.0
ADF	---	---	---	---	111	4.5
Water soluble carbohydrates	---	---	3	0.0	88	2.7
Neutral cellulase gamanase digestibility	---	---	951	12.8	---	---
Starch	---	---	198	9.0	237	13.3
Acid hydrolysis ether extract	772	6.7	83	0.3	54	2.7
Gross energy, MJ/kg	32.1	0.07	22.8	0.06	18.0	0.09

¹ Megalac® (Volac International Ltd, Royston, UK).² Standard error of the mean.³ As indicated by the manufacturer.

4 **Table 3.** Mean treatment effects on precalving DMI and measurements of BCS, BW, and loin depths of
 5 *Longissimus dorsi* (LD) and backfat of cows offered diets Hh, Hl, Lh, Ll during the dry period.

Maturity:	Young				Mature				SEM ²	Significant Factors <i>P</i> ³
	Treatments:	Ll ¹	Hl	Lh	Hh	Ll	Hl	Lh		
DMI, kg/day ⁴	12.0	12.0	11.9	12.1	12.7	12.2	11.6	14.6	0.51	P×F*; M×P×F*
Initial										
BCS	1.5	1.9	1.4	1.7	1.9	2.1	2.0	2.0	0.16	M*; P ⁺
Body weight, kg	577	644	548	604	662	690	683	707	20.5	M***; P*
LD, mm	38.8	44.3	37.6	43.8	40.0	45.5	45.2	40.5	2.37	NS
Backfat, mm	2.8	3.2	2.7	2.3	3.4	3.4	3.4	3.1	0.45	NS
Maximum⁵										
BCS	2.4	2.4	2.4	2.3	2.5	2.5	2.4	2.5	0.08	NS
Body weight, kg	670	708	666	677	691	699	677	702	6.4	P***; F*; M×P×F*
LD, mm	44.6	45.5	45.1	47.5	46.9	49.0	46.1	47.8	1.04	M*; P*
Backfat, mm	3.6	4.3	3.2	3.8	3.4	3.9	4.7	4.4	0.28	F ⁺ ; M×F**
Maximum gain (units/wk)										
BCS, units/wk	0.15	0.15	0.13	0.10	0.14	0.13	0.12	0.14	0.026	NS
Body weight, g/d	817	1405	1124	910	1315	1285	785	1505	153.9	P*; M×P×F**
LD, mm/wk	2.45	1.21	3.01	1.65	1.46	3.30	2.40	2.55	0.865	NS
Backfat, mm/wk	0.25	0.20	0.14	0.22	0.17	0.19	0.42	0.35	0.119	NS
Weeks between maximum value and calving										
BCS	1.7	2.0	0.3	1.2	1.5	2.3	2.0	1.0	0.40	F*; M×P×F ⁺
Body weight	1.5	1.0	2.0	1.0	1.2	1.2	1.7	0.8	0.35	P*
LD	3.5	2.5	3.0	2.7	2.0	3.5	3.7	1.7	0.84	NS
Backfat	4.2	3.0	3.0	1.5	4.7	4.5	3.0	2.3	0.92	F*

6
 7 ¹ Ll, Low protein, low fat, ryegrass silage; Lh, Low protein, high fat; Hl, High protein, low fat; Hh, High protein, high fat.

8 ² Standard error of the mean for n = 6.

9 ³ M, Maturity; P, Protein; F, Fat; NS, not significant; +, P ≤ 0.10; *, P ≤ 0.05; **, P ≤ 0.01; ***, P ≤ 0.001.

10 ⁴ Covariate (initial body weight) corrected means.

11 ⁵ Covariate (first homologous data recorded) corrected means.

12

13 **Table 4.** Mean treatment effects on postcalving measurements of BCS, BW, and loin depths of
 14 *Longissimus dorsi* (LD) and backfat of cows offered diets Hh, Hl, Lh, Ll¹ during the dry period.

