



An examination of late lactation management strategies to improve the body condition score of thin cows, and the effects of these strategies on cow performance during the subsequent lactation

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STRUCTURE OF REPORT

This report begins with an Executive Summary which briefly highlights the background to the overall project and provides a brief description of the work undertaken within the project.

The main body of the report comprises a detailed description of the work undertaken, including methodology, results and discussion.

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EXECUTIVE SUMMARY

The majority of Holstein cows mobilise body tissue for milk production in early lactation, and replace this body tissue in later lactation. However, on most farms, a proportion of cows end their lactation in poor body condition and with a low body condition score. This can be a problem amongst grazing herds in the summer, if the weather is poor and grazing conditions become difficult, as a significant proportion of the cows can become thin.

Cows that end lactation with a low body condition score present a real challenge for dairy farmers in the next lactation, as thin cows are more likely to have health problems following calving, and poorer fertility, and are at increased risk of being culled during the subsequent lactation.

A body condition score of 2.75 at drying off is considered appropriate for cows with a high milk yield potential, and which will be managed on a high concentrate diet post-calving. However, there is little information available on optimum strategies by which to manage thin cows to allow them to gain body condition. Recent AFBI Hillsborough research has demonstrated that cows offered diets of grass silage and concentrates throughout the dry period gain very little body condition.

However, there is some anecdotal evidence that offering a low protein/high starch concentrate to cows in late lactation may encourage gains in body condition. In addition, the use of an extended dry period also has been advocated as a strategy by which to improve the body condition score of thin cows. The latter approach is often adopted in low input grazing systems, but it is unclear if an extended dry period is suitable for cows with a high milk yield potential.

The main aim of this study was to examine the effects of offering thin cows a low protein/high starch concentrate in late-lactation, or of adopting an extended dry period, on body condition score change and performance in late lactation, the dry period, and the subsequent lactation.

The study involved 65 Holstein-Friesian dairy cows. These were grazed tightly in mid/late lactation so that cows would become thin (target body condition score range, 2.0 – 2.5) at 14 weeks pre-calving. Cows were allocated to one of three treatments at 14 weeks pre-calving, as follows:

1. **Normal Protein:** Grass silage, plus 5.0 kg/day of a 'normal protein' concentrate. This diet was offered from week-13 until week-8 pre-calving. These cows were then dried-off 8 weeks pre-calving, and offered grass silage without concentrates until calving.

2. **Low Protein:** Grass silage, plus 5.0 kg/cow/day of a 'low protein/high starch' concentrate. This diet was offered from week-13 to week-8 pre-calving. These cows were dried-off 8 weeks pre-calving, and offered grass silage without concentrates until calving.
3. **Extended Dry Period:** Cows were dried-off 13 weeks prior to their expected calving date and offered grass silage without concentrates until calving.

Following calving, cows on all three treatments were offered the same diet for 140 days. This diet comprised grass silage plus approximately 13 kg concentrate/day.

Cows had an average body condition score of 2.25 at drying off. These cows were therefore in sub-optimal body condition, and were appropriate for use in this study.

During the period from 13 weeks pre-calving until 9 weeks pre-calving, the total diet offered to cows on the normal protein treatment had a crude protein content of 15.8 % while the diet offered to cows on the reduced protein treatment had a crude protein content of 13.7 % (DM basis).

Reducing the crude protein content of the diet normally results in a reduction in dry matter (DM) intake due to rumen function being affected. This was observed in the current study, with cows on the Low Protein treatment having a silage DM intake that was 1.1 kg lower than for cows on the Normal Protein treatment.

In addition, reducing the protein content of the diet will normally result in a reduction in milk yield. While cows on the Low Protein treatment produced 1.2 kg/day less milk than those on the Normal Protein treatment, a greater reduction in milk yield had been anticipated. Cows on the Low Protein treatment had a lower milk fat content and tended to have a lower milk protein content.

Body condition score was not affected by the crude protein and starch contents of the diet offered in late lactation. This was disappointing given the reasonable and supportable hypothesis behind this treatment, namely that offering a low protein/high starch concentrate would reduce milk yield with the additional energy being diverted to body tissue. Surprisingly, however, cows on the Low Protein diet gained much less live-weight during the late lactation period than did cows on the Normal Protein diet. Part of this weight differential can be attributed to a 'rumen fill' effect associated with the lower silage intake, but this certainly cannot be the full explanation, and the primary reason remains unclear.

Cows on the Extended Dry Period treatment were offered no concentrates from 13 weeks pre-calving until nine weeks pre-calving. As a result, while their silage DM intake was similar to that of cows on the Normal Protein and Low Protein treatments, their total DM intake was significantly lower.

Adopting an extended dry period reduced full lactation milk production by approximately 350 kg (approximately 4%). However, cows on this treatment were offered 175 kg less concentrate, resulting in a financial saving.

Cows on the Extended Dry Period treatment gained marginally more body condition, from 13 weeks pre-calving until 9 weeks pre-calving, than the cows on the other two treatments, but the effect was not significant.

All cows were dry from week 8 pre-calving until calving. Cows on all three treatments were offered the same diet (grass silage with no additional concentrates) at this time.

Cows that had been offered the Low Protein diet in late lactation continued to have a lower silage intake following drying off than did the cows on either of the other two treatments. Again, the reason for this is unknown. It was expected that the intakes of the Low Protein treatment cows would have returned to match that of cows on the Normal Protein treatment, shortly after they each had moved to the same diet.

The cows on the Normal Protein and Low Protein diets gained approximately 0.1 units of body condition during their 8 week dry period. This agrees with the findings of previous Hillsborough studies that have indicated that cows offered grass silage diets tend to gain relatively little body condition during the dry period, even if supplemented with modest quantities of concentrates.

While cows on each of the Low Protein and Normal Protein treatments gained approximately 40 kg 'live-weight' during the 8-week dry period, most of this gain is likely to have been associated with foetal growth and fluids. However, cows that had been managed on the Low Protein diet in late lactation remained lighter throughout the dry period.

Cows on the Extended Dry Period treatment tended to gain more body condition during the last 8 weeks of the dry period than cows on the other two treatments. The overall effect was that these cows had a mean body condition score of 2.6 during the week pre-calving, which was approximately 0.2 units higher than for cows on the other two treatments. Thus this study has demonstrated that for cows offered a silage only diet, an extended dry period can be a reasonably effective tool to help increase the body condition of thin cows to an 'acceptable' value pre-calving.

A difficult calving can lead to a loss of milk production, poor fertility and an increased risk of health problems for both cow and calf. However, the treatments imposed during late lactation in this study had no effects on calving difficulty score or calf birth weight (Table 3).

All cows were managed on the same diet during the 19 week period post calving, and as such any differences in performance during this time were likely due to the treatments imposed pre-calving.

Cows that had been managed on the Low Protein treatment in late lactation continued to have lower intakes than those on the Normal Protein treatment following calving, although there is no obvious explanation for this. These cows also had a lower milk yield and lower milk fat + protein yield post-calving than cows on the Normal Protein treatment, with this likely to have been a consequence of their lower DM intakes.

Although cows on the Extended Dry Period treatment had a higher body condition score at calving than those on the Normal Protein treatment, this difference disappeared almost immediately after calving. Cows on each of these two treatments had similar body condition scores and liveweights throughout the 19 week period post-calving. Giving cows an Extended Dry Period resulted in no milk yield or milk composition benefits during the subsequent lactation.

A number of fertility and health parameters were examined in this study, but the numbers of cows involved were inadequate to allow a robust assessment of effects on these parameters. Nevertheless, pre-calving treatment had no effect on the time taken for cows to show first signs of oestrus, or on subsequent fertility performance.

Treatment also had no effect on the incidence of mastitis, lameness or digestive problems in the subsequent lactation.

Offering a lower protein-higher starch concentrate in late lactation cannot be recommended as a means to improve the body condition score of thin cows in late lactation. Indeed, this treatment was associated with some longer-term negative effects which cannot be easily explained.

While the use of an extended dry period improved body condition score at the time of calving, this benefit was quickly lost post-calving. An extended dry period resulted in no longer-term performance benefits post-calving.

DESCRIPTION OF STUDY

An examination of late lactation management strategies to improve the body condition score of thin cows, and the effects of these strategies on cow performance during the subsequent lactation

INTRODUCTION

Most dairy cows experience a period of negative energy balance (**NEB**) early postpartum (**pp**) when their energy intake is inadequate to service the energy demands of lactation. Even well-managed cows may mobilise up to 40% of their fat reserves at this time (Chilliard et al., 2000), while cows that are underfed relative to requirements may incur even greater losses. Postpartum NEB is more pronounced in cows of higher genetic merit (Pryce et al., 2001) and may also be more protracted, so that such cows that are already in poor condition in early or mid lactation, can end their lactation visibly thin (i.e. with a low BCS).

Thin cows are common on many dairy farms. In a recent study on 10 Northern Ireland dairy farms, Law et al. (2016) found that 88% of the 1217 cows entered the dry period (DP) in low body condition (BCS < 2.5 on a 1-5 scale). They also found that thin cows (BCS < 2.25) that were offered no concentrates during the dry period were at increased risk of being culled during the subsequent lactation. Markusfeld et al. (1997) observed that multiparous cows with a higher body condition score at calving were less likely to be culled. Thin cows are more prone to periparturient health problems, including dystocia, endometritis and retained placenta (Heuer et al., 1999; Hoedemaker et al., 2009; Roche et al., 2015), that afflict many cows at a time when their immune-competence is impaired (Sordillo, 2016). Generally, however, immune competence is more compromised in over-conditioned cows that lose condition shortly after calving (Lacetera et al., 2005). There is experimental evidence too (Pryce et al., 2000; Roche et al., 2009) that supports anecdotal observations of difficulty in re-breeding thin cows, with Hoedemaker et al. (2009) finding a higher proportion of thin German Holstein cows not cycling by 3-5 wks pp and not pregnant

at 200-d pp. Moreover, thin cows that do become pregnant are more susceptible to peripartal disease (Heuer et al., 1999), bringing added veterinary costs and, often, an extended calving-to-conception interval (Borsberry and Dobson, 1989). Typically also, cows of BCS ≤ 3 at calving are less productive in the next lactation (Roche et al., 2009; 2015), although Garnsworthy (2007) recommended 2.75 as the optimum calving BCS for high-yielding dairy cows. Improving the BCS of thin cows prior to calving is a key management objective in many herds.

Nevertheless, building body condition in higher yielding dairy cows before calving is challenging and requires either an increased level of feeding or a reduction in dietary energy use for milk production. The 50-60 d DP that is widely used in the US and Europe evolved empirically to suit the lower yielding cows of that era, and its appropriateness for today's higher genetic merit (**HGM**) cows is questionable and in need of review. Keady et al. (2001) overfed cows late in their DP and increased their body condition. However, in a 2-yr study involving > 1200 HGM cows across 10 dairy farms, Law et al. (2016) offered 2.7 kg concentrates daily across the 8-wk DP to cows in poor condition (1.0 - 2.5 BCS units) and found BCS almost unchanged. Similarly, Little et al. (2016b) found only a small but significant gain in BCS (approx. 0.2 BCS units) in cows offered 3 kg concentrates daily throughout the dry period, compared with cows that received no supplementary concentrates during this time. Cow genetics can clearly influence outcomes, as seen in a whole-of-lactation study of confined cows offered a grass silage-based TMR diet (Vance et al., 2013), in which Holstein cows gained little condition from 20 to 40 wks pp while Jersey crossbred cows gained condition from wk 20 pp onwards.

