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# Nutritional regulation of body condition score at the initiation of the transition period in primiparous and multiparous dairy cows under grazing conditions: milk production, resumption of *post-partum* ovarian cyclicity and metabolic parameters

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*The objective of this study was to investigate the effect of different body condition score (BCS) at 30 days before calving (−30 days) induced by a differential nutritional management from −100 days until −30 days on productive parameters, the interval to first ovulation and blood parameters in primiparous and multiparous Holstein cows under grazing conditions until 60 days* post partum. *The experimental arrangement was a randomized complete block design, where cows were blocked according to BW and expected calving date and then randomly assigned to different nutritional treatments from –100 to –30 days relative to calving to induce different BCS. As the assignment of cows to treatments was random, cows had to lose, maintain or gain BCS; thus, different planes of nutrition were offered with approximately 7, 14 or 20 kg dry matter per day. The BCS score was assessed every 15 days and animals were reassigned in order to achieve the desired BCS at –30 days. Only animals that responded to nutritional treatment were considered and this was defined as follows: primiparous and multiparous high cows (PH and MH) had to gain 0.5 points of BCS, primiparous low (PL) had to lose 0.5 points of BCS and multiparous low (ML) had to maintain BCS at least in two subsequent observations from −100 to −30 days. From −30 days to calving, primiparous and multiparous cows (P and M cows) were managed separately and cows were offered a diet once a day. From calving to 60 days post partum, cows of different groups grazed in separate plots a second year pasture. Cows were also supplemented individually with whole-plant maize silage and commercial concentrate. Cows had similar BCS at −100 days and differed after the nutritional treatment; however, all groups presented similar BCS at 21 days* post partum. *The daily milk production and milk yield at 60 days* post partum *was higher in M than P cows. The percentage of milk fat was higher in PH cows compared with PL cows. Concentrations of non-esterified fatty acids (NEFA) were affected by the BCS at −30 days within parity, and in PH cows the concentration of NEFA was higher than in PL cows. The concentrations of total protein were higher in M cows. A lower probability of cycling was found in PL than in PH cows ($P < 0.05$) and in ML than in MH cows ($P < 0.05$). Treatment affected various endocrine/metabolic profiles according to parity, suggesting that the metabolic reserves signal the productive/reproductive axis so as to induce a differential nutrient partitioning in adult* v. *first-calving cows.*

**keywords:** parity, hormones, metabolites

## Implications

The objective of this study was to analyze the effects of the body reserves (measured by body condition score, BCS) induced by feeding, on productive and reproductive variables, independent of other factors inherent in the environment and/or the individual animal capacity for intake. The experiment demonstrated that nutritionally regulated BCS at −30 days had positive effects on fat-corrected milk production and on days to first ovulation, which was associated with a better *pre-partum* endocrine milieu. Multiparous cows

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were more adapted to increased milk production and to reinitiate *post-partum* ovarian cyclicity earlier compared with primiparous cows.

## Introduction

During early lactation, the primary challenge faced by cows is a sudden and marked increase in nutrient requirements for milk production, at a time when dry matter intake, and thus nutrient supply, lags behind (Drackley, 1999). Moreover, the decreased intake that characterizes the transition period (3 weeks before and 3 weeks after calving) can reach up to 30% and is one of the causes of negative energy balance (NEB; Grummer, 1995; Grummer *et al*., 2004). This period of NEB is characterized by fat mobilization, which is reflected by an elevation of circulating concentrations of non-esterified fatty acids (NEFA; Pedron *et al*., 1993; Ingvartsen and Andersen, 2000; Burke and Roche, 2007), and which is often accompanied with an increase in the concentration of β-hydroxybutyrate (BHB; Whitaker *et al*., 1999).

Body condition score (BCS) at calving and the degree of BCS loss between calving and BCS nadir have profound effects on milk production (Roche *et al*., 2007). There is the nonlinear relationship between calving BCS and subsequent milk production, and the collective literature makes a compelling case for an optimum calving BCS for milk production between 3.0 and 3.5 in Holstein–Friesian dairy cows (Roche *et al*., 2009). BCS loss also has negative effects on reproduction: cows that lost one or more units of BCS during early lactation had a major risk for infertility, with a reduction in conception rates from 17% to 38% (Butler, 2000). It is accepted that the endocrine pathways that regulate the reproductive axis regarding metabolic status include insulin and insulin-like growth factor I (IGF-I; Spicer *et al*., 1993; Lucy, 2000; Kawashima *et al*., 2007).

Contradictory information is reported on the effect of parity on *post-partum* anestrus length: primiparous cows (P cows) had longer *post-partum* anestrus than multiparous cows (M cows) under confined (Tanaka *et al*., 2008) and grazing (Meikle *et al*., 2004) production systems; in addition, P cows presented shorter anestrus than M cows (Kawashima *et al*., 2006) or no effect of parity was observed (Stein *et al*., 2006; Cavestany *et al*., 2009). In addition, endocrine concentrations have been reported to differ according to parity: IGF-I concentrations after calving were greater (Taylor *et al*., 2004; Wathes *et al*., 2007) or lower (Meikle *et al*., 2004) in P than in M cows, under confined or grazing conditions, respectively. As several factors affect the endocrine milieu, these contradictory findings could be due to differences in animal management, mainly nutrition.

