Lactation and body composition responses to fat and protein supplies during the dry period in under-conditioned dairy cows

Jaurena, G.; Moorby, Jonathan

Published in:
Journal of Dairy Science

DOI:
10.3168/jds.2016-11012

Publication date:
2017

Citation for published version (APA):
Jaurena, G., & Moorby, J. (2017). Lactation and body composition responses to fat and protein supplies during the dry period in under-conditioned dairy cows. *Journal of Dairy Science*, *100*(2), 1107-1121. https://doi.org/10.3168/jds.2016-11012

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.

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

G. Jaurena*[1] and J. M. Moorby†[2]

* Cátedra de Nutrición Animal, Departamento de Producción Animal, Facultad de Agronomía – Universidad de Buenos Aires. Av. San Martín 4453, (C1417 DSE) Ciudad Autónoma de Buenos Aires, Argentina

† Institute of Biology, Environment and Rural Sciences (IBERS), Aberystwyth University, Gogerddan, Aberystwyth, SY23 3EE, UK, UK

[1] Corresponding author: Gustavo Jaurena; e-mail: gjaurena@agro.uba.ar: Tel: 00 54 11 4524-8005

ABSTRACT

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

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

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

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

INTRODUCTION

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

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

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

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

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

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

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

MATERIALS AND METHODS

General design and management

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

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

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

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

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

Feed sampling and analysis

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

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

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

Measurements and sample collection on animals

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

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

Milk yields were measured and recorded automatically at each milking and samples were taken until wk 20 of lactation: milk samples were collected from each cow at two consecutive milkings weekly and analyzed for fat, protein and lactose by infrared milk analysis (National Milk Records Central Laboratory, Somerset, UK). Gross energy of the milk samples was estimated by the formulae of Tyrrell and Reid (1965; quoted by AFRC, 1993) using milk fat, protein and lactose contents for the current lactation data, and the formulae based on milk fat and protein contents for the previous lactation data.

At wk 3 and 8 of lactation, an extra sample of milk was taken and analyzed for milk CP fractions. Milk CP (total N × 6.38) was estimated in duplicate by Kjeldahl analysis, and milk protein fractions were separated according to the International Dairy Federation Standard (FIL-IDF, 1964) into true protein (**TPr**), casein N (**CN**), non-protein N (**NPN**), and whey proteins by difference. Milk urea concentration was estimated by a Sigma kit for urea-N determination (No. 640), and read spectrophotometrically at 570 nm.

Statistical analysis

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

$$Yijk = \mu + Cov + M + Pr + F + M \times Pr + M \times F + Pr \times F + M \times Pr \times F + \varepsilon$$

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

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

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

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

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

RESULTS

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

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

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

Feed characteristics and intake

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

240 > 0.05; Hl vs. Ll ration). Ether extract concentrations were similar for Ll and Hl 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 Hl 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;

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

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

Calf birth weights, milk composition and yield

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

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

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

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

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

DISCUSSION

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

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

Feed characteristics and intake

Both silages used pre- and postcalving showed acceptable fermentation characteristics and were within the range of values commonly found in the UK (Haigh, 1996a, b). The ash content of Megalac was higher than manufacturer specifications because the ashing procedure used (combustion at 550ºC) could have retained Ca as $CaCO_3$ instead of CaO (Dedman and Owen, 1962).

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

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

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

Body composition characteristics

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

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

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

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

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

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

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

Calf birth weight, milk composition and milk yield

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

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

Animal maturity was a significant factor in this experiment as Mature cows ate more in absolute terms because they were bigger, and therefore had a larger labile body protein pool than younger cows. Overall, differences in feed intake indicated that dry period management should consider younger second calving cows as requiring diets with higher nutrient densities than older cows.

AKNOWLEDGMENTS

The authors gratefully thanks to W. J. Fisher and R. Davies for their skilful assistance in managing and sampling the experimental herd. This work was funded by the UK Ministry of Agriculture, Fisheries and Food (now the Department for Environment, Food and Rural Affairs), and G. Jaurena is grateful for scholarships provided by the British Council and “Fundación Antorchas (Argentina)”.

REFERENCES

Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83:1598-1624.

Beever, D. E. 2006. The impact of controlled nutrition during the dry period on dairy cow health, fertility and performance. Anim. Reprod. Sci. 96:212-226.

Bell, A. W., R. Slepetis, and R. A. Ehrdardt. 1995. Growth and accretion of energy and protein in the gravid uterus during late pregnancy in Holstein cows. J. Dairy Sci. 78:1954-1961.