A possible approach to improve the condition of cows ahead of calving is to offer additional concentrates in late lactation. However, high yielding cows offered additional concentrates of standard protein content in late lactation tend to partition much of the extra energy intake to milk production (Ferris and Mayne, 2003). In a previous study at this institute, offering a low protein diet (144 vs 173 g CP/kg DM) to high yielding cows in early lactation reduced their milk production and improved energy balance (**EB**) over the first 150 d of lactation, without affecting DMI (Law et al., 2009a). In a follow-on study, Gilmore et al. (2011) found that the EB of cows in early lactation was improved by short-term reductions in protein intake. Building on this, Law et al. (2011) showed that lower protein diets and a slower build-up of concentrates promoted a return to a positive EB by wk 7 pp, whereas cows offered normal allocations of a standard protein concentrate took 18 wks to attain positive EB. Similarly, in an on-farm study Dale et al. (2016) obtained small BCS increases by reducing diet protein content and delaying the introduction of concentrates to 21-d pp. However, there is no substantial research evidence that offering cows diets low in protein and high in energy in late lactation improves their BCS in late gestation.

Extending the DP, as is commonly practised in New Zealand's grazing-based dairy systems, is an alternative option for improving cow condition in late lactation. About 20% of high-yielding Holstein cows in US dairy herds have a DP > 70-d, while 8% have a DP > 90-d, mostly as a consequence of fertility problems (Kuhn et al., 2006). While it is axiomatic that a longer DP reduces milk yield in that lactation, allowing the diversion of dietary energy to tissue gain, there is an expectation also of a higher milk yield in the next lactation. Weber et al. (2015) found that cows given a 90-d DP produced more milk to 200 DIM in the next lactation than cows given a 28-d DP. In

contrast, Smith and Becker (1995) found that a DP > 70 d negatively affected subsequent milk production, though that study may lack relevance to today's higher yielding cows. However, Pinedo et al. (2011) also found both shorter (< 31 d) and longer (77-250 d) DPs negatively associated with early lactation and 305-d milk yields. These two studies, and similar studies, used a meta-analysis of potentially confounded data taken from retrospective herd records rather than generating data through controlled experiments. The value of such studies was questioned by Bachman and Schairer (2003) but there have been few attempts to systematically evaluate the use of planned extended DPs for dairy cows. Even in the study by Weber et al. (2015), cows were allocated to the notional 90-d DP treatment because their milk yield had fallen to < 15kg/d, while cows allocated to the 28-d and 56-d DPs were randomly selected from a separate cohort that had maintained 'normal' levels of production. However, none of these studies focused exclusively on thin cows.

Few controlled studies have evaluated the effects of varying DP length or DP nutrition on cow BCS at calving and subsequent lactation performance, and fewer still have focused exclusively on cows that are thin at drying-off (Little et al., 2016b). The benefits for BCS at calving and for subsequent lactation performance, of offering thin cows a lower protein diet in late lactation or extending the DP markedly, are essentially un-researched and were the focuses of this study.

MATERIALS AND METHODS

All animal procedures were approved by the animal research ethics committee at the Agri-Food and Biosciences Institute (AFBI), Hillsborough. The experiment was conducted at AFBI Hillsborough under an experimental licence granted by the

Department of Health, Social Services & Public Safety for Northern Ireland, and in compliance with the *United Kingdom Animals (Scientific Procedures) Act (1986)*.

Pre-experimental management

Commencing in mid-July, a dynamic group of mid-late lactation autumn calving Holstein-Friesian dairy cows (new cows being added as the season progressed) was managed with the objective of achieving a mean BCS of 2.25 (target range: 2.0 –2.5) at wk-13 prior to expected calving date. Cows were grazed tightly (target post-grazing sward height, less than 4.5 cm) and offered 3.0 kg concentrate per cow per day (1.5 kg at each milking) through an in-parlour concentrate feeding system until 13 wks prior to their expected calving date. Cows remaining within this dynamic group on 5th November were moved indoors and offered medium quality grass silage (ad-libitum) plus 5.0 kg concentrate daily via an in-parlour feeding system (2.5 kg at each milking) until they reached 13 wks prior to their expected calving date.

Cows and housing

A total of sixty-five cows, each with a BCS of 2.5 or less, were selected from the group described above 14 weeks prior to their expected calving date. Cows selected prior to 5th November were moved indoors at this time. Selected cows were placed in a free-stall house with access to individual rubber matted cubicles (2.20 m × 1.25 m). The mats were dusted with lime once weekly and the sawdust bedding was refreshed three times weekly. The house had a concrete floor which was scraped every three hours using an automated system. Cows remained in this house for the full experimental period (13 weeks pre-calving, until wk 19 post-calving). These cows (23 primiparous and 42 multiparous; mean parity, 2.0, s.d., 0.87) had a mean

Predicted Transmitting Ability (PTA₂₀₁₅) for milk and milk fat-plus-protein yield of 79 (s.d., 135.9) and 16.7 (s.d., 8.04) kg, respectively, and a mean Profitable Lifetime Index (PLI) of £219 (s.d., 91.9) (December 2015 proof run).

Treatments

Cows were allocated to one of three treatments 13 wks prior to their expected calving date, at which time they had a mean BCS of 2.25 (s.d., 0.158). Cows within each treatment group were balanced for parity (1, 2 and >2), BCS, BW and milk yield (during previous week), milk composition (as recorded on one occasion during the previous 4 wk period), and PTA for fat + protein (kg), PLI, and their previous lactation 305-d milk yield.

The three experimental treatments were as follows:

1. **Normal protein (NP):** *ad libitum* grass silage, plus 5.0 kg/cow per d of a 'normal protein' concentrate (target: 230 g crude protein/kg DM). This diet was offered from wk 13 to wk 9 (5 wk period) prior to the expected calving date for each cow. Cows were 'dried-off' 8 weeks prior to their expected calving date.
2. **Low-protein (LP):** *ad libitum* grass silage, plus 5.0 kg/cow per d of a 'low protein' concentrate (target: 150 g crude protein/kg DM). This diet was offered from wk 13 to wk 9 (5 wk period) prior to the expected calving date for each cow. Cows were 'dried-off' 8 wks prior to their expected calving date.
3. **Extended dry period (EDP):** cows were 'dried-off' 13 wks prior to their expected calving date and offered grass silage *ad libitum* without concentrate supplementation.

During wks 13 to 9 prior to their expected calving date, cows on the NP and LP treatments were kept in a single group, while cows in the EDP group were housed in a separate pen. All cows accessed the forage portion of their diet via a series of feed-boxes mounted on weighing platforms with access controlled by a Calan-gate feeding system (American Calan; NH, USA) linked to an automatic cow-identification system (Griffith Elder; Bury St Edmunds, UK), which recorded the weight of food consumed by each cow each day. Silage for all three treatments was placed in a complete diet mixer wagon (Redrock, Armagh, UK) and mixed for approximately 5 minutes, before being placed in the feed-boxes. Cows on the NP and LP diets accessed their silage via separate feed boxes. Fresh silage was offered daily between 09.00 and 10.30 h. Uneaten silage was removed the following day at approximately 08.30 h. The silage offered during wks 13 to 9 pre-partum was produced from a combination of primary growth (harvest dates: 16 - 22 May) and secondary re-growth of grass (harvest date: 17 September). The concentrate portion of the diet (ingredient composition presented in Table 1) was offered via an in-parlour concentrate feeding system, at 2.5 kg fresh weight/cow at each milking.

After drying off (at 8 wks prior to expected calving) cows from the NP and LP treatments joined those from the EDP treatment, and all cows were offered the same grass silage as was offered during wks 13 to 9 pre-calving, until calving. No concentrates were offered during this period. The silage offered throughout the DP was supplemented with pre-calving minerals, and with additional calcined magnesite over the final 3 weeks pre-partum. Both supplements were mixed with the silage. Target intakes were 100 and 50 g/cow per d, respectively.

Cows were moved to a straw-bedded maternity pen 24 – 48 h prior to their expected calving date, based on physical observations. Post-calving (normally within 12 – 24 h) cows were returned to the house described earlier, and placed in a group comprising freshly calved cows.

Post-calving management

All cows calved between 16 October 2014 and 8 March 2015 (mean calving date, 9 January 2015; s.d., 35 d). Cows from all three treatments were offered a common diet for the first 133 d (19 wks) pp. The diet comprised a mixed ration of grass silage and concentrates (60:40 ratio, on a DM basis) plus 5 kg/cow/day fresh weight of the same concentrates, offered via an in-parlour concentrate feeding system. The mixed ration was offered at 1.07 of the previous day's intake. The concentrates offered in-parlour were introduced into the diet incrementally over the first nine days post-calving, starting at 1.0 kg/d immediately pp and increasing to 5.0 kg/d at d 9 pp. The mixed ration portion of the diet was prepared daily using a complete diet mixer wagon as described previously, and was offered between 09.00 and 10.30 h each day. Cows accessed the mixed ration via the feeding system described previously. Uneaten food was removed at approx 08.30 h each day. The silage offered postpartum was produced from a primary grass re-growth (harvest dates: 9 and 29 July 2014). The ingredient compositions of the concentrate offered is presented in Table 1.

Animal measurements

Calving difficulty was recorded using a 1 - 4 scale according to the following criteria: unobserved/unassisted calving (1); assisted calving but without a calving aid (2); assisted calving with use of a calving aid (3); and veterinary assisted calving (4). Calves were weighed immediately after birth. Throughout the experiment, cows were milked twice daily (between 06.00 and 08.00 h, and between 15.00 and 17.00 h) using a 50-point rotary milking parlour. Milk yields were recorded automatically at each milking, allowing the total milk daily yield for each cow to be calculated.

Colostrum samples

During each of the first four milkings, colostrum was sampled and analysed for fat, protein, lactose, casein nitrogen (**N**) and urea N contents using a Milkoscan 605 (Foss, Warrington, UK). Immunoglobulin G (**IgG**) concentrations were determined using a bovine-specific ELISA kit (Bio-X Diagnostics, Jemelle, Belgium), after centrifugal removal of fat. Kit components were brought to 21°C before use. Wash buffer was diluted 20-fold with distilled water. Samples were diluted in PBS, as per the manufacturer's instructions, and analysed in duplicate. After incubation at 21°C for 1 hour, the test plate was washed 3 times with wash buffer, before addition of chromogen solution (100 µL) to each well, and incubation away from direct light, for approx 10 minutes. Finally, stop solution (50 µL) was added to each well and the optical density of each was measured using a micro-plate spectrophotometer with a 450 nm filter. The concentration of IgG in samples was determined from a calibration curve generated using standards supplied with the kit. If a sample result fell outside the range of the standard curve, the sample was re-tested, after dilution if necessary, and according to kit instructions. A weighted IgG content was calculated for the first

and second milking pp, and for the third and fourth milking pp. The inter-assay CV was < 0.15.

Throughout the remainder of the experiment (and during the period from wk 13 to wk 9 pre-partum), milk samples were collected during two consecutive milkings each week and analysed for fat, protein, and lactose concentrations as described above. A weighted milk composition was calculated (based on morning and afternoon milk yields) for each 24-h sampling period. Once per month, samples from two consecutive milkings, combined in proportion to yield, were collected and SCS was measured using a SomaScope MK II somatic cell counter, Model CA-3A4 (Delta Instruments, Drachten, The Netherlands).