Most reports on the effect of BCS on productive and reproductive parameters are based on a classification of the cows according to BCS at calving (Gallo *et al*., 1996; Meikle *et al*., 2004; Patton *et al*., 2007) and/or at the moment of the initiation of the transition period (Shrestha *et al*., 2005; Chagas *et al*., 2006). We did not find reports on the effect of different nutritionally regulated BCS at the initiation of the transition period in dairy cows; thus, productive and reproductive responses to BCS could be the result of a differential animal capacity to face NEB during this period. For that reason, the objectives of this study were to evaluate the effect of nutritionally regulated differential BCS one month before the expected calving date on milk production, metabolic and endocrine profiles and days to first ovulation in primiparous and multiparous Holstein cows under grazing conditions.

Our hypothesis was that both parity and nutritionally regulated BCS one month before calving affect both productive and reproductive performance, and that this would be associated with a differential endocrine/metabolic environment.

## Material and methods

Animal experimentation was approved by the Animal Experimentation Committee of the University of Uruguay.

### *Animals and experimental design*

Holstein cows with two to five parturitions (M cows; $n = 32$) and cows without previous parturitions (P cows; $n = 30$; average 305 days corrected milk yield: 6000 and 4800 kg, respectively for herd of Uruguay) with parturitions during autumn (March, April and May), dry period 127 ± 82 days and interval between *partum* of 419 ± 128 days, were selected from the herd (200 dairy cows) of the experimental dairy farm of the Faculty of Agronomy Sciences (Paysandú, Uruguay).

Only animals that responded to nutritional treatment were considered and this was defined as follows: primiparous and multiparous high cows (PH, $n = 13$ of 15 and MH, $n = 9$ of 16) had to gain 0.5 points of BCS, primiparous low (PL, $n = 9$ of 15) had to lose 0.5 points of BCS and multiparous low had to maintain BCS (ML, $n = 8$ of 16) at least in two subsequent observations (every 15 days) of BCS from −100 to −30 days. The BCS were assessed according to a scale of 1 to 5 (1 = thin, 5 = fat; Edmonson *et al*., 1989) and the observations were made by the same observer. The experimental arrangement was a randomized complete block design, where cows were blocked according to BW and expected calving date, and then randomly assigned to different nutritional treatments from –100 to –30 days relative to calving (days) to induce different BCS. As the assignment of cows to treatments was random, cows had to lose, maintain or gain BCS; thus, different planes of nutrition were offered with approximately 7, 14 or 20 kg/DM (estimated chemical composition: 12.7% CP, 56.7% NDF, 24.3% ADF and 1.25 Mcal/kg DM of net energy of lactation (NEL)) of a long-term established pasture with a maximal herbage mass of 1200 kg DM/ha. The animals grazed on different areas to determine the forage offer above. The food in this period was similar for all animals, and differed only in quantity.

From −30 days to calving, P and M cows were managed separately. Cows were offered once a day a diet that included whole-plant maize silage (4.2 and 5.1 kg DM) and commercial concentrate (3.7 and 4.6 kg DM), for P and M cows, respectively, and had *ad libitum* access to *Setaria italica* hay.

This resulted in an estimated chemical composition of 9.5% CP, 54.2% NDF, 30.8% ADF and 1.33 Mcal/kg DM of NEL, for both groups. From calving to 60 days *post partum*, cows of different groups grazed in separate plots – a second-year pasture, mixture of *Festuca arundinacea*, *Trifolium repens* and *Lotus corniculatus* – with a forage allowance of 30 kg DM/cow in weekly plots grazed twice daily (0930 to 0230 and 0530 to 0400). Herbage mass available composition for the whole period was on average 25% DM, 14.2% CP, 49.0% NDF and 24.5% ADF. Cows were also supplemented individually with 3.1 kg DM of whole-plant maize silage (31.1% DM, 6.9% CP, 65.0% NDF and 33.0% ADF) and 3.7 kg DM of a commercial concentrate (89% DM, 18.1% CP, 19.2% NDF and 12% ADF) after the morning milking. In addition, cows received 1.3 kg DM of the same commercial concentrate in each milking (morning and evening).

For the first 60 days of lactation, cows were milked twice a day (0500 and 1500), milk production was determined daily and milk samples were obtained weekly from four consecutive milking (milking of morning and evening) and combined for fat, protein and lactose determinations. From 0 to 60 days, BCS was assessed weekly and BW was measured at 30-day intervals. Blood samples were collected once a week *pre partum* and twice *post partum*, from the coccygeal vein in heparinized tubes approximately 1 h before the morning milking and at 0700 *pre partum*. Samples were centrifuged immediately and plasma was stored at $-20°C$ until assayed.