Choi, B. R. and D. L. Palmquist. 1996. High fat diets increase plasma cholecystokinin and pancreatic polypeptide, and decrease plasma insulin and feed intake in lactating cows. J. Nutr. 126:2913-2919.

Crowe, M. A. 2008. Resumption of ovarian cyclicity in post-partum beef and dairy cows. Reprod. Domest. Anim., 43 (Suppl. 5): 20–28

Dawson, J. M., C. I. Bruce, P. J. Buttery, M. Gill, and D. E. Beever. 1988. Protein metabolism in the rumen of silage-fed steers: effect of fishmeal supplementation. Br. J. Nutr. 60:339-353.

Dedman, A. J. and A. J. Owen. 1962. Calcium cyanamide synthesis. Part 4. - The reaction $CaO + CO_2 = CaCO_3$. Trans. Faraday Soc. 58:2027-2035.

DePeters, E. J. and J. D. Ferguson. 1992. Non protein nitrogen and protein distribution in the milk of cows. J. Dairy Sci. 75:3192-3209.

Dewhurst, R. J. and C. H. Knight. 1993. An investigation of the changes in sites of milk storage in the bovine udder over 2 lactation cycles. Anim. Prod. 57:379-384.

Dewhurst, R. J., A. M. Mitton, and C. H. Knight. 1993. Calibration of polyurethane foam cating technique for estimating the weight of bovine udders. Pages 101-102 in Proc. Br. Society of Anim. Prod.. Winter Meeting 13-17 March 1993, Scarborough, UK.

456 Dewhurst, R. J., J. M. Moorby, M. S. Dhanoa, R. T. Evans, and W. J. Fisher. 2000. Effects of altering
457 energy and protein supply to dry dairy cows. 1. Intake, body composition and milk production. J.
458 Dairy Sci.. 83:1782-1794.
459 Dewhurst, R. J., J. M. Moorby, M. S. Dhanoa, and W. J. Fisher. 2002. Effects of level of concentrate
460 feeding during the second gestation of Holstein-Friesian dairy cows. 1. Feed intake and milk
461 production. J. Dairy Sci. 85:169-177.
462 Douglas, G. N., T. R. Overton, H. G. Bateman Ii, H. M. Dann, and J. K. Drackley. 2006. Prepartal plane
463 of nutrition, regardless of dietary energy source, affects periparturient metabolism and dry matter
464 intake in Holstein cows. J. Dairy Sci. 89:2141-2157.
465 Drackley, J. K. 1999. Biology of dairy cows during the transition period: The final frontier? J. Dairy Sci.
466 82:2259-2273.
467 Faverdin, P., D. M'Hamed, and R. Verite. 2003. Effects of metabolizable protein on intake and milk
468 production of dairy cows independent of effects on ruminal digestion. Anim. Sci. 76:137-146.
469 FIL-IDF. 1964. International Dairy Federation Standard. Vol. 29. FIL-IDF.
470 Fronk, T. J., L. H. Schultz, and A. R. Hardie. 1980. Effect of dry period overconditioning on subsequent
471 metabolic disorders and performance of dairy cows. J. Dairy Sci. 63:1080.
472 Garnsworthy, P. C. 1988. The effect of energy reserves at calving on performance of dairy cows. Pages
473 157-169 in Nutrition and lactation in the dairy cow. Vol. 1. P. C. Garnsworthy, ed. Butterworths,
474 London.
475 GenStat-Committee. 2000. Genstat for Windows (5th Edition). VSN International, Oxford.
476 Grum, D. E., J. K. Drackley, R. S. Younker, D. W. LaCount, and J. J. Veenhuizen. 1996. Nutrition during
477 the dry period and hepatic lipid metabolism of periparturient dairy cows. J. Dairy Sci. 79:1850-
478 1864.

Grummer, R. R. 1993. Etiology of lipid-related metabolic disorders in periparturient dairy cows. J. Dairy Sci. 76:3882-3896.

Grummer, R. R. 1998. Transition cow energy, protein nutrition examined. Feedstuffs 70:11-13, 23.

Haigh, P. M. 1996a. The effect of dry matter content and silage additives on the fermentation of bunker-made grass silage on commercial farms in England 1984-91. J. Agric. Eng. Res. 64:249-259.

Haigh, P. M. 1996b. The effect of dry matter content and silage additives on the fermentation of bunker-made grass silages on commercial farms in Wales 1987-93. J. Agric. Eng. Res. 64:261-270.