Individual cow BWs were recorded twice daily (immediately after each milking) using an automated weighbridge, and a mean weekly BW was determined for each cow. Non-lactating animals were weighed once weekly prior to being offered fresh food, between 09.00 and 10.30 h. The BCS of each cow was estimated weekly by a trained technician, and according to Edmondson et al. (1989), using a 5 point scale. Milk samples for progesterone analysis were collected twice weekly during the first 70 d pp, and a Lactab Mark III preservative tablet (Thompson and Cooper Ltd., Runcorn, UK) was added. Samples were stored at 4°C for up to 4 wks pending analysis. Progesterone concentrations were determined using an ELISA kit (Ridgeway Science, St Briavels, Glos., UK) based on the method of Sauer et al. (1986), with the day of onset of luteal activity defined as the first day when at least two consecutive daily milk progesterone concentrations were ≥ 3 ng/ml. All cows were bred using AI conducted by trained stockpersons, and pregnancy was

confirmed using an ultrasound scan carried out by a veterinarian. All individual cow health events and treatments were recorded. Any incidence of displaced abomasum, dilated caecum, decreased rumen motility or diarrhoea was recorded as a digestive upset. Cows identified with mastitis or lameness were treated by trained stockpersons.

The mean daily energy balance (MJ/cow per day) was calculated for each cow using the equation of Agnew et al. (2004) below:

$$\text{mean EB} = ([\text{ME}_{\text{main+milk}} \times \text{BW}^{0.75}] + [(0.0013 \times \text{BW}) / \text{K}_m] + \text{ME}_c - 10) - \text{ME}_i$$

where $\text{ME}_{\text{main+milk}}$ is the ME required for maintenance and milk production (MJ/kg metabolic weight), $\text{BW}^{0.75}$ is the metabolic BW, K_m is the efficiency of utilization of ME for activity (calculated as $0.35 \times \text{ME}/\text{gross energy} + 0.503$), ME_c is the ME required for pregnancy, and ME_i is the ME intake (MJ/cow per d). Data for mean daily milk yield, milk fat, protein and lactose concentrations, and mean BW were used in the calculations for the EB variables.

Blood samples were collected from the coccygeal vein of each cow prior to feeding in wks 9, 6 and 3 pre-partum (± 3 days), and in weeks 2, 4, 6, 8 and 10 (± 3 days) pp. Samples were collected in evacuated tubes (Becton Dickinson, Oxford, UK) coated with either a clot activator (for serum) or fluoride oxalate (for plasma). The blood samples were centrifuged (1800 g, 17°C, 30 minutes) to recover serum and plasma which were stored at -20°C until analysed. Plasma was analysed for glucose concentrations, while serum was analysed for βHB , NEFA, urea and haptoglobin

concentrations. Plasma glucose concentrations were determined by the hexokinase method using a clinical auto-analyser kit (Roche Diagnostics Ltd., Burgess Hill, UK); serum BHB concentrations were determined according to McMurray et al. (1984); serum urea concentrations were analysed using a kinetic UV kit (Roche Diagnostics Ltd., Burgess Hill, UK); and serum NEFA concentrations were determined using a WaKo kit (Wako Chemicals GmbH, Neuss, Germany). Serum haptoglobin was determined using a Tridelta PHASE Haptoglobin Assay kit (Tridelta, Maynooth, Kildare, Ireland). All analyses were conducted on an Olympus AU640 analyser.

Feed analysis

The grass silages were sampled daily throughout the experiment. Samples were dried at 85°C for 18 h to determine oven DM content. Subsamples of the dried samples were taken twice weekly and milled to pass through a 0.8 mm sieve, and composited every 28 d and analysed for NDF, ADF, and ash concentrations. The concentrate feeds (pellets and meal) were sampled every 14 d, dried at 100°C for 24 h, and milled to pass through a 0.8 mm sieve. Milled samples were composited every 28 days, and analysed for CP ($N \times 6.25$), NDF, ADF, ash, and gross energy (GE) concentrations. An additional concentrate sample was taken at the same sampling frequency, dried at 60°C for 48 h and milled to pass through a 0.5 mm sieve, prior to analysis for starch concentration. Concentrations of NDF and ADF were determined using a Fibertec analyser (Foss, Warrington, UK) based on the method of Van Soest (1976). Ash concentrations were determined following combustion at 550°C in a muffle furnace for approximately 10 h. Starch concentrations were determined using a Megazyme kit (Megazyme International, Bray, Ireland). A fresh sample of each grass silage offered was taken weekly and

analysed for concentrations of CP ($N \times 6.25$), ammonia-N (NH_3-N), fermentation acids (lactic and acetic), ethanol, propanol, and for GE and pH. Silage fermentation acids, ethanol, and propanol were determined using a single-column Varian Star 3400CX gas chromatograph (Agilent Technologies Ltd., Cheadle, UK) with on-column injection of samples and flame-ionisation detection. Crude protein was determined using a Tecator Kjeltac Auto 2400/2460 Analyser and Sampler System (Foss, Warrington, UK). The gross energy concentrations of fresh silages and dried concentrates were determined using a Parr 6300 Isoperibol Bomb Calorimeter (Parr Instrument Co., Moline, IL). In addition, the ME concentration of fresh silage was predicted weekly by NIRS, as described by Park et al. (1998).

Statistical analysis

Data for milk production and composition, BW, BCS and DMI were analysed within three distinct periods, namely wk 13 to wk 9 pre-partum, wk 8 to wk 1 pre-partum and wk 1 to wk 19 post-partum. These data were analysed using ANOVA. Appropriate pre-experimental data were included as co-variates in the model when analysing corresponding dependent variables, namely milk yields recorded during wks 14 and 15 pre-partum (for milk yield) and milk composition recorded on one occasion during the 4-wk period prior to the start of the experiment (for milk composition), and mean BW and BCS recorded during the 2-wk period prior to the start of the experiment (for BW and BCS). In the case of total DMI, pre-experimental BW was used as a covariate, as pre-experimental intake data was unavailable. In addition, parity (1, 2, and > 2) was also included as a factor within the analysis. For variables for which late lactation treatment had a significant effect ($P < 0.05$), differences between treatments were tested using Fisher's-protected adjusted

comparisons. Sixty-five cows were allocated to the late lactation treatments and remained on the study throughout the period from wk 13 pre-partum to calving. Five cows did not complete the 133-d pp period, with four cows being removed from the study immediately pp due to issues unrelated to the treatments (fatal digestive upset, n = 1; and calving injury, n = 3). One further cow was removed from the study at 68 d pp due to a leg injury that resulted in poor locomotion. Post-partum data for these cows were treated as missing values in the analysis. Data for calving difficulty, calf birth weight, colostrum quality, calving interval, days to onset of corpus luteum (CL) activity, and the maximum progesterone concentration during the first CL were analysed using ANOVA, with the model including parity as a covariate. Binomial data relating to the health and fertility parameters were analysed using a regression model, with the model including parity as a covariate. Weekly data for mean daily milk yield, mean BW, mean BCS and mean total daily DMI were analysed using REML analysis, with weekly measurements included as the repeated measure in the model. Parity (1, 2, and > 2) and the corresponding pre-experimental co-variate, as described above (for milk yield, BW, BCS and intake), were included in the model. The model for REML analysis included the effects of late lactation treatment, Week (either pre-partum or post-partum) and late lactation treatment x Week, with data for each of the three experimental periods described above analysed separately. Weekly data for pre-partum serum and plasma biochemistry were analysed by ANOVA for individual weeks (weeks 9, 6 and 3 pre-partum), with the post-partum data analysed using REML (weeks 2, 4, 6, 8 and 10), with parity (1, 2, and > 2) used as a covariate and using the REML model described above. All data were analysed using GenStat Version 16.2 (VSN International) as described by Payne et al. (2013).

RESULTS

Diet composition

The NP concentrate had protein and starch contents of 228 and 207 g/kg DM, respectively, while the corresponding values for the LP concentrate were 153 and 293 g/kg DM (Table 2). The concentrate offered postpartum had average CP and starch contents of 215 and 245 g/kg DM, respectively. Mean volatile corrected DM, CP and ME concentrations of the silage offered pre-partum were 235 g/kg of fresh weight (FW), 130 g/kg DM and 10.9 MJ/kg DM, respectively, while the corresponding values for the silage offered postpartum were 326 g/kg FW, 119 g/kg DM and 11.4 MJ/kg DM. The diets offered between wk 13 and wk 9 pre-partum to cows on the NP, LP and EDP treatments had mean CP contents of 158, 137 and 130 g/kg DM, respectively, while the NP and LP diets had starch contents of 60 and 92 g/kg DM, respectively. The mean CP contents of the postpartum diets were 165, 166 and 165 g/kg DM for the NP, LP and EDP treatments, respectively.

Treatment effects during wk 13 to wk 9 pre-partum

Cows on the LP treatment had a lower ($P = 0.004$) silage DMI than those on the NP and EDP treatments (Table 3) while total DMI was lowest for the EDP treatment and highest for the NP treatment ($P < 0.001$). Cows on the NP treatment tended to have a greater milk yield and milk protein content ($P = 0.05$ and $P = 0.094$, respectively) while their milk had a greater fat content and they had a greater milk fat-plus-protein yield ($P = 0.003$ and 0.014 , respectively).

Cows on the LP treatment gained less BW over the 5 wk period ($P = 0.004$) and had lower BW at wk 9 pre-partum ($P = 0.004$), and the lowest mean BW during this

period ($P = 0.005$). There were no significant treatment effects on either mean BCS ($P = 0.0320$) or on BCS change over the period ($P = 0.103$).

When examined using repeated measures analysis, total DMI (Figure 1), BW (Figure 2) and daily milk yield (Figure 4) changed with time during this 5 wk period (all $P < 0.001$), while BCS (Figure 3) tended to change with time ($P = 0.051$). There was no treatment x week interaction for BCS, milk yield or total daily DMI (all > 0.10). Treatment did not affect plasma glucose concentration ($P = 0.266$) at wk 9 pre-partum (Figure 5), but serum BHB, NEFA and urea concentrations were all lower in cows on the EDP treatment than for those on the NP treatment ($P < 0.001$, $P < 0.001$ and $P = 0.004$, respectively).

Treatment effects during wk 8 to wk 1 pre-partum

During this period (i.e. the conventional dry period) cows which had been managed through late lactation on the LP treatment had lower silage DMI ($P < 0.001$) than cows managed on either the NP or EDP treatments (Table 4). Treatment did not affect BW change during this period ($P = 0.588$), but LP cows had the lowest mean BW during this period ($P = 0.001$) and the lowest BW during the final week pre-partum ($P = 0.020$), with the respective BW values of cows on the other two treatments being similar. Mean BCS and BCS during the final week pre-partum were higher ($P = 0.021$ and $P = 0.006$, respectively) for EDP cows than for cows on the other two treatments.

When examined using repeated measures analysis, DMI (Figure 1) decreased ($P < 0.001$), while BW (Figure 2) and BCS (Figure 3) both increased ($P = 0.001$ and $P =$

0.002, respectively) over the 8 wk period pre-partum. There a late lactation treatment x week interaction during this period for DMI and for BW ($P < 0.001$) but not for BCS ($P = 0.923$). Treatment did not affect ($P > 0.10$) plasma glucose or serum BHB, NEFA, urea and haptoglobin concentrations in wks 6 or 3 pre-partum (Figures 5 and 6).