*Metabolite and hormone determination*

The metabolic profiles were determined in 118 samples from $-30$ to 30 days *post partum* and included NEFA, BHB, cholesterol, total protein, albumin and urea. All samples were analyzed in one assay in the Laboratory DILAVE of Uruguay. Blood biochemistry was analyzed according to the following colorimetric methodologies: cholesterol: CHOD-PAP (Wiener Lab 861231904); total protein: Biuret reaction (Wiener Lab 864102502); albumin: Bromocresol green (Wiener Lab 861250000); and urea: Urease UV (Wiener Lab 861237004). For these determinations, commercial kits from Weiner Laboratory (Rosario, Argentina) were used and calibrated with control calibrator serum (Wiener Lab 861244507) on a Vitalab Selectra 2 autoanalyzer (Vital Scientific, Dieren, The Netherlands). For quality controls, Lyotrol N (Ref. 62373) and P (Ref. 62373), and internal controls of the Laboratory DILAVE were used. Concentrations of NEFA were determined by the method ACS-ACOD (kit NEFA-C, Wako Chemicals, Richmond, VA, USA) and BHB by the d-3-hydroxybutyrate kit (Randox Laboratories Ltd, Ardmore, UK). The intra-assay coefficient of variability (CV) were 6.8%, 4.8%, 0.1%, 3.8%, 4.0% and 18% for protein, albumin, urea, cholesterol, NEFA and BHB assays, respectively.

Concentrations of insulin, IGF-I and progesterone (P4) were determined in the Laboratory of Nuclear Techniques, Veterinary Faculty, Montevideo, Uruguay. Insulin concentration was determined from $-37$ to 30 days *post partum* by a $^{125}$I-Insulin RIA kit (Diagnostic Products Co., Los Angeles, CA, USA). The sensitivity of the assay was 2.2 μIU/ml and the intra-assay CV was 8.2% and 9.4% for low concentration (2.2 μIU/ml) and medium (12.6 μIU/ml) controls, respectively.

Concentrations of IGF-I were determined $-37$, $-30$, $-21$, $-15$, 0, 15 and 30 days *post partum* using immunoradiometric assay with a commercial kit (IGF-I-RIACT Cis Bio International, Gif-sur-Yvette, France). The kit contains two monoclonal antibodies against two different antigenic sites of IGF-I molecule, one that is coated on the solid phase, the other radio labeled with $^{125}$I. Samples are first treated with an acidic solution to strip the carrier proteins, which are then saturated with IGF-II to avoid the reassociation between IGF-I and the carrier protein. The sensitivity of the assay was 0.7 ng/ml and the intra-assay CV for control 1 (74 ng/ml) and control 2 (535 ng/ml) were 6.9% and 7.2%, respectively.

Progesterone was determined three times per week in all plasma samples from calving until 60 days *post partum* using a commercial kit (Diagnostic Products Co., Los Angeles, CA, USA). The sensitivity of the assay was 0.1 ng/ml and the intra-assay CV for the low (0.5 ng/ml) and medium (2 ng/ml) controls were 15.6% and 8.1%, respectively. A day to first ovulation was defined as the first day after calving in which the concentration of P4 was greater than 1 ng/ml. The probability to first ovulation was estimated by the proportion of cows with first ovulation, in total animals per group, estimated every 5 days from 15 days *post partum*.

*Statistical analyses*

Milk production, BCS, metabolite and hormonal concentrations were analyzed with repeated measures using a statistical model that included the effects of parity (P or M cows), BCS at $-30$ days (low or high BCS) nested in parity, days and their interactions as fixed effects and block random effect (Mixed procedure, SAS Institute Inc., Cary, NC, USA). The covariance structure was autoregressive order 1 and degrees of freedom were adjusted by the Kenward–Rogers method. The probability of cows cycling was analyzed using a generalized linear model with a binomial distribution of the variable (Genmod procedure of SAS). Tukey–Kramer tests were conducted to analyze differences between groups except for probability of cows cycling where likelihood ratio tests were used. The means are considered different when $P < 0.05$. The data are presented as least squares means $\pm$ s.e.m.