Factors:	Maturity ²			Protein		Fat			Significant Factors
	Y	M	SEM ³	Low	High	Low	High	SEM ⁴	P ⁵
n	16	24	---	20	20	20	20	---	
Minimum⁶									
BCS	1.8	1.8	0.06	1.7	1.9	1.8	1.8	0.06	Cov***
Body weight, g/d	569	583	9.0	569	586	581	574	6.6	Cov***
LD, mm	34	37	0.6	35	37	37	34	0.7	Cov***; M*; P*; F**
Backfat, mm	1.1	1.5	0.14	1.3	1.4	1.4	1.3	0.14	M ⁺
Postpartum loss									
BCS, units/wk	0.10	0.08	0.010	0.08	0.09	0.10	0.08	0.011	NS
Body weight, g/d	1213	1107	160	1223	1076	1210	1090	175	M×P ⁷
LD, mm/wk	1.5	1.3	0.12	1.6	1.2	1.2	1.6	0.14	P ⁺ ; F*; M×P ⁸
Backfat, mm/wk	0.14	0.20	0.036	0.21	0.14	0.16	0.19	0.039	NS ⁸
Wk between calving and minimum value									
BCS	5.6	6.6	0.91	5.7	6.6	5.5	6.9	1.0	NS
Body weight	3.3	4.7	0.78	3.7	4.6	3.6	4.7	0.86	NS ⁹
LD	4.9	6.0	0.45	5.0	6.0	5.5	5.5	0.49	NS
Backfat	12.6	10.0	1.21	9.6	12.4	11.7	10.3	1.33	M×P ¹⁰

15
 16 ¹ Ll, low protein, low fat, ryegrass silage; Lh, low protein, high fat; Hl, high protein, low fat; Hh, high protein, high fat.
 17 ² Y, young cows; M, mature cows.
 18 ³ Standard error of the mean for n = 24.
 19 ⁴ The same SEM for P and F factors.
 20 ⁵ Cov, covariate; M, Maturity; P, protein; F, fat; NS, not significant; +, P ≤ 0.10; *, P ≤ 0.05; **, P ≤ 0.01; ***, P ≤ 0.001.
 21 ⁶ Statistical model with covariate of first homologous data recorded during the dry period.
 22 ⁷ M×P⁺ for body weight loss, M-Low P = 1366, M-High P = 849, LSD (5 %) = 656; Y-Low P = 1010, Y-High P = 1417, LSD
 23 (5 %) = 803 g/d.
 24 ⁸ M×P* for LD loss, M-low P = 1.7, M-high P = 1.0, LSD (5 %) = 0.51; Y-low P = 1.4, Y-high P = 1.6, LSD (5 %) = 0.63
 25 mm/d.
 26 ⁹ Failed Bartlett's test.
 27 ¹⁰ M×P^{**} for wk since calving to minimum backfat, M-low P = 6.5, M-high P = 13.5, LSD (5 %) = 4.9; Y-low P = 14.2, Y-
 28 high P = 10.9, LSD (5 %) = 6.0 wk.
 29

30 **Table 5.** Mean treatment effects on daily milk yield, composition and component yields of the first 20
 31 wk of lactation of cows fed with Ll, Lh, Hl and Hh diets¹ during the pre-calving period. Values are
 32 covariate adjusted means for the M×F interaction.

Maturity:	Young			Mature			Significant factors	
	Fat:	Low Fat	High Fat	SEM ²	Low Fat	High Fat	SEM	P ³
n		8	7 ⁴		11 ⁴	12		
Yield, kg/d		27.5	26.3	1.21	26.3	28.7	0.99	M×F ⁺
Fat, g/d		1038	1036	43.4	1084	1128	35.4	Cov ⁺
Protein g/d		841	788	24.9	837	899	20.3	M ⁺ ; M×F ^{**} ; F×P ^{*5}
Lactose, g/d		1272	1218	59.9	1242	1331	48.9	NS
Fat, g/kg		39	39	0.8	41	40	0.6	Cov ^{***} ; M ⁺
Protein, g/kg		31	31	0.4	32	31	0.5	Cov ^{***} ; P ⁺⁶
Lactose, g/kg		46	46	0.4	47	46	0.5	NS

33

34 ¹ Ll, Low protein, low fat, ryegrass silage; Lh, low protein, high fat; Hl, high protein, low fat; Hh, high protein, high fat.