Holter, J. B., M. J. Slotnick, H. H. Hayes, C. K. Bozak, W. E. Urban, and M. L. McGilliard. 1990. Effect of prepartum dietary energy on condition score, postpartum energy, nitrogen partitions, and lactation production responses. J. Dairy Sci. 73:3502-3511.

Hutjens, M. F. 1996. Practical approaches to feeding the high producing cow. Anim. Feed Sci.Technol. 59:199-206.

Huyler, M. T., R. L. Kincaid, and D. F. Dostal. 1999. Metabolic and yield responses of multiparous Holstein cows to prepartum rumen undegradable protein. J. Dairy Sci. 82:527-536.

Ingvartsen, K. L. and J. B. Andersen. 2000. Integration of metabolism and intake regulation: A review focusing on periparturient animals. J. Dairy Sci. 83:1573-1597.

Jaurena, G. 2003. Effect of dry period protein nutrition on subsequent milk production from dairy cows. Page 374 in Institute of Rural Studies - Institute of Grassland and Environmental Research (IGER). Vol. Philosophy Doctor. University of Wales (Aberystwyth), Aberystwyth, UK.

Jaurena, G., J. M. Moorby, W. J. Fisher, and R. Cantet. 2005. Association of body weight, loin longissimus dorsi and backfat with body condition score in dry and lactating holstein dairy cows. Anim. Sci. 80:219-224.

Jonker, J. S., R. A. Kohn, and R. A. Erdman. 1998. Using milk urea nitrogen to predict nitrogen excretion and utilization efficiency in lactating dairy cows. J. Dairy Sci. 81:2681-2692.

503 Kehoe, S. I., B. M. Jayarao, and A. J. Heinrichs. 2007. A survey of bovine colostrum composition and
504 colostrum management practices on Pennsylvania dairy farms. J. Dairy Sci. 90:4108-4116.
505 Kronfeld, D. 1982. Major metabolic determination of milk volume, mammary efficiency, and
506 spontaneous ketosis in dairy cows. J. Dairy Sci. 65:2204-2212.
507 Mee, J. F. 2008. Prevalence and risk factors for dystocia in dairy cattle: A review. Vet. J. 176:93-101.
508 Moorby, J. M., R. J. Dewhurst, R. T. Evans, and W. J. Fisher. 2002a. Effects of level of concentrate
509 feeding during the second gestation of Holstein-Friesian dairy cows. 2. Nitrogen balance and
510 plasma metabolites. J. Dairy Sci.. 85:178-189.
511 Moorby, J. M., R. J. Dewhurst, R. T. Evans, and W. J. Fisher. 2002b. Effects of varying the energy and
512 protein supply to dry cows on high-forage systems. Livest. Prod. Sci. 76:125-136.
513 Moorby, J. M., R. J. Dewhurst, and S. Marsden. 1996. Effect of increasing digestible undegraded protein
514 supply to dairy cows in late gestation on the yield and composition of milk during the subsequent
515 lactation. Anim. Sci. 63:201-213.
516 Moorby, J. M., N. M. Ellis, and D. R. Davies. 2016. Assessment of dietary ratios of red clover and corn
517 silages on milk production and milk quality in dairy cows. J. Dairy Sci. *in press.*
518 Mulvany, P. 1977. Dairy cow condition scoring. Paper No. 4468. No. Paper No. 4468. National Institute
519 for Research in Dairying, Reading, UK.
520 Murphy, J. J. 1999. Effect of dry period protein feeding on post-partum milk production and composition.
521 Livest. Prod. Sci. 57:169-179.
522 NRC. 2001. National Research Council. Nutrient Requirements of Dairy Cattle. 7th Rev. ed. National
523 Academy Press, Washington, DC,.
524 Orskov, E., R. 1982. Protein nutrition in ruminants. Academic Press, London.
525 Otto, K. L., J. D. Ferguson, D. G. Fox, and C. J. Sniffen. 1991. Relationship between body condition
526 score and composition of 9^{th} to 11^{th} rib tissue in holstein dairy-cows. J. Dairy Sci. 74:852-859.

Palmquist, D. L. 1993. Meeting the energy needs of dairy cows during early lactation. Page 43 in Proc. Tri-State dairy nutrition conference. Ohio State University, Michigan State Univ., and Purdue Univ., Ft. Wayne.

Prior, R. L. and D. B. Laster. 1979. Development of the bovine fetus. J. Anim. Sci. 48:1546-1553.

Putnam, D. E. and G. A. Varga. 1998. Protein density and its influence on metabolite concentration and nitrogen retention by Holstein cows in late gestation. J. Dairy Sci. 81:1608-1618.