Treatment effects during wks 1 to 19 pp

Pre-partum treatment did not affect calving difficulty score ($P = 0.329$) or calf birth weight ($P = 0.209$), with the values for NP, LP and EDP treatments being 1.4, 1.2 and 1.2 (s.e.d., 0.18) and 41.4, 38.6 and 41.3 kg (s.e.d., 1.83), respectively. The first and second colostrums taken from cows on the LP treatment had significantly lower urea N contents ($P = 0.033$) than those from cows on either of the other two treatments (Table 5). Cows on the LP treatment had lower third and fourth colostrum yields than cows on the other two treatments ($P = 0.046$) but no other colostrum characteristic was affected by pre-partum treatment (Table 5).

Cows on the LP treatment from wks 13 to 9 pre-partum had a lower concentrate DMI ($P < 0.009$), a lower silage DMI ($P < 0.017$) and a lower total diet DMI ($P = 0.010$) than cows on either the NP or EDP treatments during the subsequent lactation (Table 6). However, EDP cows had greater wks 1-19 milk yields than those on the LP treatment ($P = 0.040$). The milk yield of cows on the NP treatment did not differ from those of cows on the other two treatments, while milk composition was unaffected by treatment ($P > 0.1$). Cows on the LP treatment had the lowest BW in the first wk postpartum ($P = 0.022$) only, but treatment did not affect BW at wk 10 pp ($P > 0.10$) or wk 19 pp ($P = 0.075$) nor mean BW through wks 1-19 pp ($P = 0.090$).

Neither the nadir BW through wks 1-19 pp ($P > 0.10$), nor days to nadir BW ($P > 0.01$), nor BW loss to nadir ($P = 0.081$) were affected by treatment.

When examined using repeated measures analysis, total DMI (Figure 1), BW (Figure 2), BCS (Figure 3) and daily milk yield (Figure 4) changed over the period from wks 1-19 postpartum ($P < 0.001$). There was no treatment x wk interaction during this period for daily DMI, BW or milk yield (all $P > 0.1$) but there was an interaction between late lactation treatment and wk postpartum for BCS ($P = 0.004$; Figure 3).

When blood metabolites measured during wks 2, 4, 6, 8 and 10 pp were examined using a repeated measures analysis, plasma glucose concentrations were higher ($P = 0.002$) in cows on the LP treatment, but mean serum BHB, NEFA and urea concentrations were unaffected (Figure 5). Concentrations of plasma glucose increased ($P < 0.001$) over the 10 week period postpartum, while serum NEFA ($P < 0.001$), BHB ($P = 0.001$) and urea ($P = 0.013$) decreased over the 10 week measurement period. No treatment x week interactions were identified during this period ($P > 0.1$) for any of these metabolites. For serum haptoglobin concentrations measured during wks 2, 4 and 6 postpartum (Figure 6), there were no treatment, week or treatment x week interactions ($P > 0.1$).

Treatment did not affect ($P > 0.1$) any milk progesterone variable (Table 7) or pregnancy rate to 1st and 2nd service ($P = 0.665$). Cows on the LP treatment tended to have a shorter calving interval ($P = 0.072$) than cows on either of the other two treatments, but treatment did not affect the prevalence of key fertility or health indicators during the first 19 wks postpartum ($P > 0.1$). The proportion of cows

treated for fertility problems was unaffected by treatment, as was the proportion of cows treated for mastitis, digestive upset and lameness ($P > 0.05$).

DISCUSSION

The problems associated with thin dairy cows in early lactation are well documented. Improving the BCS of thin cows prior to calving is an important management goal on many dairy farms, but the optimum strategy by which this might be achieved is unclear. This study investigated two approaches to improving the BCS of thin cows prior to calving, namely, the adoption of an extended dry period, and offering a diet with a 'lower' protein and 'higher' starch content in late lactation.

Cow performance from wk 13 to wk 8 pre-partum

Studies with early/mid lactation cows have demonstrated that feed intake can be sustained provided dietary CP levels do not fall to excessively low levels, although milk yields are normally reduced with lower protein diets. For example, Cunningham et al. (1996) and Olmos Colmenero and Broderick (2006) found no difference in DMI between diets decreasing incrementally in CP content from 185 to 145 g/kg DM and from 194 to 135 g/kg DM, respectively, while fat-corrected milk yields were reduced in these studies by 5.6 kg/d ($P = 0.04$) and by 2.5 kg/d ($P = 0.10$) respectively. Law et al. (2009a) offered diets containing 114, 144 and 173 g CP/kg DM to cows in early lactation and found DMI significantly reduced only on the lowest protein diet while milk yield differed significantly between all three diets. The concept of reducing diet protein level so as to reduce milk yield without a corresponding reduction in DMI, thereby improving EB in early lactation (d1 - 150), was extended by Gilmore et al.

(2011) who showed that even short-term reductions in dietary CP content could be used to quickly improve the EB of dairy cows in early lactation.

Maintaining feed intake while reducing milk yield, was a key premise of the LP treatment in the current study, with cows on this treatment expected to partition dietary energy not used for milk production to body tissue. The reduction in diet CP content from 158 to 137 g/kg DM for the NP and LP treatments respectively was sufficient to result in a significantly greater plasma urea concentration in cows on the NP treatment during week 9 pre-calving. However, this reduction in diet CP content resulted in a 1.1 kg/d reduction in silage DMI and an associated late lactation milk yield reduction of 1.2 kg/d (12.6 vs 11.4 kg/d respectively). Based on previous studies involving early lactation cows, a reduction in diet CP content in excess of 20 g/kg DM was expected to result in a more substantial reduction in milk yield. For example, Ipharraguerre and Clark (2005) reviewed data from 112 trials involving dairy cows in early-mid lactation with diets ranging from 121 to 258 g CP/kg DM. Using their response curve a difference of 2.2 kg milk/d was predicted between the NP and LP diets. Olmos Colmonero and Broderick (2006) reasoned that diets \leq 160 g CP/kg provide insufficient MP for maximal milk synthesis, making milk yield more sensitive to small changes in diet CP content at lower diet protein levels. Nevertheless, it is recognised that cows in late lactation are less responsive to changes in diet protein level than are cows in early-mid lactation (Kalscheur et al., 1999) and these authors noted a reduction in DMI of 0.9 kg per cow per d, with no effect on milk yield, when the CP content of late lactation diets of similar RUP status was reduced from 142 to 125 g/kg DM. Law et al. (2009a) offered diets of 114, 144 and 173 g CP/kg DM to Holstein-Friesian cows in later lactation (d-151 to d-305) and

found differences in DMI (16.8, 17.8 and 19.3 kg/d respectively; $P < 0.05$) between all treatments, while milk yield was only significantly reduced with the lowest protein diet (23.0 vs 28.8 and 29.8 kg/d respectively). These data highlight inconsistencies in 'late' lactation responses to a reduction in dietary CP levels.

The reduction in late lactation milk fat content in cows on the LP diet was consistent with the depressive effect of a low protein diet on fibre digestibility (Faverdin, 1999), and is likely also to be associated with an impairment in ruminal acetate production. Both factors may have contributed to the unexpected reduction in DMI, with this compounded also by the higher levels of starch in this diet.

Energy balance data for the 5 week period prior to drying off showed no significant difference between the NP and LP treatments, and is supported by the absence of differences in BCS responses, and in plasma glucose, NEFA and BHB concentrations between cows on these two treatments. However, cows on the NP treatment showed an unexpectedly large increase in BW during the period from wk 13 to wk 9 pre-partum, compared to those on the LP diet (56 vs 29 kg respectively). Differential 'rumen-fill' effects may have been a factor in this, as forage DMI during this period was lower for the LP diet. Rumen volume is affected by forage NDF content (Van Soest, 1994) and while the NP and LP diets had similar NDF contents, the 1.1 kg/d difference in silage DMI between the NP and LP diets equates to a difference of almost 600 g NDF per d between NP and LP cows. Nevertheless, in summary, offering a lower protein, higher starch diet in late lactation did not improve BCS in late lactation, contrary to expectations.

By definition, cows on the EDP treatment produced no milk during the period from wk 13 to wk 9 pre-partum, resulting in a reduction in milk yield during this lactation of approximately 380 kg (compared with cows on the NP and LP treatments). The loss in milk was associated with a saving in concentrates of 175 kg/cow, while forage DM intake was similar to that of cows on the NP treatment. Thus the immediate economic impact of an extended dry period will be determined by current milk price-concentrate cost ratios.

The calculated EB values for this period highlight that EDP cows tended to have an improved energy status relative to cows on the LP treatment ($P=0.098$), while not being different from cows on the NP treatment, with this largely reflected in the lower serum BHB and NEFA concentrations with EDP at wk 9 pre-partum. While there was a trend for cows on the EDP treatment to gain more BCS than cows on either of the other two treatments, there was no treatment x time interaction for BCS gain, so that cows managed on the extended DP treatment had similar BW gain to those on the NP treatment, perhaps reflecting the short duration of the observation period. In the study by Weber et al. (2015), both BW and BCS differed before calving between cows given a 90-d DP and cows with DPs of 28- and 56-d. However, in that study, cows were not randomly selected for the 90-d DP but were allocated to it because of their already low milk production (≤ 15 kg/d) around 90 d before calving. These same cows already had greater BW and BCS at dry off.

Cow performance from wk 8 pre-calving until calving

While cows on all treatments were offered a common diet from wk 8 pre-partum until calving, cows on the LP diet continued to have a lower forage intake than either NP

or EDP cows. The reasons for this 'carry-over' effect are unclear as it would have been expected that any negative effects on rumen function would have been short-lived after these cows moved on to a common diet. While the BW of cows on the LP treatment remained lower throughout the pre-calving period than that of cows on the other two treatments, BW gain was similar across all treatments. Weight gain in cows at this stage of gestation largely reflects exponential foetal growth rather than maternal weight gain, consistent with the modest increases in BCS across all treatments during this 8 wk period (range, 0.11 to 0.19 units). These relatively small gains are consistent with the outcomes of previous studies at this institute (Law et al., 2016; Little et al., 2016b) which showed that cows offered moderate quality grass silage-based diets, either supplemented or un-supplemented, gain little body condition during the 8 wk period pre calving, reflecting the move towards NEB during the last few weeks pre calving (Little et al., 2016b) as observed in many similar studies.

There is a paucity of published data on the effects of adopting a dry period of more than 56-60 d on BCS and BW at calving, reflecting the reluctance to adopt longer DPs as they are considered prejudicial to lifetime productivity (Kuhn et al., 2006). However, it is unclear if this holds true for thin cows as their increased risk of being culled during the subsequent lactation (Law et al., 2016) is clearly also prejudicial to lifetime performance. Cows on the EDP treatment ended the 8-wk pre-calving period with a significantly higher BCS than cows on the other two treatments, having gained approximately 0.4 BCS units during the 13 wks up to calving. Thus, the adoption of an extended DP allowed the thin cows in the current study to gain more body condition than was possible with a conventional 8-wk DP, and to move closer

to a pre-calving BCS of 2.75, regarded as optimal by Garnsworthy (2007). The between-treatment similarity of plasma glucose and serum BHB, NEFA and urea concentrations at 6 wks and 3 wks pre-calving suggested a similar metabolic status of cows across all treatments at this time, in agreement with the calculated EB data.