35 ² Standard error of the mean.

36 ³ Cov, Covariate (previous lactation's similar variable); M, maturity; P, Protein; F, Fat; NS, non-significant; +, P ≤ 0.10; *, P
 37 ≤ 0.05; **, P ≤ 0.01; ***, P ≤ 0.001.

38 ⁴ Data from 2 cows were removed for this analysis due to very low production.

39 ⁵ F × P* Ll = 858, Hl = 820, Lh = 832, Hh = 877, SEM (5 %) = 20.8 g/d.

40 ⁶ P⁺ low protein = 31, high protein = 32, SEM (5 %) = 0.92 g/kg.

41

42 **Table 6.** Mean treatment effects on milk N fractions at 3 wk postcalving of cows fed with Hh, Hl, Lh, Ll
 43 diets during the pre-calving period. Values are covariate adjusted means.

Maturity:	Young				Mature				SEM ²	Significant factors	
	Treatments ¹ :	Ll	Hl	Lh	Hh	Ll	Hl	Lh			Hh
n		4	4	3	4	5	5	4	5		
Milk protein fraction concentrations, g/kg											
Crude protein		32.7	34.8	32.3	32.9	34.5	33.3	32.7	33.4	0.89	Cov ⁺
Non protein N		0.36	0.34	0.34	0.30	0.33	0.34	0.36	0.39	0.020	M×F*
Urea N		0.03	0.04	0.04	0.04	0.04	0.04	0.03	0.04	0.002	M×F*
True protein		30.4	32.5	30.0	31.0	32.4	31.1	30.4	30.9	0.83	Cov**
Casein		25.4	26.5	23.8	24.2	27.3	25.7	24.3	25.2	0.72	F**
Whey protein		5.0	6.0	6.2	6.7	5.0	5.4	6.1	5.7	0.62	Cov ⁺

44
 45 ¹ Ll, low protein, low fat, ryegrass silage; Lh, low protein, high fat; Hl, high protein, low fat; Hh, high protein, high fat.

46 ² Standard error of the mean for n = 6.

47 ³ Cov, covariate (previous lactation average milk protein content); M, maturity; P, protein; F, fat; NS, non-significant; +, P≤
 48 0.10; *, P≤ 0.05; **, P≤ 0.01

Table 7. Mean treatment effects on milk N fraction yields (values in g/d unless otherwise stated) at 3 and 8 wk postcalving of cows fed with Hh, Hl, Lh, Ll diets during the pre-calving period. Values are covariate adjusted means.

Maturity:	Young				Mature				SEM ²	Significant factors
	Ll	Hl	Lh	Hh	Ll	Hl	Lh	Hh		
Treatments ¹ :									P ³	
N	4	4	3	4	6	6	6	6		
Wk 3 of lactation										
Milk yield, kg/d	30.5	26.3	27.0	26.0	31.9	28.4	34.1	37.0	1.88	Cov ^{**} ; M ^{**} ; M×F [*]
Crude protein	998	914	877	862	1092	948	1100	1224	60.3	M ^{**} ; M×F [*] ; P×F [*]
Non protein N	11	9	9	8	11	10	12	15	0.9	M ^{**} ; M×F ^{**}
Urea N	1.0	1.1	1.0	1.0	1.2	1.2	1.2	1.3	0.08	M [*]
True protein	929	856	816	812	1023	884	1022	1131	55.5	M ^{**} ; M×F [*] ; P×F [*]
Casein	774	695	642	633	864	732	821	929	47.2	M ^{**} ; M×F [*] ; P×F [*]
Whey protein	155	161	174	178	159	152	201	201	20.8	F [*]
Wk 8 of lactation										
Milk yield, kg/d	28.4	26.6	24.8	25.5	28.6	26.8	30.0	32.9	1.39	Cov ⁺ ; M; M×F ^{**}
Crude protein	980	893	844	896	955	880	912	1035	65.9	NS
Non protein N	11	14	11	11	13	12	12	12	1.0	NS
Urea N	1.4	1.2	1.1	1.4	1.2	1.2	1.1	1.2	0.06	P×F ⁺ ; M×P×F ⁺
True protein	912	807	778	831	935	807	830	954	57.7	F×P [*]
Casein	716	630	638	634	683	606	655	719	52.3	NS
Whey protein	194	176	138	196	149	201	224	235	29.5	NS