## Results

*BCS and BW*

The body condition was affected by days *post partum* ($P < 0.001$), body condition *pre partum* ($P < 0.001$) and parity ($P < 0.001$), and there was interaction between BCS and parity ($P < 0.001$) and days *post partum* and parity ($P < 0.001$). P cows had greater BCS than M cows at the initiation ($-100$ days) of the experiment ($3.80 \pm 0.74$ *v.* $2.91 \pm 0.68$; $P <$ 0.001). There were no differences between BCS within parity groups before the nutritional

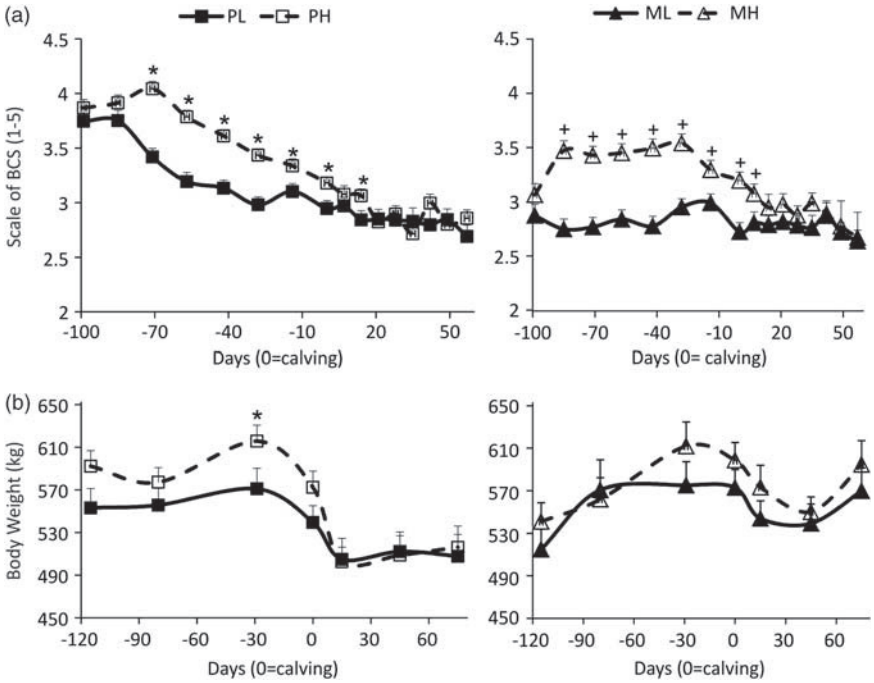


**Figure 1** Body condition score (BCS) from −100 to 56 days of calving (a) and body weight (BW) (b; least squares means ± pooled s.e.). PL = primiparous cows (P cows) in low BCS, PH = P cows in high BCS, ML = multiparous cows (M cows) in low BCS, MH = M cows in high BCS. *Indicates the difference between low and high BCS for a given day, for P and for M (+) cows, respectively.

treatments were applied, but they did differ from −85 to −40 days (Figure 1). Whereas in PH cows BCS increased from −100 to −75 days ($P = 0.01$) and decreased thereafter to 21 days ($P < 0.001$), PL cows lost BCS from −75 to −30 days ($P < 0.001$) and from −15 to 15 days ($P = 0.01$). The BCS of MH cows increased from −100 to −75 days ($P = 0.002$), remained constant until −30 days and decreased thereafter until 15 days *post partum*, whereas BCS of ML cows was maintained from −100 days to 60 days *post partum*, except for the decrease found from −15 to 0 days ($P < 0.001$). A month before calving, BCS was different between high and low cows within parity: P cows ($3.43 \pm 0.05$ *v.* $2.98 \pm 0.07$; $P < 0.001$) and M cows ($3.44 \pm 0.07$ *v.* $2.89 \pm 0.07$; $P < 0.001$), respectively.

BW was affected by days *post partum* ($P < 0.001$) and parity ($P < 0.01$). BW at calving was $539 \pm 15$, $572 \pm 10$, $594 \pm 17$ and $610 \pm 18$ kg for PL, PH, ML and MH cows, respectively, with M cows being on average heavier than P cows from −100 to 60 days *post partum* (Figure 1b). At 75 days *post partum*, after the end of the experimental period, only the MH group gained weight compared with 45 days ($P < 0.05$).

*Milk production and composition*

Milk production and accumulated milk production at 60 days *post partum* were affected by parity ($P < 0.001$ for both) and were greater for M cows but were not affected by BCS at −30 days within parity. The milk of PH cows had a greater fat percentage than that of PL ($3.72 \pm 0.05$ *v.* $3.38 \pm 0.06$; $P < 0.01$), but no differences were found in M cows. Percentages of fat ($P < 0.001$), protein ($P < 0.001$) and lactose

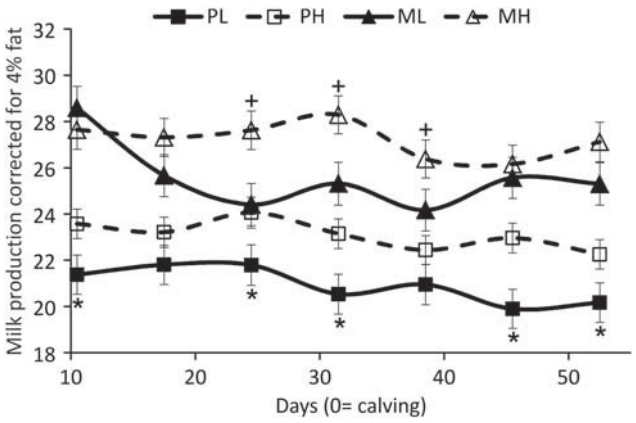


**Figure 2** Milk production corrected for 4% fat (least squares means ± pooled s.e.) during 60 days after calving. PL = primiparous cows (P cows) in low body condition score (BCS), PH = P cows in high BCS, ML = multiparous cows (M cows) in low BCS, MH = M cows in high BCS. *Indicates the difference between low and high BCS for a given day, for P and for M (+) cows, respectively.