Cow performance during wks 1 – 19 postpartum

Colostrum quality was essentially unaffected by treatment. Colostrum yields were lower in milkings 3 and 4 (only) from cows on the LP diet and these cows also had a lower urea-N content in milkings 1 and 2, but there were no obvious explanations for either outcome, and it is difficult to attach any biological significance to them.

While all cows were managed on a common diet post-calving, cows on the LP treatment continued to have a significantly lower DMI than cows on the NP treatment over the first 19 wks of lactation, similar to the 8 wk period pre-partum. There was no obvious explanation for this long term 'carry over' effect, other than that these cows were lighter in BW at calving, again a carry-over from the pre-partum period. While neither mean milk yield nor mean milk fat or protein content differed between treatments NP and LP over the 19 wk period, both were numerically lower with the LP treatment so that milk fat + protein yield was significantly lower with the LP treatment, in line with the lower DMI with this treatment. The combined effects of a lower DM intake and lower fat plus protein yield with the LP treatment was that mean EB was unaffected by treatment. This was reflected in the absence of a treatment effect on BCS or on serum BHB and NEFA concentrations during the post-calving period.

Evidence relating to the effects of an extended dry period on dairy cow performance is inconsistent. For example, Weglarzy (2009) found that cows that had DPs from 61 to 90 d had 12.6% greater 305-d milk yields in the subsequent lactation than cows given a DP < 60 d or > 90 d. In contrast, Kuhn et al. (2006) analysed 7 yrs of US Dairy Herd Improvement Association data and concluded that a DP > 60 d was associated with lower milk production in the next lactation and, often, a loss of lifetime milk production also, with cows receiving a DP \geq 90 d typically yielding > 4,000 kg less lifetime milk than those on a 40-60 d DP. Yield losses were substantial even in cows that received a 70-90 d DP. However both of these retrospective studies included data from cows with DPs that differed naturally in length as a consequence of reactive management decisions, rather than as a pre-arranged consequence of experimental design, as was the case in the current study. Similarly, Weber et al. (2015) compared the effects of a 28-d, 56-d and 90-d DP for dairy cows and found that neither total milk production nor milk composition over the first 200 d of lactation differed between cows given a 90-d DP and those given a 56-d DP. However, while cows on the 28-d and 56-d DPs in their study were randomly selected from a common pool, cows given the extended 90-d DP were dried off earlier because, and when, their milk yield had declined to \leq 15 kg/d. In one of the few studies to have imposed pre-planned dry periods of variable length on dairy cows, Sorensen and Enevoldsen (1991) assessed the effect of DPs of 4, 7 and 10 wks on subsequent lactation yields across 8 herds (n=366 cows) and found that a 10 wk DP resulted in an increase of 0.5 kg/d of 4% FCM up to d-84 of lactation, and 0.4 kg/d up to d-184 of lactation. However, in the current study, neither the DMI nor milk yield of cows allocated to the extended DP treatment differed from those of cows on the NP treatment.

A significant risk associated with the imposition of an extended DP is that cows may become overly fat. Garnsworthy (2007) and Drackley and Cardoso (2014) advised that cows given a greatly extended DP should be managed individually in order to avoid over-conditioning as it is well known that cows with a higher BCS at calving lose more BCS early postpartum (Garnsworthy and Topps, 1982; Bjerre-Harpoth et al., 2014) and that lower pre- and post-calving DMI (Roche et al., 2013), impaired immune function (Lange et al., 2016) and poorer reproductive performance (Garnsworthy et al., 2008; Hoedemaker et al., 2009) are associated with cows that mobilize more fat postpartum. In the current study, cows on the EDP treatment had a higher BCS at calving than those on the NP treatment (2.60 vs 2.44), but all cows in the study were demonstrably 'thin' at drying off, and were at no risk of becoming excessively fat on the grass silage diets offered to them during the dry period. As occurred also in the study of Weber et al. (2015), the advantage of a higher BCS at calving in cows on the EDP treatment was quickly lost postpartum. Indeed, the BCS of all cows began to merge shortly after calving and did not differ at 19 wks into lactation. Cows that are nutritionally manipulated to make them either fatter or leaner at calving experience a change in the rate of BCS loss post-calving that helps re-establish normal body fatness levels by 3 to 4 mo postpartum (Friggens, 2003; Garnsworthy, 2007; Law et al., 2011). In the current study, extending the DP gave no longer-term performance benefits despite clearly allowing significantly greater BCS gains before calving than in cows with a conventional DP.

Cow reproductive performance and cow health

Higher CP intakes can be detrimental to cow reproductive performance (Butler, 1998). Rather less is known about the impact of low CP diets and lower CP intakes on dairy cow fertility, although Law et al. (2009b, 2009c) found that a number of measures of fertility performance were unaffected by dietary protein content, even though a higher diet protein content reduced the average daily EB of the cows. The impact of offering low CP diets in late lactation on fertility performance during the subsequent lactation does not appear to have been examined previously. Similarly, the impact of extending the DP on fertility performance has not been extensively investigated through planned experiments. Pinedo et al. (2011) retrospectively examined the association between DP lengths ranging from 0 to 250-d on a variety of cow performance parameters (including reproductive performance) in Chilean dairy herds and found only that extending the DP beyond 143 d had a negative effect on reproductive performance in the subsequent lactation.

Milk progesterone measurements in the current study provided no evidence that cyclicity onset was influenced either by offering a low CP diet in late lactation or by extending the length of the DP. Nevertheless, cows with a lower BCS at calving are more likely to have a longer calving interval. Pryce et al. (2001) and Roche et al. (2007) found difficulty re-breeding thin dairy cows within an acceptable period, probably due to the recognised delay in the resumption of ovarian luteal activity in thinner cows (Markusfeld et al., 1997; Reksen et al., 2002). The current study did not involve a 'normal' BCS group, but cows on the EDP treatment gained more body condition during their extended dry period, and had a higher BCS at calving than those on either of the other two treatments. Nevertheless, the key metric of

conception rates to 1st and 2nd service was unaffected by treatment, although the number of cows involved was inadequate to robustly assess fertility outcomes.

Thin cows have reduced immune competence and an associated increased disease incidence (Hoedemaker et al., 2009) and are more likely to be culled during early lactation than cows with a higher BCS (Law et al., 2016). However, treatment had no effect on immune status pre-partum or postpartum, as assessed by serum haptoglobin (Hp) levels in the current study. Increases in serum Hp level occur in response to inflammatory stimuli and are exacerbated by mastitis, lameness, metritis and metabolic stress (Little et al., 2016a). While Hp provides only one assessment of immune function, cow health (incidence of cows requiring uterine washouts, mastitis, lameness and digestive upset) was unaffected by treatment in our study despite an improved BCS at calving for cows on the EDP treatment. This supports the fertility data above. For example, the incidence of uterine infection is a key driver of fertility outcomes (Walsh et al., 2011; Little et al., 2016b) with Hoedemaker et al. (2009) observing that thin (BCS < 3) cows were at greater risk of dystocia, retained placenta and endometritis than cows in better condition (BCS \geq 3). The proportion of cows treated for uterine washouts did not differ in the current study. In a recent study, Roche et al. (2015) concluded that thin cows should be fed to their prescribed requirements in order to maintain or improve their immunity, with mean energy requirements during the dry period met with all treatments in the current study, as demonstrated by the EB data. Overall, there was no discernible benefit of either a low protein diet (LP) or an extended dry period (EDP) on the health and fertility of the thin cows in this study.

CONCLUSIONS

Offering a lower protein, higher starch diet to thin cows in late lactation reduced DMI and milk fat plus protein yield, but had no effect on BCS at calving. The lower feed intakes persisted throughout the subsequent lactation, while there were no beneficial effects on cow fertility or health. While the adoption of an extended dry period resulted in an improved BCS at calving compared to cows offered a normal protein diet, this difference was small, and disappeared after calving. The extended dry period had no beneficial effects on cow performance, health or fertility during the subsequent lactation.

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TABLES

Table 1: Ingredient composition (kg/t, fresh basis) of the concentrates offered during the pre- and post-partum periods.

	Pre-partum ¹		Post-partum ²
	Normal protein	Low protein	
Wheat	137	98	125
Maize	98	293	170
Sugar beet pulp	102	102	-
Palm kernel	-	-	95
Wheat feed	-	-	65
Maize Gluten	-	175	124
Soya bean meal	298	50	130
Soya hulls	204	205	100
Rapeseed meal	100	-	125
Maxfat CS ³	22	28	-
Palm oil	-	-	9
Molasses ⁴	17	21	30
Minerals ³	22	28	17
Lime No. 1 flour	-	-	10

¹ Concentrates offered during five week period, commencing at thirteen weeks pre-partum

² Concentrates offered during nineteen week period post-partum

³ Trow Nutrition, 36 Ship Street, Belfast

⁴ United Molasses Ireland Ltd, Duncrue St, Belfast

Table 2 Chemical composition of the grass silages (g/kg volatile-corrected DM, unless stated otherwise) and concentrates (g/kg DM) offered throughout this study (standard deviations are presented in parentheses).

	Grass silage		Concentrate		
	Pre-partum ¹	Post-partum ²	Pre-partum ³		Post-partum ⁴
			Normal protein	Low protein	
Neutral detergent fibre	605 (45.3)	519 (22.2)	310 (37.7)	321 (45.4)	317 (8.4)
Acid detergent fibre	363 (36.9)	294 (13.7)	161 (14.7)	161 (28.2)	152 (7.1)
Crude protein	130 (18.0)	119 (16.7)	228 (27.2)	153 (10.6)	215 (2.3)
Starch	-	-	207 (22.7)	293 (40.9)	245 (3.4)
Ash	80 (8.7)	92 (9.7)	83 (3.9)	75 (6.7)	74 (2.5)
Gross energy (MJ/kg of DM)	19.6 (1.96)	18.9 (2.98)	18.4 (0.15)	18.3 (0.3)	18.7 (0.09)
Oven dry matter (g/kg FW)	226 (54.9)	306 (44.7)	896 (7.3)	897 (7.0)	898 (0.1)
Volatile corrected DM (g/kg FW)	235 (43.3)	326 (44.7)	-	-	-
Silage fermentation variables					
Lactic acid	90 (50.0)	92 (52.0)	-	-	-
Acetic acid	40.0 (17.80)	21.3 (7.53)	-	-	-
Ethanol	16.0 (6.27)	6.6 (2.45)	-	-	-
Propanol	7.6 (7.10)	2.1 (2.90)	-	-	-
pH	3.94 (0.277)	3.84 (0.152)	-	-	-
Ammonia N (g/kg of total N)	88 (11.7)	67 (11.6)	-	-	-
Metabolisable energy (MJ/kg of DM) ⁵	10.9 (0.46)	11.4 (0.40)	-	-	-

¹ Silage offered across all treatments throughout the thirteen weeks pre-partum

² Silage offered across all treatments throughout a nineteen week period post-partum

³ Concentrates offered during five week period, commencing at thirteen weeks pre-partum

⁴ Concentrates offered during nineteen week period post-partum

⁵ Determined using near-infrared reflectance spectroscopy (NIRS)