Robinson, P. H., J. M. Moorby, M. Arana, and T. Graham. 2004. Effect of feeding a high- or low-rumen escape protein supplement to dry Holstein cows and heifers within 3 weeks of calving on their productive and reproductive performance in the subsequent lactation. Anim. Feed Sci. Technol. 114:43-57.

Robinson, P. H., J. M. Moorby, and D. D. Gisi. 2009. Colostrum production by primiparous and multiparous Holstein dairy cows and its usefulness as an estimator of full lactation milk yield. Livest. Sci. 125:323-325.

Santos, J. E. P., E. J. DePeters, P. W. Jardon, and J. T. Huber. 1999. Effect of prepartum crude protein level on performance of primiparous Holstein cows. J. Dairy Sci. 82(Suppl. 1):120 (Abstr.).

Santos, J. E. P., E. J. DePeters, P. W. Jardon, and J. T. Huber. 2001. Effect of prepartum dietary protein level on performance of primigravid and multiparous Holstein dairy cows. J. Dairy Sci. 84:213-224.

Schepers, A. J. and R. G. M. Meijer. 1998. Evaluation of the utilization of dietary nitrogen by dairy cows based on urea concentration in milk. J. Dairy Sci. 81:579-584.

Staples, C. R., J. M. Burke, and W. W. Thatcher. 1998. Influence of supplemental fats on reproductive tissues and performance of lactating cows. J. Dairy Sci. 81:856-871.

Studer, E. 1998. A veterinary perspective of on-farm evaluation of nutrition and reproduction. J. Dairy Sci. 81:872-876.

551 Tesfa, A. T., M. Tuori, L. Syrjala-Qvist, R. Poso, H. Saloniemi, K. Heinonen, K. Kivilahti, T. Saukko,
552 and L. A. Lindberg. 1999. The influence of dry period feeding on liver fat and postpartum
553 performance of dairy cows. Anim. Feed Sci. Technol. 76:275-295.
554 Tomar, S. S. 1973. Udder and teat mesurements andd their relation with milk prodution in Hariana cattle.
555 Indian J. Dairy Sci. 26:25.
556 Van Saun, R. J., S. C. Idleman, and C. J. Sniffen. 1993. Effect of undegradable protein amount fed
557 prepartum on postpartum production in first lactation holstein cows. J. Dairy Sci. 76:236-244.
558 Van Saun, R. J. and C. J. Sniffen. 1996. Nutritional management of the pregnant dairy cow to optimise
559 health, lactation and reproductive performance. Anim. Feed Sci. Technol. 59:13-26.
560 Waltner, S. S., J. P. McNamara, D. Brown, and J. K. Hillers. 1992. The validation of body condition
561 score, fat cell size and deuterium oxide dilution as indirect body fat measures in lactatin, Holstein
562 dairy cattle. J. Dairy Sci. 74 (Suppl. 1):252 (Abstr.).
563 Wu, Z., R. J. Fisher, C. E. Polan, and C. G. Schwab. 1997. Lactational performance of cows fed low or
564 high ruminally undegradable protein prepartum and supplemental Methionine and Lysine
565 postpartum. J. Dairy Sci. 80:722-729.
566 Zhu, W.-Y., M. K. Theodorou, A. C. Longland, B. B. Nielsen, J. Dijkstra, and A. P. J. Trinci. 1996.
567 Growth and survival of anaerobic fungi in batch and continuous-flow cultures. Anaerobe 2:29-
568 37.
569

1

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.

2

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.

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

Maturity:	**Young**				**Mature**					**Significant Factors**
Treatments:	**Ll[1]**	**Hl**	**Lh**	**Hh**	**Ll**	**Hl**	**Lh**	**Hh**	**SEM[2]**	***P*[3]**
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
Maximum[5]										
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*

[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 for n = 6.
[3] M, Maturity; P, Protein; F, Fat; NS, not significant; $^{+}$, P≤ 0.10; *, P≤ 0.05; **, P≤ 0.01; ***, P≤ 0.001.
[4] Covariate (initial body weight) corrected means.
[5] Covariate (first homologous data recorded) corrected means.