¹Ll, Low protein, low fat, ryegrass silage; Lh, Low protein, high fat; Hl, High protein, low fat; Hh, High protein, high fat.

²Standard error of the mean for n = 6.

³Cov, covariate (previous lactation average milk protein content); M, maturity; P, protein; F, fat; NS, non-significant; +, P ≤ 0.10; *, P ≤ 0.05; **, P ≤ 0.01.

Jaurena - Figure 1 a and b

Figure 1. Daily dry matter intake (DMI) of Young (a; $R^2 = 0.69$; $P < 0.001$) and Mature (b; $R^2 = 0.80$; $P < 0.001$) cows fed with the experimental diets during the dry period. Hh, high protein, high fat (■, —); Hl, high protein, low fat (◆; —••—); Lh, low protein, high fat (○; —•—); Ll, low protein, low fat (△, ---). Scatter symbols correspond to data, and lines to the fitted exponential model $DMI (kg) = a + b \times (1 - e^{-k \times d})$, where d is days before calving.

Jaurena - Figure 2 a and b

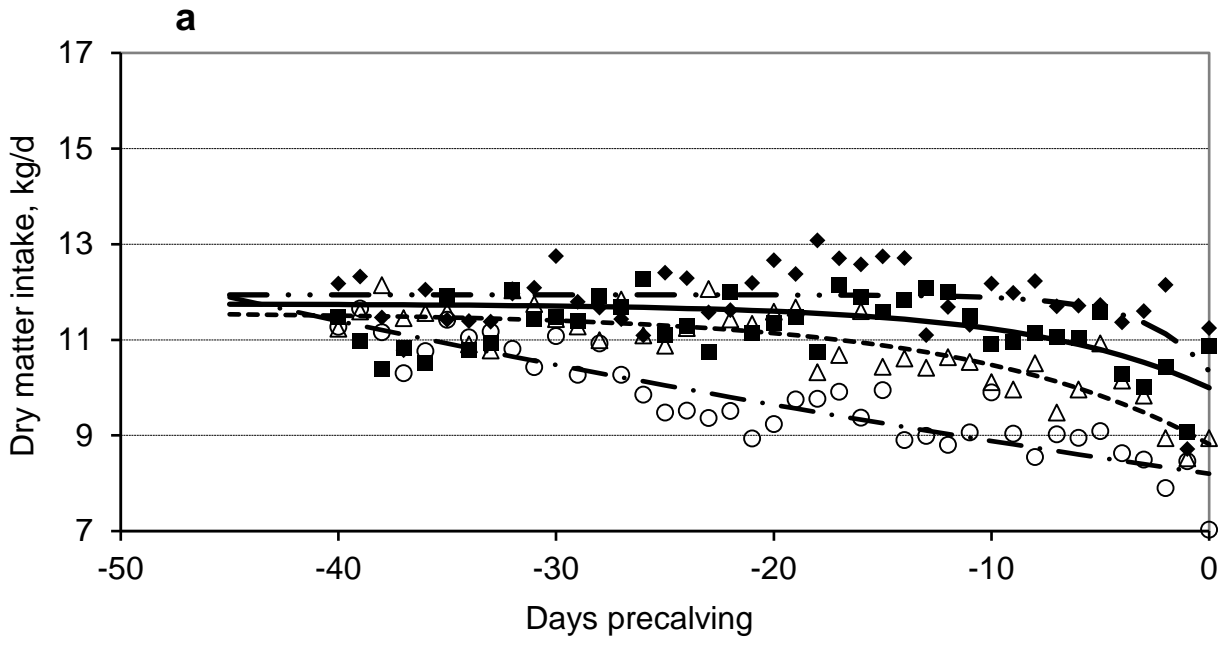
Figure 2. Body condition score (BCS) of Young (a) and Mature (b) cows fed with the experimental diets during the precalving period. Hh, high protein, high fat (■—); Hl, high protein, low fat (◆; —••—); Lh, low protein, high fat (○; —•—); Ll, low protein, low fat (△, ---). Markers represent treatment means; lines are fitted 4th degree polynomials. Vertical bars equal 1 pooled standard deviation.