Table 3 Effect of concentrate protein level in late-lactation and dry period length on food intake, milk yield, milk composition, body condition score (BCS), body weight (BW) and energy balance over a five week period, commencing thirteen weeks pre-partum

	Late lactation treatment ¹			s.e.d	P-value
	Normal protein	Low protein	Extended dry period		
Silage DMI (kg/cow per d)	10.9 ^b	9.8 ^a	10.8 ^b	0.37	0.004
Total DMI (kg/cow per d) ²	15.4 ^c	14.3 ^b	10.8 ^a	0.37	<0.001
Milk yield (kg/cow per d)	12.6	11.4	-	0.63	0.050
Milk composition (g/kg)					
Fat	41.8	38.8	-	0.91	0.003
Protein	35.8	34.5	-	0.80	0.094
Lactose	43.7	43.5	-	0.36	0.604
Milk fat + protein yield (kg/cow per d)	0.97	0.83	-	0.055	0.014
Somatic cell count (000/ml log ^e)	11.7	11.6	-	0.14	0.397
BW at week 9 pre-partum (kg)	628 ^b	601 ^a	628 ^b	9.3	0.004
BW change (kg) ³	56 ^b	29 ^a	56 ^b	9.3	0.004
Mean BW (kg)	613 ^b	594 ^a	615 ^b	7.1	0.005
BCS at start of study	2.27	2.26	2.22	0.049	0.585
BCS at week 9 pre-partum	2.33	2.30	2.41	0.054	0.103
BCS change ³	0.08	0.04	0.16	0.054	0.103
Mean BCS	2.30	2.28	2.33	0.033	0.320
Mean Energy Balance (MJ/cow per d)	30.7	23.7	36.1	5.53	0.098

¹ Treatments imposed over five week period, commencing thirteen weeks pre-partum

² Includes 4.49 kg concentrate DM with the Normal and Low protein treatments

³ Change in BW and BCS between week 13 and week 9 pre-partum

Table 4 Effect of concentrate protein level in late-lactation and dry period length on silage intake, bodyweight (BW) and body condition score (BCS) from 8 weeks pre-partum until the week pre-partum.

	Late lactation treatment ¹			s.e.d.	P-value
	Normal protein	Low protein	Extended dry period		
Silage DMI (kg/cow per d)	10.8 ^b	9.5 ^a	10.6 ^b	0.33	<0.001
BW during the week pre-partum	669 ^b	642 ^a	671 ^b	11.4	0.020
BW change ²	41	39	46	7.2	0.588
Mean BW (kg)	650 ^b	619 ^a	655 ^b	10.2	0.001
BCS during the week pre-partum	2.44 ^a	2.42 ^a	2.60 ^b	0.061	0.006
BCS change ²	0.11	0.12	0.19	0.057	0.274
Mean BCS	2.37 ^a	2.37 ^a	2.50 ^b	0.053	0.021
Mean Energy Balance (MJ/cow per d)	21.0	15.0	18.3	4.37	0.403

¹ Treatments imposed over a five week period, commencing thirteen weeks pre-partum

² Difference between week 8 and week pre-partum

Table 5 Effect of concentrate protein level in late-lactation and dry period length on colostrum yield and composition during the first and second milkings post-partum, and during the third and fourth milkings post-partum.

	Late lactation treatment ¹			s.e.d	P-value
	Normal protein	Low protein	Extended dry period		
First and second milkings					
Yield (kg/cow per d)	10.3	10.3	11.6	2.11	0.770
Casein (g/kg)	72.0	67.4	72.4	5.09	0.532
Fat (g/kg)	54.7	49.4	65.6	9.29	0.213
Protein (g/kg)	122.0	112.4	121.8	9.89	0.517
Lactose (g/kg)	31.6	33.8	31.5	1.69	0.296
Urea Nitrogen (mg/kg)	336 ^b	250 ^a	339 ^b	38.8	0.033
Immunoglobulin (mg/ml)	46.2	41.3	47.5	5.16	0.429
Third and fourth milkings					
Yield (kg/cow per d)	18.0 ^{ab}	14.6 ^a	19.3 ^b	1.93	0.046
Casein (g/kg)	40.7	38.2	39.1	2.61	0.592
Fat (g/kg)	50.3	44.8	46.4	4.69	0.432
Protein (g/kg)	61.6	56.9	58.4	4.93	0.576
Lactose (g/kg)	38.6	40.0	39.6	1.08	0.352
Urea Nitrogen (mg/kg)	119	115	114	18.2	0.951
Immunoglobulin (mg/ml)	13.2	13.9	14.7	1.72	0.645

Table 6 Effect of concentrate protein level in late-lactation and dry period length on food intake, milk yield, milk composition, body weight (BW) and body condition score (BCS) during the first nineteen weeks of lactation.

	Late lactation treatment ¹			s.e.d	P-value
	Normal protein	Low protein	Extended dry period		
Concentrate DMI (kg/cow per d)	11.2 ^b	10.8 ^a	11.3 ^b	0.19	0.009
Silage DMI (kg/cow per d)	11.7 ^b	11.0 ^a	11.8 ^b	0.29	0.017
Total DMI (kg/cow per d)	22.9 ^b	21.8 ^a	23.1 ^b	0.47	0.010
Milk yield (kg/cow per d)	37.8 ^{ab}	35.6 ^a	38.4 ^b	1.14	0.040
Milk composition (g/kg)					
Fat	40.5	39.1	39.0	0.97	0.232
Protein	33.6	33.3	33.1	0.60	0.707
Lactose	46.6	46.9	46.5	0.24	0.238
Milk fat + protein (kg/cow per d)	2.78 ^b	2.55 ^a	2.75 ^b	0.08	0.009
Somatic cell count (000/ml log ^e)	10.7	10.5	10.6	0.23	0.785
BW during first week post-partum (kg)	609 ^b	586 ^a	616 ^b	11.2	0.022
BW at week 10 post-partum (kg)	619	606	625	10.2	0.189
BW at end of experimental period (kg)	632	620	646	11.1	0.075
Nadir BW (kg)	587	571	589	10.7	0.182
BW loss to nadir (kg)	21	14	27	5.5	0.081
Days to nadir BW (days)	28	20	27	5.0	0.243
Mean BW (kg)	618	604	625	9.6	0.090
BCS during the first week post-partum	2.46	2.39	2.47	0.074	0.475
BCS at week 10 post-partum	2.33	2.34	2.35	0.061	0.947
BCS at end of experimental period	2.44	2.45	2.44	0.064	0.984
Mean BCS	2.37	2.37	2.37	0.054	0.991
Mean Energy Balance (MJ/cow per d)	-3.0	-1.1	-3.9	6.05	0.903

¹ Treatments imposed over a five week period, commencing at thirteen weeks pre-partum

Table 7 Effect of concentrate protein level in late-lactation and dry period length on cow health and fertility during the first nineteen weeks of lactation.

	Late lactation treatment ¹			S.E.M	P-value
	Normal protein	Low protein	Extended dry period		
Progesterone parameters					
Onset of Corpus Luteal activity (d)	33	30	34	4.0	0.641
Corpus Luteal activity <42 d (proportion of cows)	0.72	0.91	0.71	0.063	0.208
Maximum progesterone concentration during the first Corpus Luteum (ng/mL)	22.6	22.7	22.2	3.46	0.988
Fertility outcomes ²					
Pregnancy rate to first and second service (proportion of cows)	0.55	0.68	0.64	0.076	0.665
Calving interval (d)	383	361	379	10.4	0.072
Fertility treatments (proportion of cows treated with) ²					
Washout	0.18	0.15	0.17	0.061	0.968
Progesterone	0.09	0.15	0.08	0.050	0.764
Prostaglandin	0.53	0.36	0.49	0.077	0.522
Health treatments (proportion of cows treated for) ²					
Mastitis	0.20	0.08	0.15	0.052	0.374
Digestive upset	0.22	0.35	0.16	0.069	0.393
Lameness	0.24	0.26	0.10	0.064	0.390

¹ Treatments imposed over a five week period, commencing at thirteen weeks pre-partum

² Performance monitored over a 19-week period post-partum

FIGURES

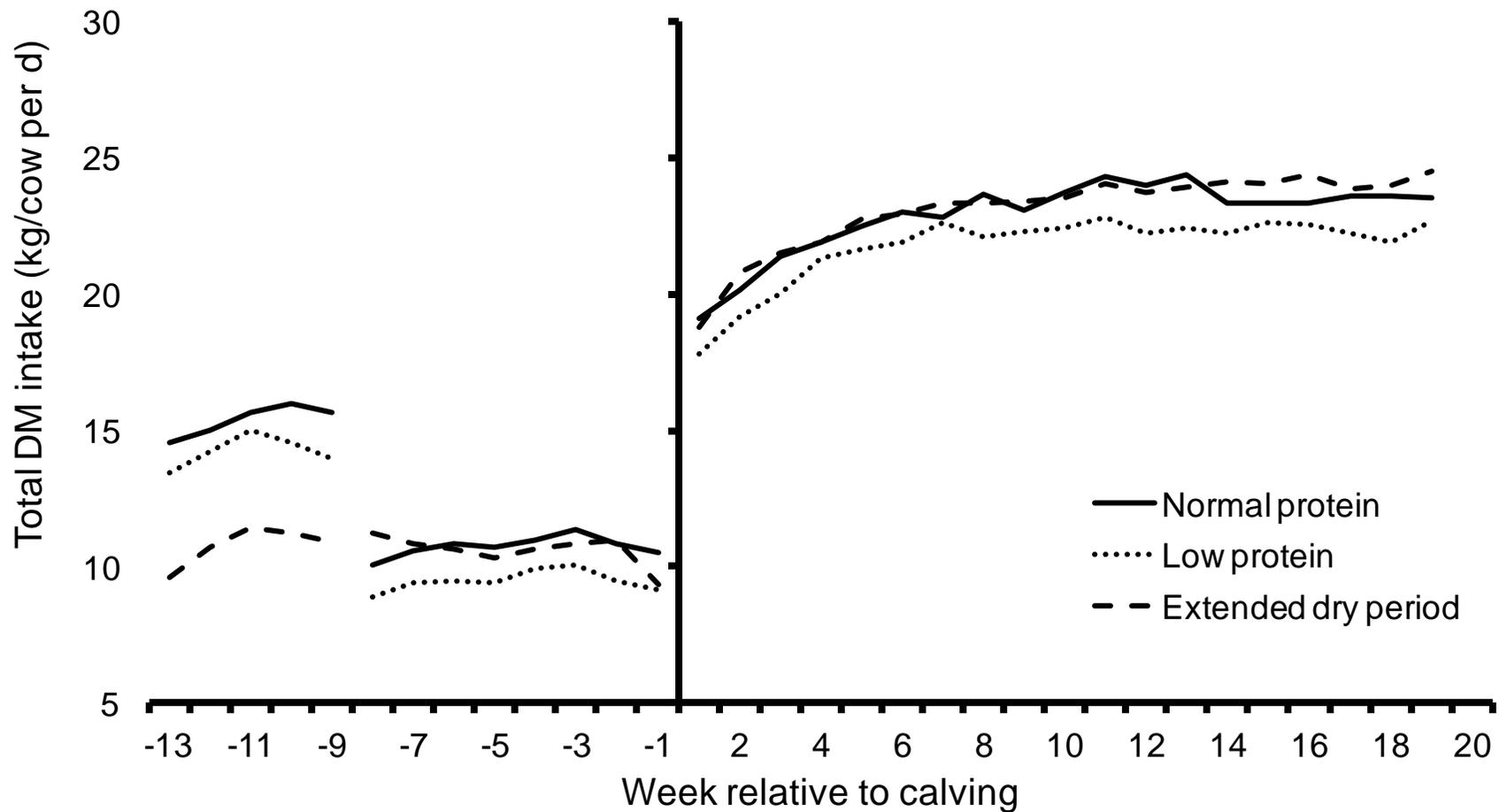


Figure 1 Effect of concentrate protein level in late-lactation and dry period length on total dry matter intake throughout the experiment. Main effects of treatment, week, and treatment x week interactions are as follows: wks 13 to 9 pre-partum (s.e.d., 0.32, 0.22, 0.45, respectively; P-value, <0.001, <0.001, 0.623, respectively); wks 8 to 1 pre-partum (s.e.d., 0.32, 0.24, 0.48, respectively; P-value, <0.001, <0.001, 0.027, respectively); wks 1 to 19 post-partum (s.e.d., 0.38, 0.36, 0.70, respectively; P-value, <0.001, <0.001, 0.720, respectively).