Table 4. Mean treatment effects on postcalving measurements of BCS, BW, and loin depths of *Longissimus dorsi* (LD) and backfat of cows offered diets Hh, Hl, Lh, Ll[1] 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	---	
Minimum[6]									
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]

[1] Ll, low protein, low fat, ryegrass silage; Lh, low protein, high fat; Hl, high protein, low fat; Hh, high protein, high fat.
[2] Y, young cows; M, mature cows.
[3] Standard error of the mean for n = 24.
[4] The same SEM for P and F factors.
[5] Cov, covariate; M, Maturity; P, protein; F, fat; NS, not significant; $^{+}$, P ≤ 0.10; *, P ≤ 0.05; **, P ≤ 0.01; ***, P ≤ 0.001.
[6] Statistical model with covariate of first homologous data recorded during the dry period.
[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 (5 %) = 803 g/d.
[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 mm/d.
[9] Failed Bartlett's test.
[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-high P = 10.9, LSD (5 %) = 6.0 wk.

Table 5. Mean treatment effects on daily milk yield, composition and component yields of the first 20 wk of lactation of cows fed with Ll, Lh, Hl and Hh diets[1] during the pre-calving period. Values are 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

[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] Cov, Covariate (previous lactation's similar variable); M, maturity; P, Protein; F, Fat; NS, non-significant; +, $P \leq 0.10$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.
[4] Data from 2 cows were removed for this analysis due to very low production.
[5] F ×P* Ll = 858, Hl = 820, Lh = 832, Hh = 877, SEM (5 %) = 20.8 g/d.
[6] P+ low protein = 31, high protein = 32, SEM (5 %) = 0.92 g/kg.

Table 6. Mean treatment effects on milk N fractions at 3 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**
Treatments[1]:	**Ll**	**Hl**	**Lh**	**Hh**	**Ll**	**Hl**	**Lh**	**Hh**		***P*[3]**
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^{+}

[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 for n = 6.
[3] Cov, 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

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**
Treatments[1]**:**	**Ll**	**Hl**	**Lh**	**Hh**	**Ll**	**Hl**	**Lh**	**Hh**		***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

[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 for n = 6.

[3] Cov, 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.

Jaurena - Figure 1 a

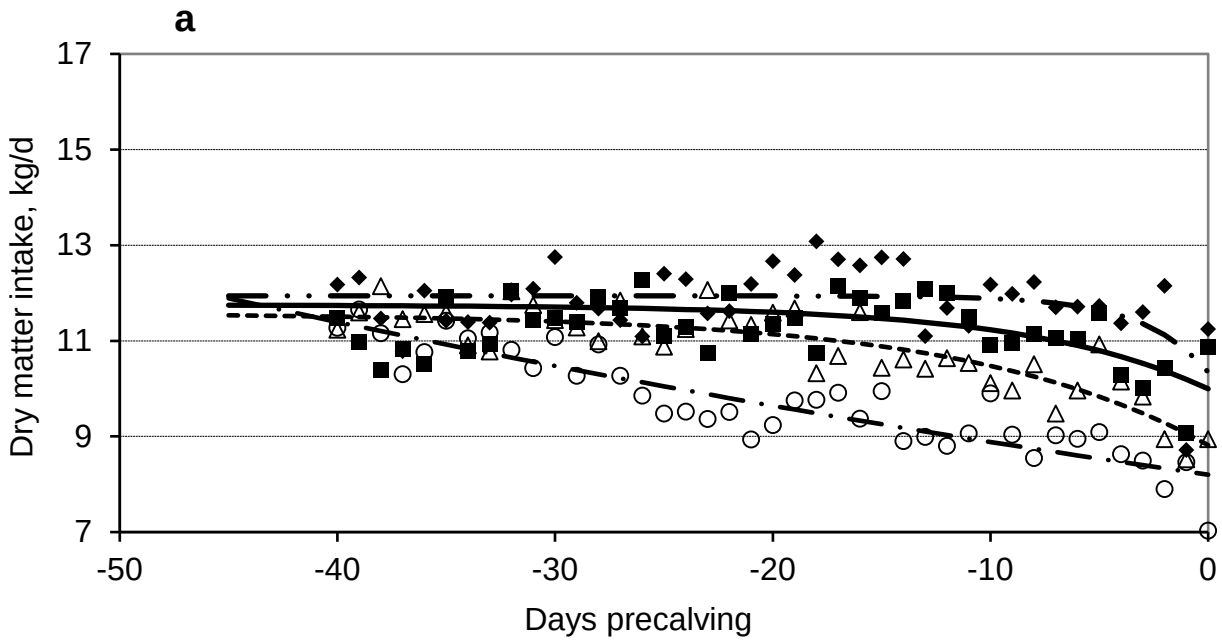
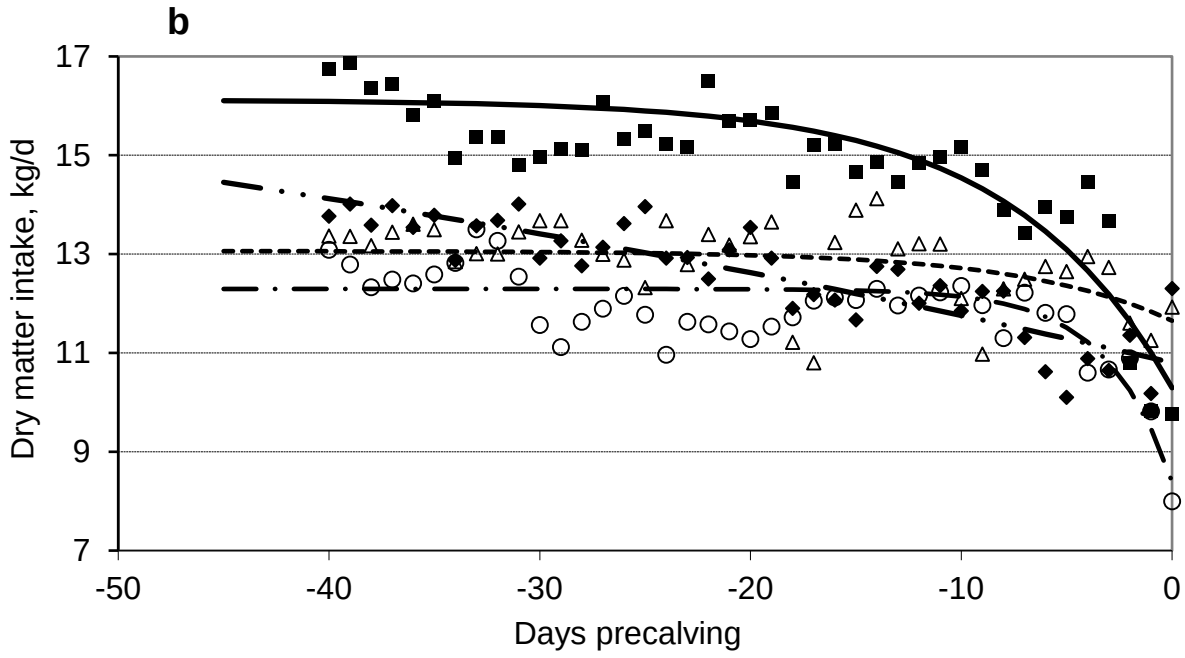