Jaurena Figure 3 a and b

Figure 3. *Longissimus dorsi* depth of Young (a) and Mature (b) cows fed with the experimental diets during the precalving period. Hh, high protein, high fat (■—); Hl, high protein, low fat (◆; —••—); Lh, low protein, high fat (○; —•—); Ll, low protein, low fat (△, ---). Markers represent treatment means; lines are fitted 4th degree polynomial. Vertical bars equal 1 pooled standard deviation.

1 Jaurena - Figure 1 a

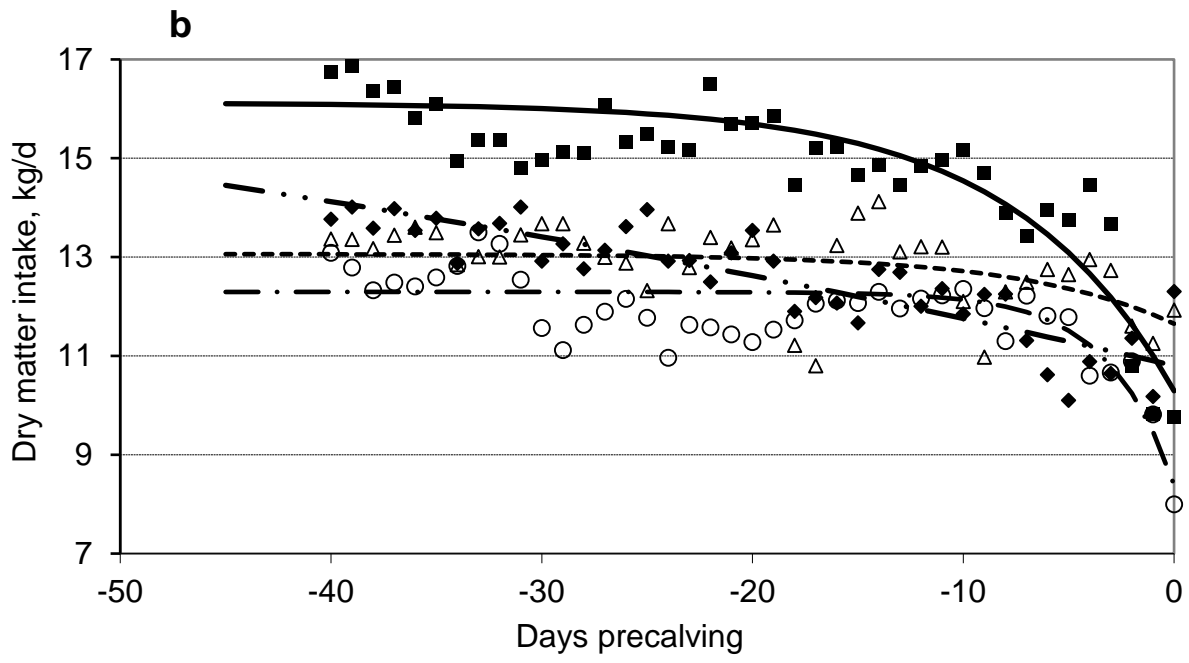
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7 **Figure 1 b**