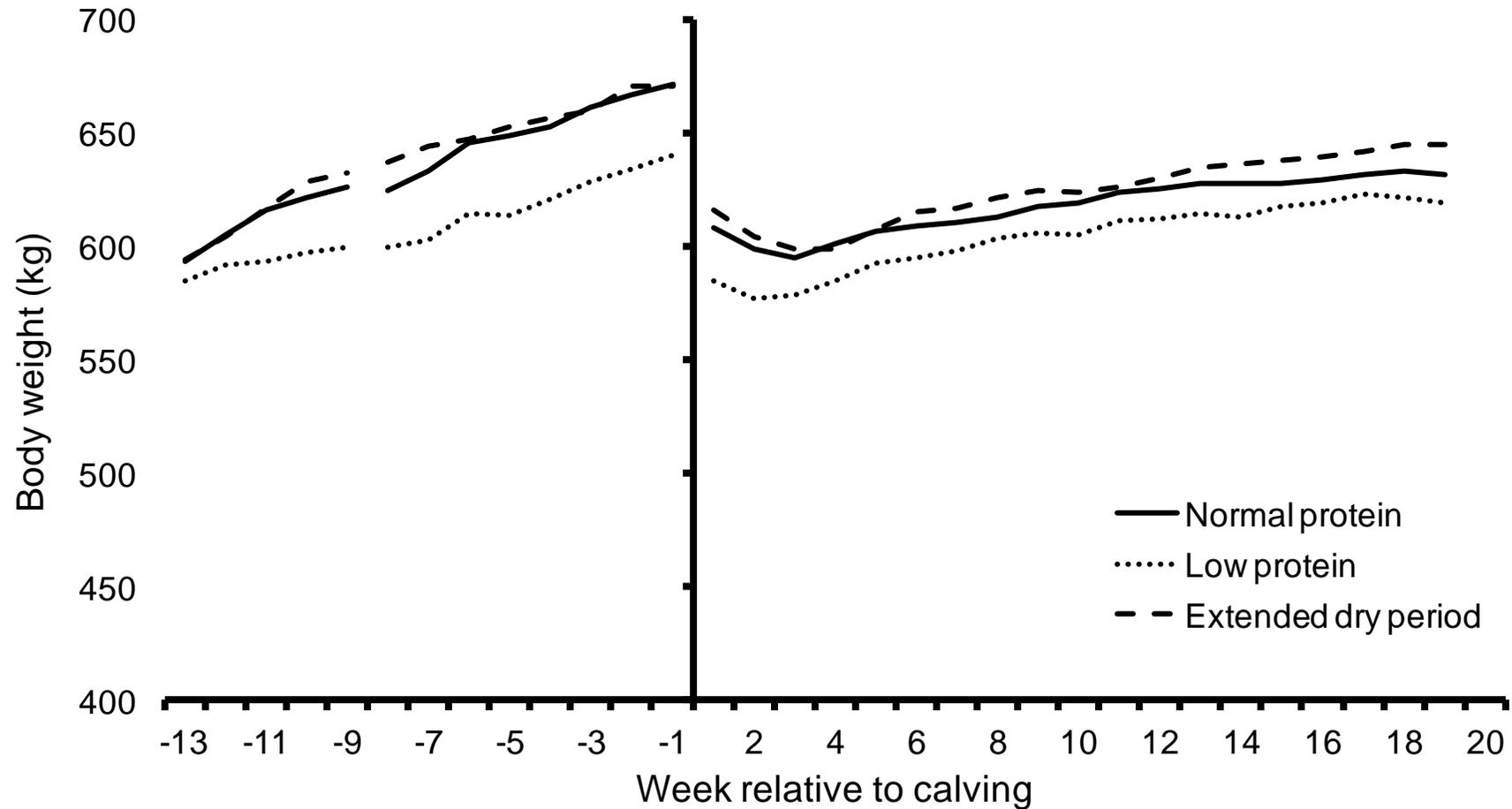


Figure 2 Effect of concentrate protein level in late-lactation and dry period length on body weight throughout the experiment. Main effects of treatment, week, and treatment x week interactions are as follows: wks 13 to 9 pre-partum (s.e.d., 7.19, 2.04, 6.64, respectively; P-value, 0.008, <0.001, 0.021, respectively); wks 8 to 1 pre-partum (s.e.d., 9.74, 2.25, 8.46, respectively; P-value, 0.001, <0.001, 0.042, respectively); wks 1 to 19 post-partum (s.e.d., 9.82, 2.84, 9.06, respectively; P-value, 0.028, <0.001, 0.669, respectively).

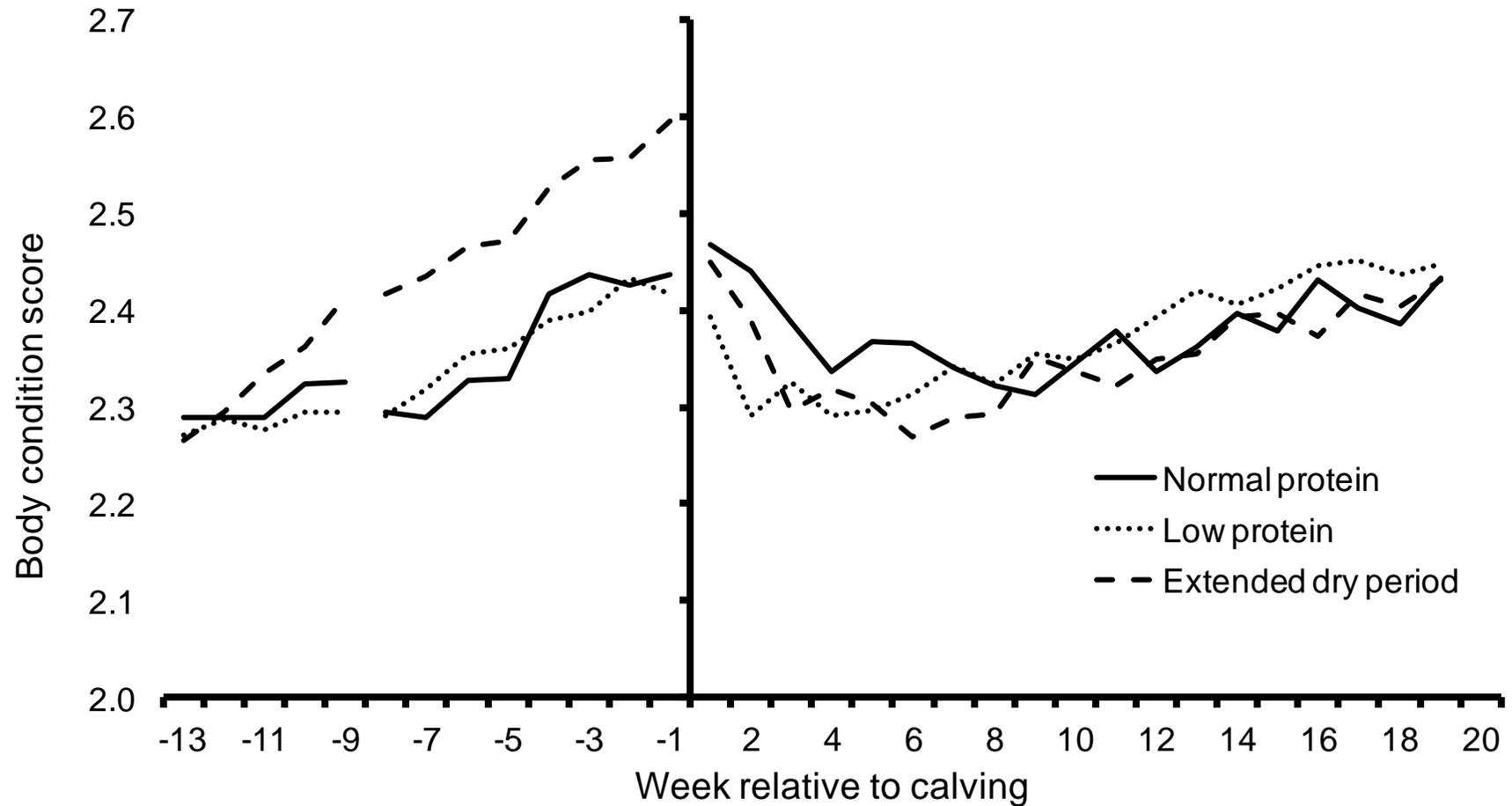


Figure 3

Effect of concentrate protein level in late-lactation and dry period length on body condition score throughout the experiment. Main effects of treatment, week, and treatment x week interactions are as follows: wks 13 to 9 pre-partum (s.e.d., 0.03, 0.02, 0.04, respectively; P-value, 0.249, 0.051, 0.491, respectively); wks 8 to 1 pre-partum (s.e.d., 0.05, 0.03, 0.06, respectively; P-value, 0.002, <0.001, 0.923, respectively); wks 1 to 19 post-partum (s.e.d., 0.05, 0.04, 0.07, respectively; P-value, 0.906, <0.001, 0.004, respectively).