Figure 1 b


b
17
15
13
11
9
7
Dry matter intake, kg/d
-50
-40
-30
-20
-10
0
Days precalving

Jaurena Figure 2 a

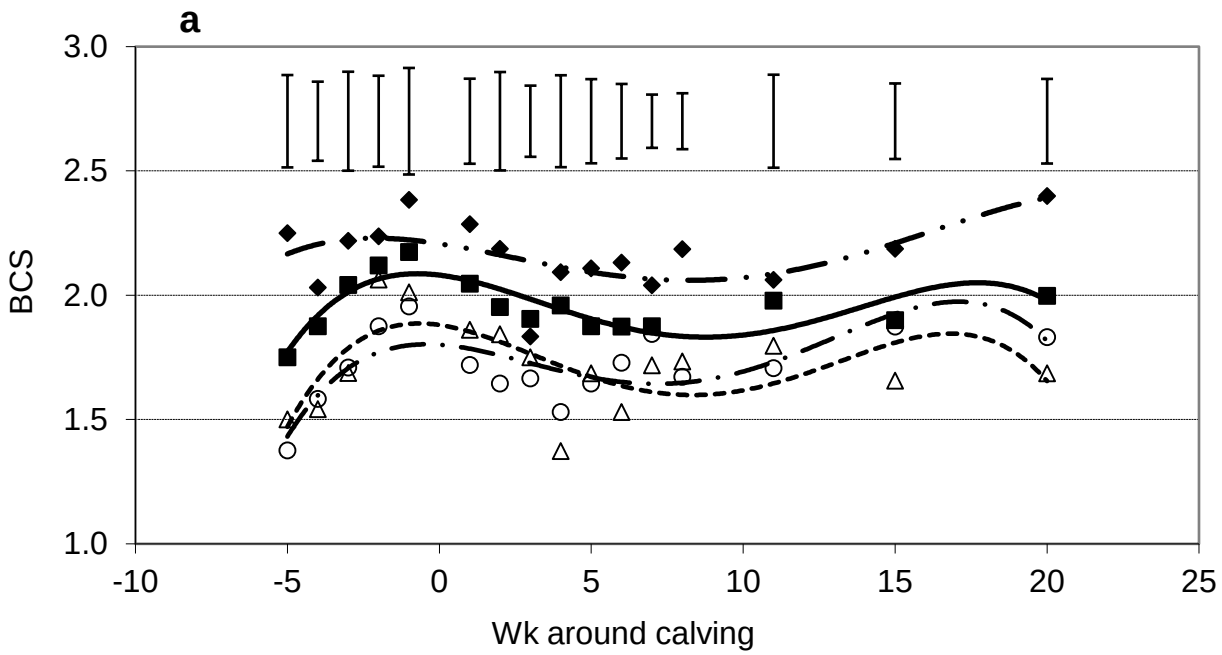
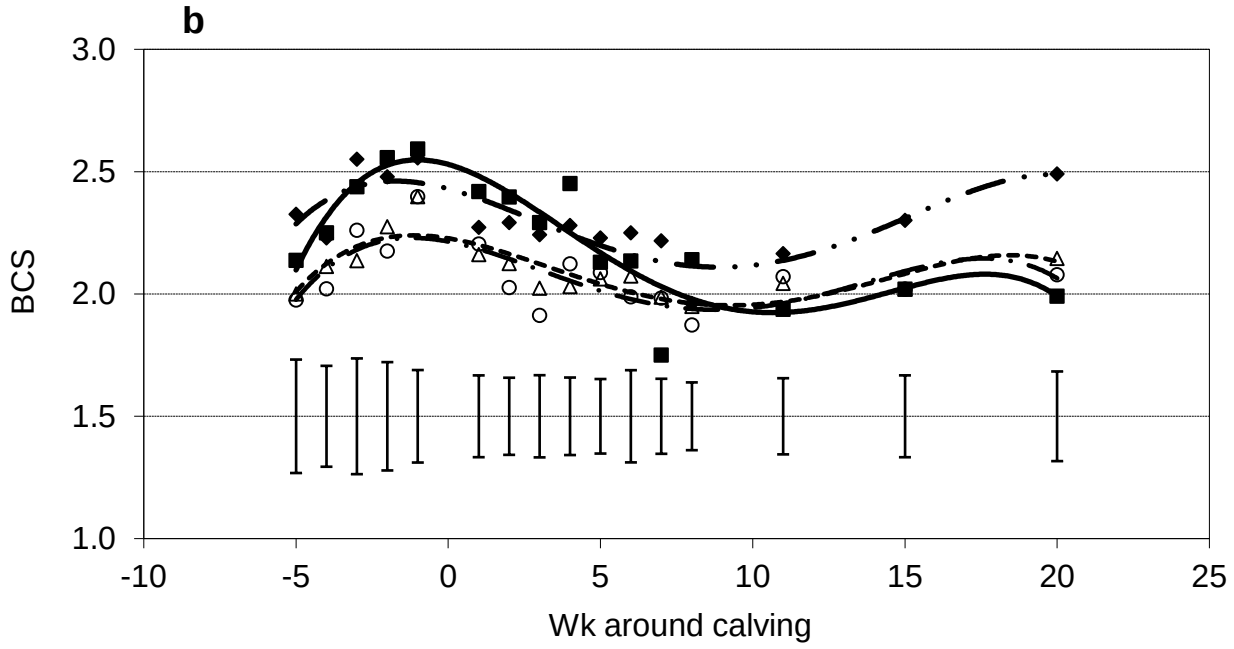