($P = 0.03$) in milk decreased from week 2 to 8. Fat-corrected milk (FCM) was affected by days *post partum* ($P < 0.001$) and parity ($P < 0.001$), and tended to be affected by BCS ($P < 0.10$). The P and M cows with high BCS produced 4% FCM than cows with low BCS at several times during the first 60 days *post partum* (Figure 2).

*Plasma NEFA and BHB concentrations*

The concentrations of NEFA were affected by the BCS at −30 days within parity ($P < 0.01$) and days ($P < 0.001$), but there was no effect of parity. Non-esterified fatty acid concentrations

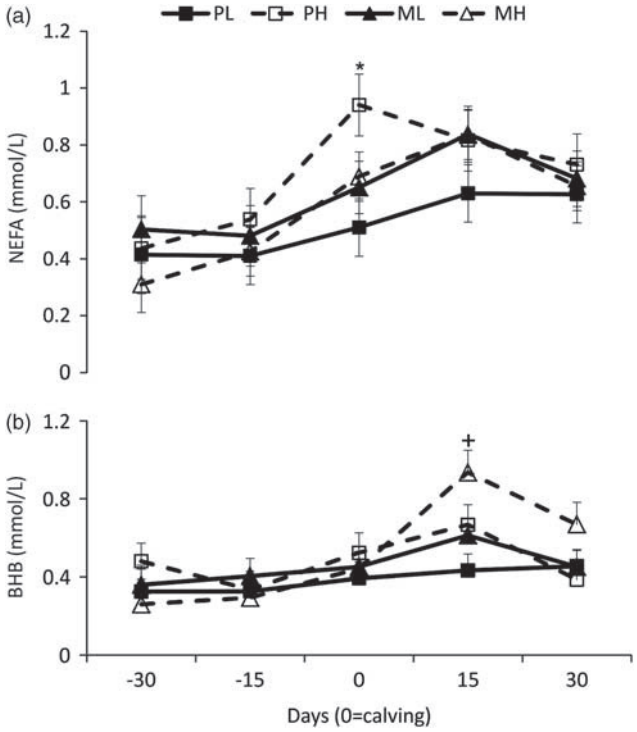


**Figure 3** Concentration (least squares means ± pooled s.e.) of non-esterified fatty acids (a) and β-hydroxybutyrate (b) in plasma. PL = primiparous cows (P cows) in low body condition score (BCS), PH = P cows in high BCS, ML = multiparous cows (M cows) in low BCS, MH = M cows in high BCS. *Indicates the difference between low and high BCS for a given day, for P and for M (+) cows, respectively.

increased at calving (Figure 3a). Concentration of NEFA was higher in PH cows than in PL cows ($P = 0.003$). The BHB concentration was affected by days ($P < 0.01$), increasing at 15 days in all groups except PL cows (Figure 3b).

*Plasma total protein, albumin, urea and cholesterol*

The total protein concentration of plasma was affected by parity ($P < 0.05$) and days ($P < 0.001$). The protein concentration decreased from −30 days to calving in the PL, PH and ML groups ($P = 0.001$, $P = 0.02$ and $P < 0.001$, respectively) and remained constant in MH groups ($P = 0.16$). The concentration increased from calving to 15 days *post partum* in PH ($P = 0.08$), ML ($P = 0.002$) and MH cows ($P = 0.0006$; Figure 4a). M cows had greater protein concentrations than P cows ($P = 0.01$). The albumin concentrations tended to be affected by the BCS at −30 days within parity ($P < 0.10$) and days ($P < 0.01$; Figure 4b), with minimum levels found at calving and greater concentrations in PL than in PH cows ($P < 0.05$). Urea concentrations were affected by BCS at −30 days within parity groups ($P < 0.05$) and days ($P < 0.01$). The ML cows had a greater urea concentration than MH cows, $P = 0.007$ (Figure 4d). In PH cows, the concentration of urea remained constant in the period −30 to 30 days *post partum*, whereas in PL cows the concentration decreased from −30 days calving ($P = 0.0016$). In ML cows, urea concentrations decreased sharply at −15 days ($P = 0.012$), whereas in MH cows it remained constant