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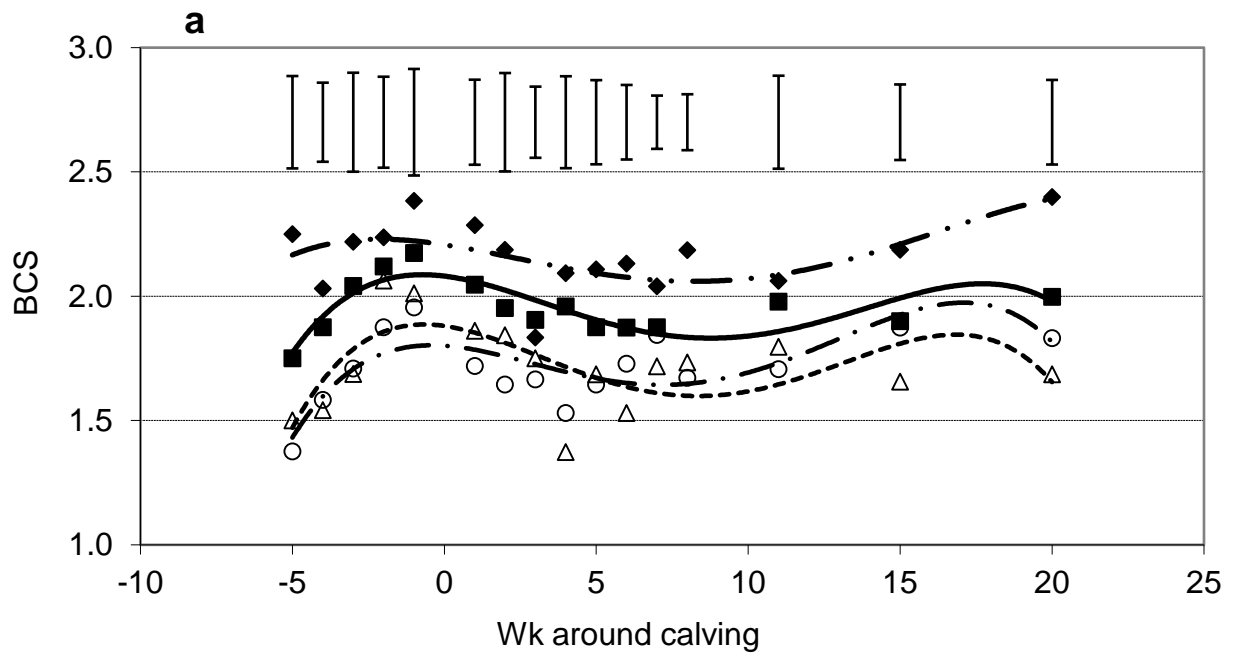
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12 **Jaurena Figure 2 a**