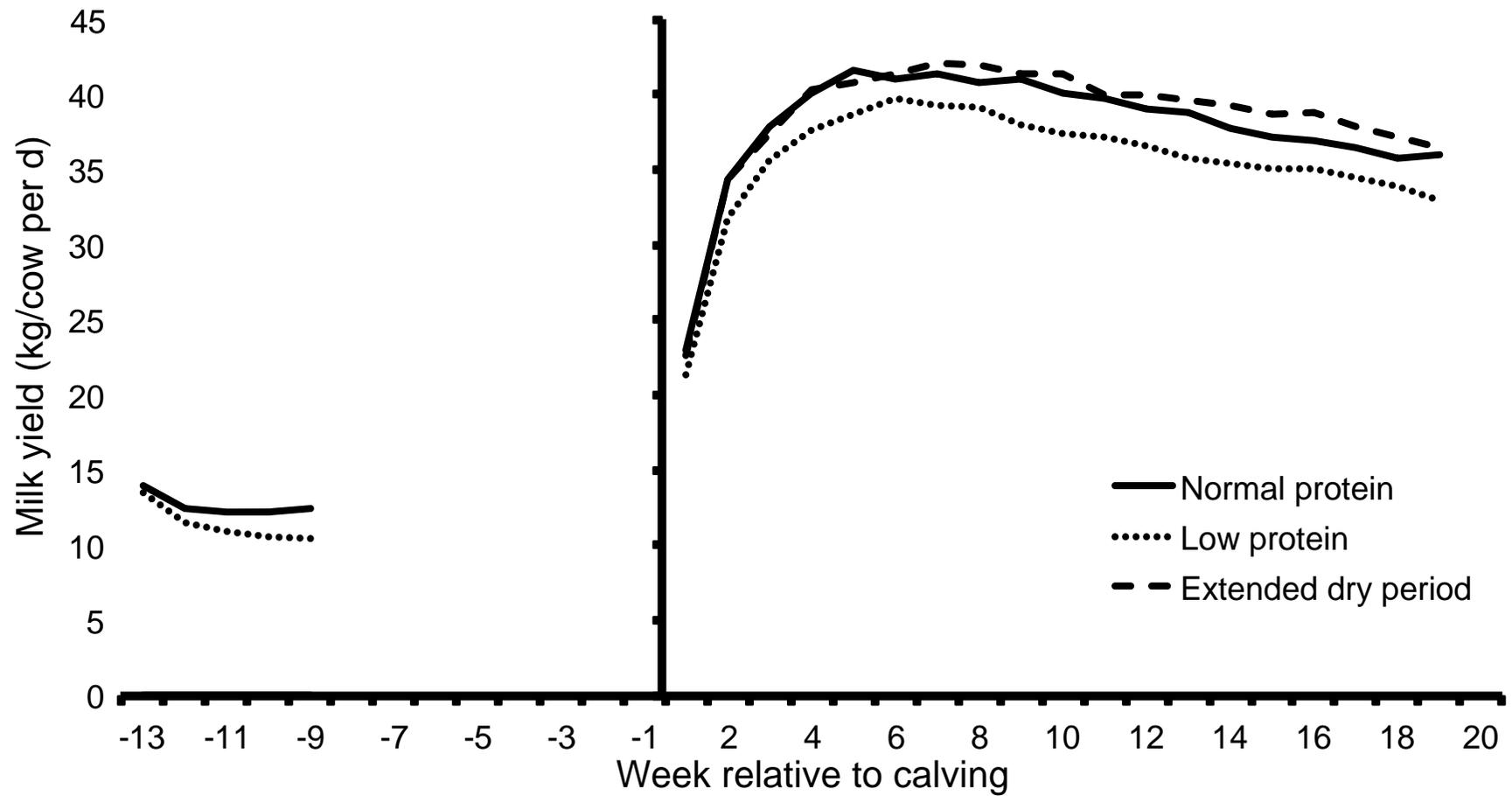


Figure 4 Effect of concentrate protein level in late-lactation and dry period length on daily milk yield throughout the experiment. Main effects of treatment, week, and treatment x week interactions are as follows: wks 13 to 9 pre-partum (s.e.d, 0.68, 0.25, 0.57, respectively; P-value, 0.064, <0.001, 0.309, respectively); wks 1 to 19 post-partum (s.e.d., 0.98, 0.55, 1.23, respectively; P-value, 0.010, <0.001, 0.358, respectively).

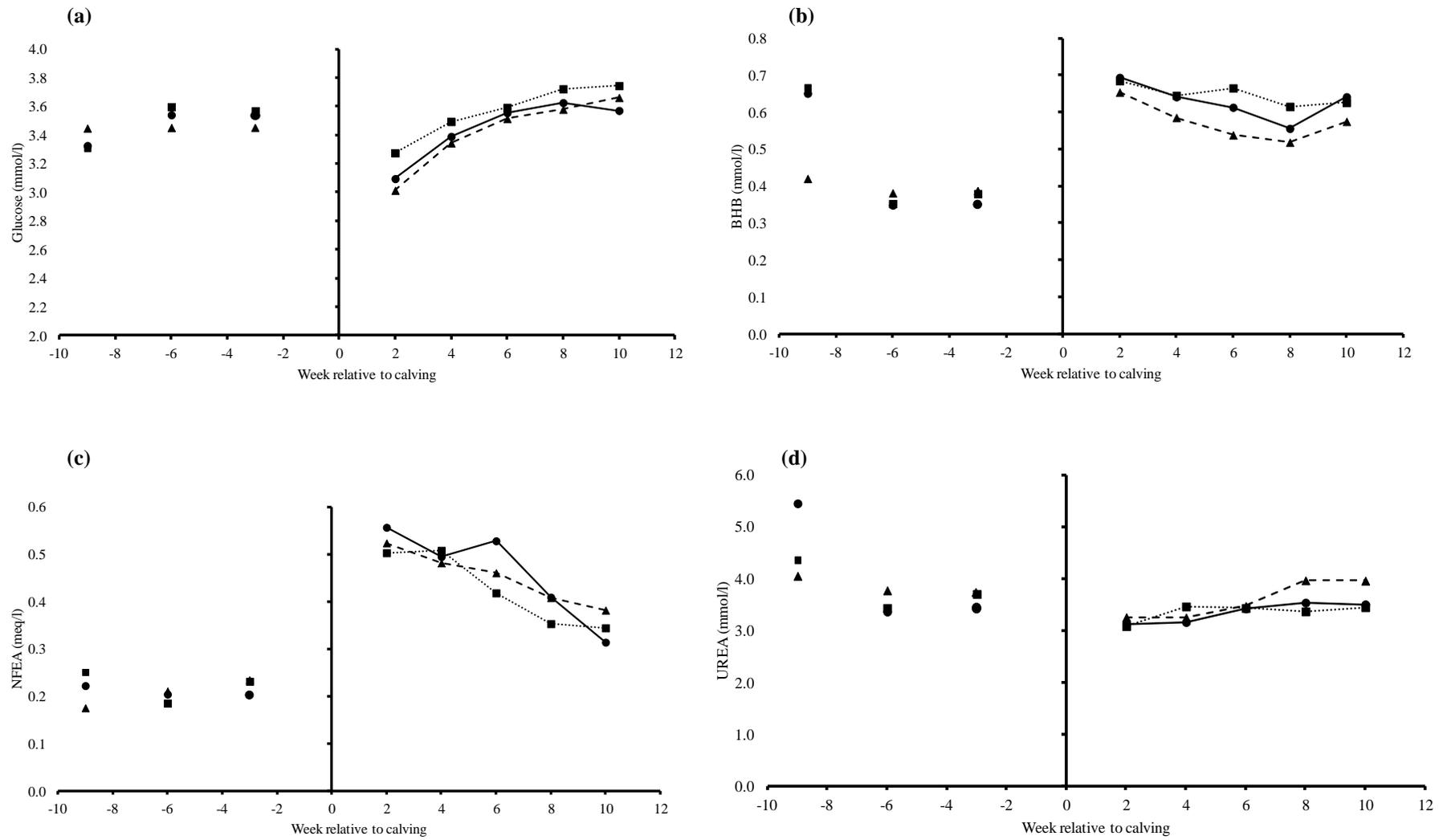


Figure 5. Effect of concentrate protein level in late lactation and dry period length on (a) plasma glucose, (b) serum BHB, (c) serum NEFA and (d) serum urea concentrations pre-partum and in the first 10 wks postpartum (• normal protein; ■ low protein; ▲ extended dry period)

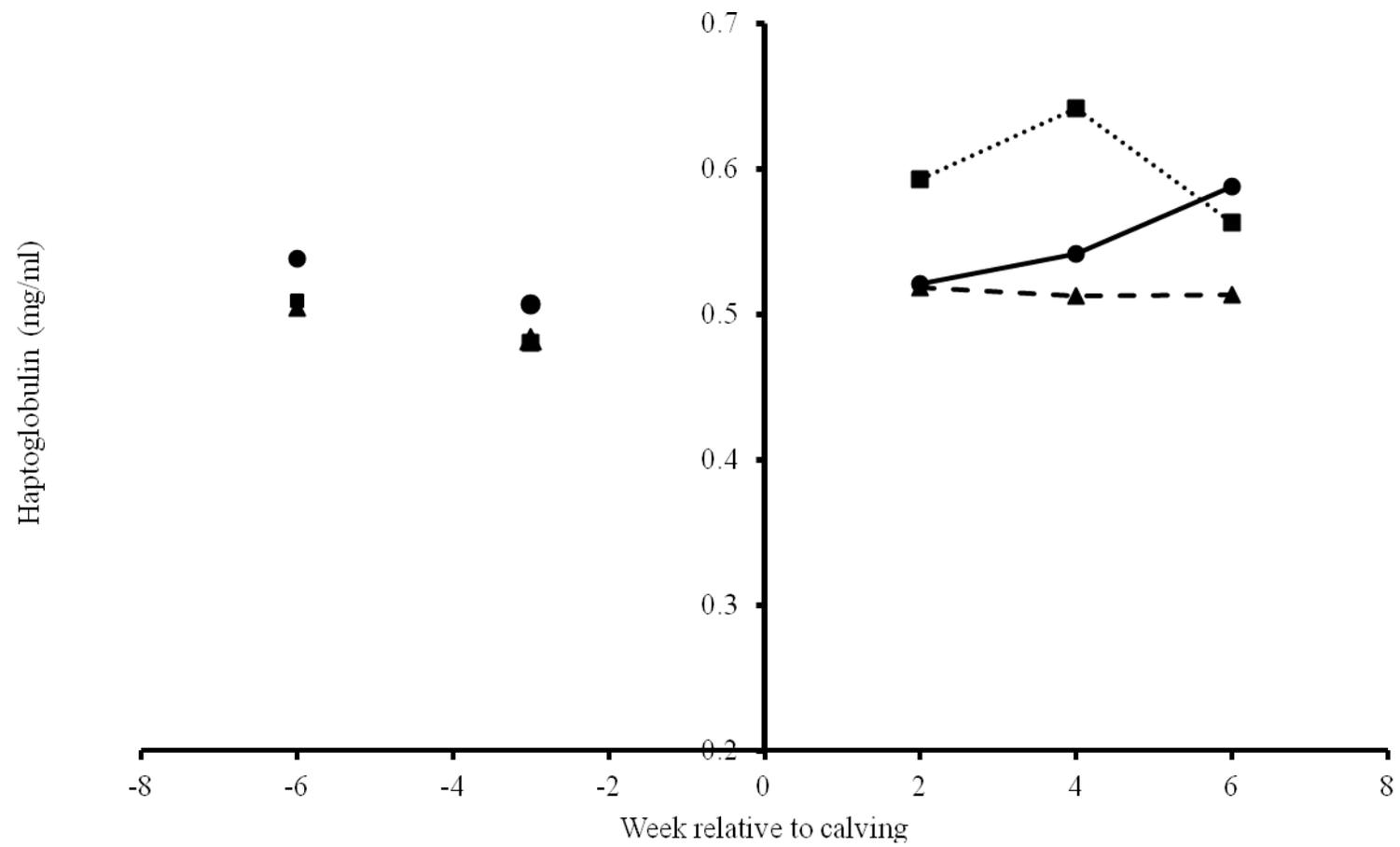


Figure 6. Effect of concentrate protein level in late-lactation and dry period length on serum haptoglobin concentrations prepartum and at wks 2, 4 and 6 postpartum (● normal protein; ■ low protein; ▲ extended dry period)