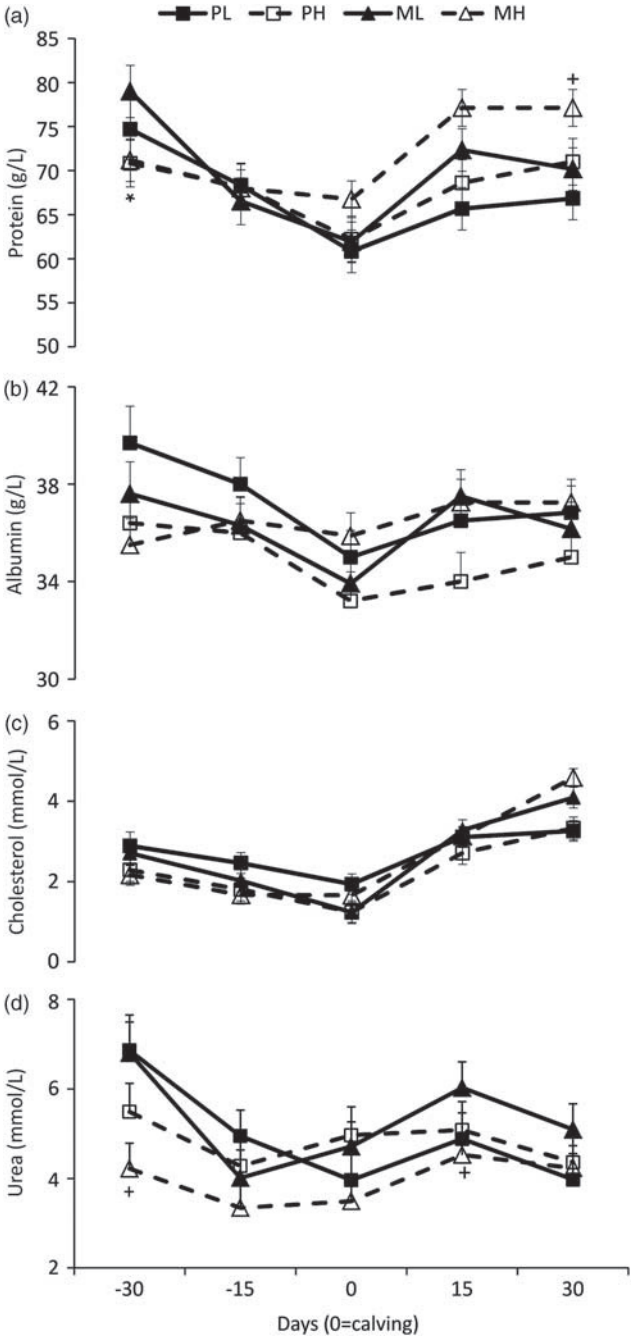


**Figure 4** Concentration (least squares means ± pooled s.e.) of protein (a), albumin (b), cholesterol (c) and urea (d) in plasma. PL = primiparous cows (P cows) in low body condition score (BCS), PH = P cows in high BCS, ML = multiparous cows (M cows) in low BCS, MH = M cows in high BCS. *Indicates the difference between low and high BCS for a given day, for P and for M (+) cows, respectively.

until calving. Urea concentrations tended to increase at 15 days in MH ($P = 0.09$) and ML ($P = 0.06$) cows. The cholesterol concentrations were affected by days ($P < 0.001$) and there was an interaction between parity and days ($P < 0.01$), as concentrations decreased at calving and increased again at 15 days; M cows had greater concentrations than P cows at 30 days (Figure 4c).

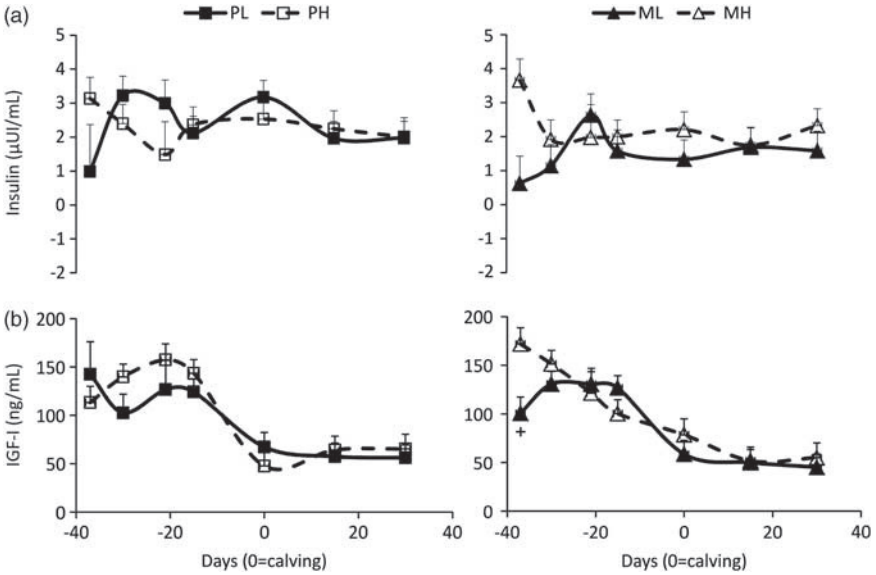


**Figure 5** Concentration (least squares means ± pooled s.e.) of insulin (a) and insulin-like growth factor I (b) in plasma. PL = primiparous cows (P cows) in low body condition score (BCS), PH = P cows in high BCS, ML = multiparous cows (M cows) in low BCS, MH = M cows in high BCS. (+) Indicates the difference between low and high BCS for a given day for P cows.