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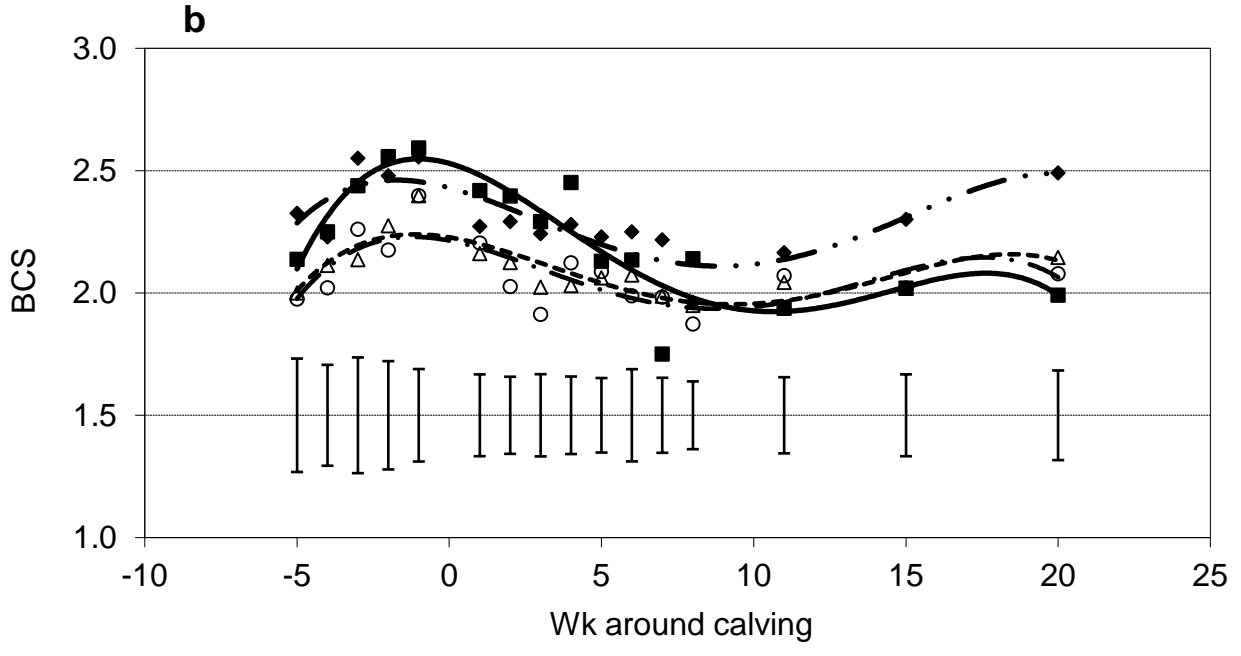
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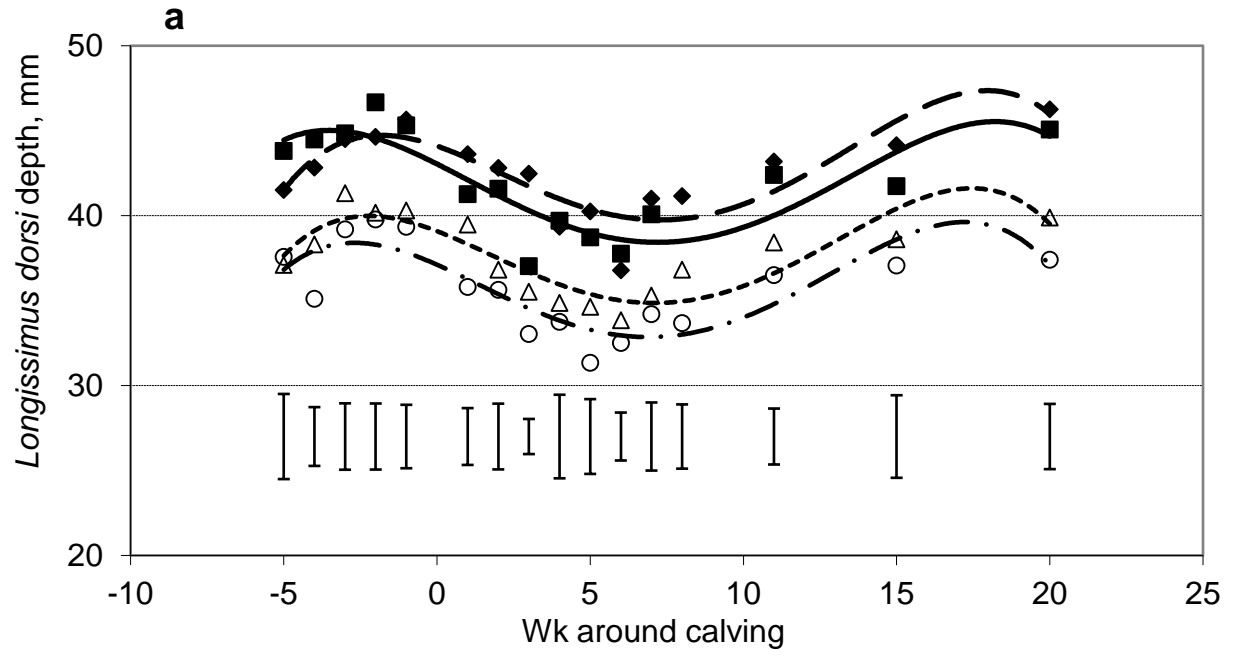
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17 **Figure 2 b**

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Figure 3 b.