Figure 2 b



Jaurena Figure 3 a

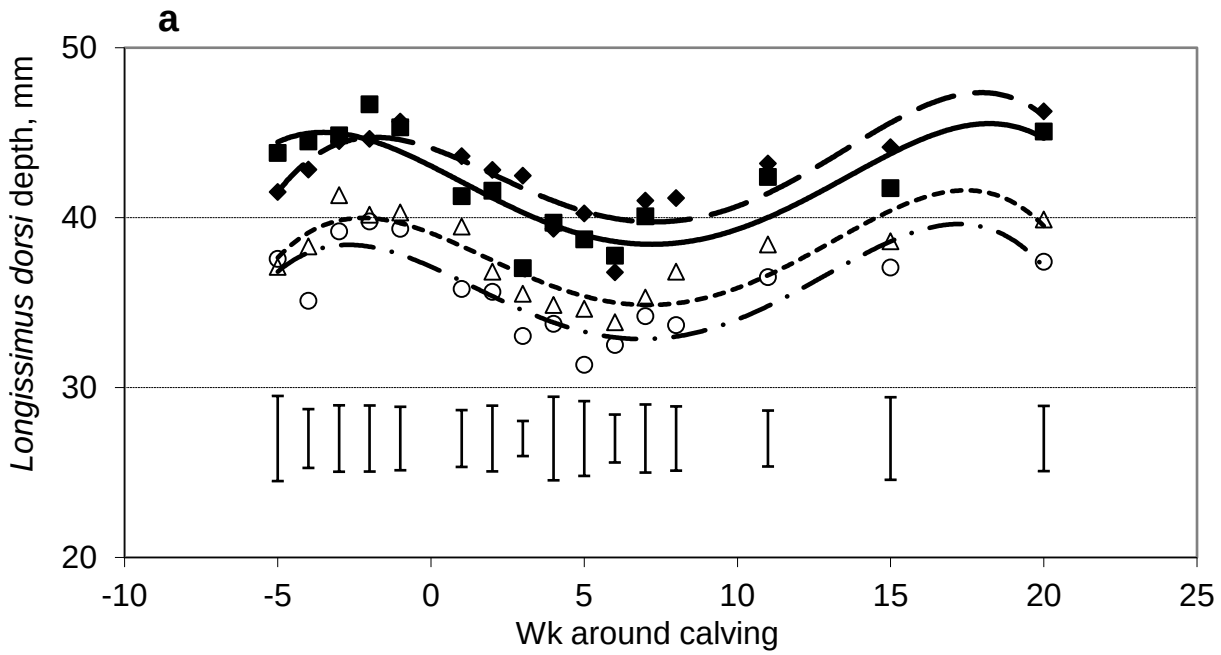
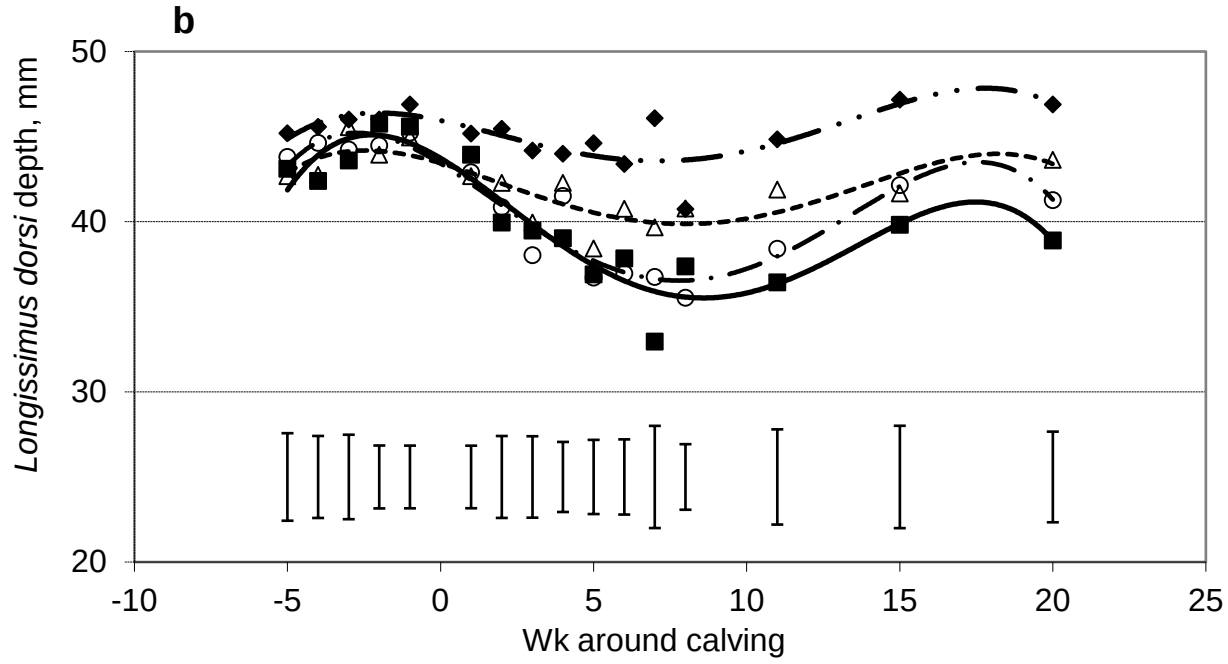


Figure 3 b.


b
50
40
30
20
Longissimus dorsi depth, mm
-10
-5
0
5
10
15
20
25
Wk around calving