*Insulin*

The body condition tended to affect the concentration of insulin ($P = 0.14$). In PL and ML cows, insulin concentrations were already reduced at −37 days (end of the nutritional treatment) and increased during the *pre-partum* period, whereas the opposite was found in PH and MH cows. The PH and MH cows maintained insulin concentrations during the *post-partum* period, whereas concentrations of insulin tended to decrease at calving in ML ($P = 0.11$) and 15 days in PL cows ($P = 0.07$), respectively (Figure 5a). When only *post-partum* data were considered, P cows had greater insulin concentration than M cows ($P < 0.05$).

*IGF-I*

The concentration of IGF-I was affected by days ($P < 0.001$), and a tendency for an interaction between days and BCS was observed ($P = 0.07$). MH cows presented greater IGF-I concentrations than ML cows at −37 days *post partum* (dpp; $P < 0.01$). In PL cows, IGF-I concentrations were observed at calving ($P = 0.0005$), whereas in PH cows the decrease was already observed at −21 dpp ($P = 0.04$; Figure 5b). In the ML cows, IGF-I concentrations increased from days −37 to −30 ($P < 0.05$) and decreased at calving ($P < 0.001$), whereas in MH cows IGF-I concentrations decreased already at −21 dpp ($P < 0.05$).

*First post-partum ovulation*

The time between calving and first ovulation tended to be affected by parity ($P = 0.06$), and was 16 days longer for P cows than for M cows (47.1 days for P cows and 31.4 days for M cows). The days to first ovulation occurred at 40.6 ± 2 and 55.2 ± 2 days for PH and PL and 22.1 ± 2 and 39.1 ± 2 for MH and ML cows, respectively. The probability of cows cycling was affected or tended to be affected by BCS within parity at 50 dpp ($P = 0.05$) and 30 dpp ($P = 0.06$; Figure 6), presenting a lower probability of cycling in PL than in PH cows ($P < 0.05$) and in ML than in MH cows ($P < 0.05$).

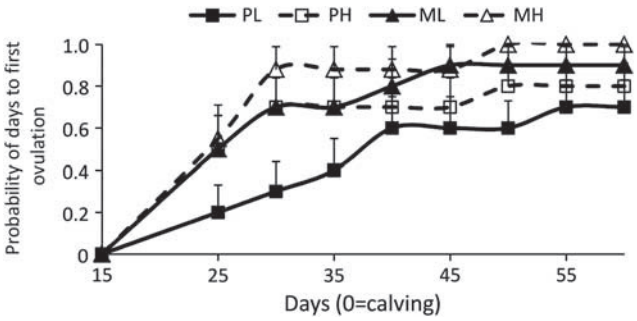


**Figure 6** Probability of days to first ovulation ($P$ ± confidence interval). PL = primiparous cows (P cows) in low body condition score (BCS), PH = P cows in high BCS, ML = multiparous cows (M cows) in low BCS, MH = M cows in high BCS.

## Discussion

Differences in BCS induced by the treatment (high *v.* low BCS) were maintained during the *pre-partum* period and at calving, but no differences were found 21 days after calving. Similarly, cows under different *pre-partum* nutritional treatments (*ad libitum v.* restrictive intakes) reached the same nadir of BCS between 4 and 6 weeks *post partum* (Douglas *et al.*, 2006).

The 0.5-point difference in BCS at −30 days affected FCM yield during the first 40 to 60 days of lactation in M or P cows, respectively. Pedron *et al.* (1993) and Agenas *et al.* (2003) did not find any effect of BCS at calving on milk yield in confined systems. However, Stockdale (2008), using fat

and thin animals at calving (4.36 and 3.87 points of BCS in a 1 to 8 scale), produced 33.7 *v.* 31.6 kg/day, respectively, in the first 92 days of lactation. It was established that the BCS has a nonlinear effect on milk production, which determines that at certain ranges of BCS milk yield would increase and at others it may decrease (Roche *et al.*, 2009). The greater milk and fat yields with BCS up to 3.5 are probably a result of a greater availability of energy for the cow, thereby sparing glucose production for lactose synthesis (Roche *et al.*, 2009).

Milk production curves did not present a peak in milk yield, which has already been observed in other studies conducted with cows calving in autumn (Meikle *et al.*, 2004; Cavestany *et al.*, 2005). One possible reason for these results could be that cows present the lactation peak in winter, when pasture quality is lower, an aspect that should be taken into account when comparison with well-fed cows is performed.

Concentrations of NEFA reflected the mobilization of reserves and were greater in PH cows, which were consistent with the greater losses of BCS, as well as the increased milk fat percentage in early lactation in this group. The lower NEFA concentrations in PL cows may be the result of the smaller body reserves that could be mobilized for milk production. Moreover, BHB in this group did not increase during the *post-partum* period. Although no differences were observed according to treatment in NEFA concentrations in M cows, BCS nadir was reached later during the *post-partum* period for MH than for ML cows. This was associated with the greater FCM yields found from approximately 3 to 5 weeks *post partum* in the former group. Indeed, dairy cows can mobilize fat reserves up to 12 weeks *post partum* (Komaragiri and Erdman, 1997); this was reflected in this study by the concentrations of preformed fatty acids ($C > 16$) in milk fat, which were greater in MH cows than in ML cows (Artegoitia V *et al.*, 2011, unpublished data).

Total plasma protein nadir found at calving reflected the mobilization of proteins toward the mammary gland principally for the immunoglobulin secretion in colostrum. The decrease in albumin, urea and cholesterol concentrations at calving may reflect the decreased intake in the *peri partum* period, as has been reported earlier (Cavestany *et al.*, 2005; Seifi *et al.*, 2007). Total plasma protein concentrations were greater in MH cows, suggesting the better energy/nitrogen balance. This idea is reinforced by the greater urea concentrations found in ML cows, which peaked at 2 weeks *post partum*, possibly suggesting an increase in protein mobilization to maintain milk production. According to Komaragiri *et al.* (1998), the capacity for protein mobilization is limited and occurs mainly during the first 5 weeks *post partum* (Komaragiri and Erdman, 1997). Interestingly, the FCM production decreased sharply in ML cows during the first 4 weeks, whereas MH cows maintained FCM production in the experimental period, which may be attributed to the greater available body reserves to maintain milk production.

Insulin profiles around calving differed between high and low BCS groups: whereas insulin concentrations were already decreasing one month before calving in high BCS cows, an increase was observed in low BCS cows, which could be the result of a better nutrient allowance during the close-up dry period (the nutritional treatment finished at −30 dpp). A decrease in insulin at calving was observed in all groups, which is a metabolic adaptation to cope with the energy demands of lactation, as reported earlier (Taylor *et al.*, 2003; Wathes *et al.*, 2007), as low insulin levels favor gluconeogenesis and lipolysis (Herdt, 2000; e.g. homeorhetical effect). Wathes *et al.* (2007) reported that insulin concentrations are lower in adult animals, which is consistent with *post-partum* data of the present study. These reduced insulin concentrations in M cows may also explain the better capacity for protein/fat reserve mobilization for milk synthesis.

In the present experiment, no differences were found in IGF-I concentrations between P and M cows, which is in contrast with Wathes *et al.* (2007) who reported that both insulin and IGF-I concentrations were greater in young animals associated with their role in growth. *Pre-partum* IGF-I concentrations tended to be greater in MH cows than in ML cows, which was consistent with the better BCS of these animals. Concentrations of IGF-I decreased at calving, as reported previously (Lucy *et al.*, 2001; Taylor *et al.*, 2003; Meikle *et al.*, 2004). It is interesting to note that IGF-I profiles reflected more likely the changes in BCS, in contrast with insulin profiles, which were associated more with the day-to-day effects of the nutritional input as observed in the *pre-partum* increases in low BCS cows. In addition, cholesterol is positively associated with concentrations of glucose, insulin and IGF-I (Francisco *et al.*, 2003), and in our study cholesterol increased with days *post partum* and was greater in MH cows.

The probability of cows cycling was affected by BCS at −30 days and parity, showing that cows in lower BCS and P cows had longer anestrus, which was consistent with other reports (Spicer *et al.*, 1990; Meikle *et al.*, 2004; Sakaguchi *et al.*, 2004; Burke and Roche, 2007; Tanaka *et al.*, 2008). A possible explanation of the delayed reinitiating of ovarian cyclicity in P cows may be their extra requirements for growth. A decrease in BCS of $\geqslant$1 unit at 7 weeks after calving increased the occurrence of delayed first ovulation (Shrestha *et al.*, 2005). Kawashima *et al.* (2007) demonstrated that IGF-I concentrations were a key determinant of time of ovulation. However, in this study, *post-partum* IGF-I concentrations did not explain the differences found in the probability of cows resuming cyclicity. Chagas *et al.* (2006) reported that *pre-partum* IGF-I concentrations were greater in high BCS cows that had unrestricted allowance *v.* restricted cows; however, even if *post-partum* IGF-I concentrations were similar, most of high BCS cows had ovulated by 77 days, whereas only 8% of low BCS cows did so.

In summary, the nutritionally regulated BCS at −30 days had positive effects on FCM production and on days to first ovulation, and it was associated with a better *pre-partum* endocrine milieu. M cows had better productive result, produced more milk and started cyclicity before P cows.

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