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Studies of the Transition Cow Under a Pasture-based Milk Production System: Metabolic Profiles

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With 6 figures and 3 tables

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Summary

This study describes the effect of parity (multiparous versus primiparous) and body condition score (BCS) at calving (< 3 or ≥3; scale 1–5) on variations of BCS, body weight (BW) and metabolic profiles in Holstein cows grazing on improved pastures. Forty-two cows were studied (21 multiparous and 21 primiparous) from 2 months before to 3 months after calving. BCS, BW and milk production were measured every 2 weeks. Blood samples were taken every 2 weeks to determine total protein, albumin, urea, non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB), cholesterol, aspartate aminotransferase (AST), calcium, phosphorus and magnesium. Primiparous cows had lower BCS during the early postpartum (PP) period and produced less milk than multiparous. In primiparous cows NEFA concentrations were higher during the early postpartum period; BHB levels were similar in both categories during this period. Primiparous cows showed a more unbalanced metabolic profile than multiparous cows, reflecting that they are recovering from the loss of BCS after calving with less success.

Introduction

During the transition period (3 weeks before to 3 weeks after calving) cows must adapt their metabolism to the high demands for lactation and to a different diet in order to meet their new requirements (Drackley, 1999), which results in a negative energy balance (for review see Chilliard, 1999). This is characterized by fat mobilization and elevation of circulating concentrations of non-esterified fatty acids (NEFA) (Ingvartsen and Andersen, 2000), which in most cases is paralleled by an increased production of β-hydroxybutyrate (BHB) and other ketone bodies (Whitaker et al., 1999). The significance of blood cholesterol concentrations at this stage is somehow controversial. Some authors associate a rise in cholesterol to a better energy balance or fat intake (Wittwer et al., 1987), while others postulate that is the result of energy deficiency (Bruss, 1997). Urea nitrogen concentrations are influenced by a wide variety of interrelated factors, including dietary protein intake and rumen degradability, dietary amino acid composition, liver and kidney function, muscle tissue breakdown, and amount of rumen degradability of dietary carbohydrate (Godden et al., 2001). Liver function can be assessed through enzymes in blood (e.g. gamma-glutamyltransferase, aspartate aminotransferase, Reid et al., 1979), and fatty infiltration is a natural process for the high-producing dairy cow.

Mechanisms for maintaining blood calcium levels perform efficiently most of the time (Goff, 2000). Phosphorus has no direct mechanism of regulation, although calcium-regulating hormones directly affect its blood concentration. Calcium and phosphorus have important bone reserves, while the magnesium reserve is very low and has no primary hormonal response for compensation (Martens and Schweigel, 2000).

The interrelationships between nutritional and productive factors and the variations in metabolites and energy balance in the dairy cow are not completely understood. Moreover, while most of the scientific information regarding metabolic physiology of the transition cow has been generated in confined or free stall production systems with TMR feeding systems, studies on dairy cows under grazing are scarce. Thus, the main objective of this work was to describe the body condition and metabolic changes during prepartum and early postpartum periods in Holstein dairy cows under grazing conditions. Additionally, we aimed to evaluate the effect of parity and body condition score at parturition on metabolic profiles and productive parameters.

Materials and Methods

Animals

From the herd of the experimental dairy farm of the Agronomy College (Paysandú, Uruguay), 21 multiparous (two to five lactations and average BW of 609 ± 5.7 kg) and

21 primiparous (average BW 575 ± 5.8 kg) Holstein cows with normal calvings were selected. Animal experimentation was performed in compliance with regulations set by the Veterinary Faculty (University of Uruguay). Cows were grazing on improved pastures (mixture of grasses and legumes) and 3 weeks before parturition they were offered a diet consisting of a mixture of 12 kg (fresh basis) of corn silage (33% DM, 6.8% CP) and 4 kg of a commercial concentrate (14% CP, 7.075 MJ Nel/kg) which were given once a day, and hay (bales of Setaria Itálica) was offered *ad libitum*. After parturition cows had access to a daily strip of pasture (mixture of grasses and legumes), 15 kg (fresh basis) of the same prepartum corn silage and 6 kg (DM basis) of a commercial concentrate (17% CP, 7.075 MJ Nel/kg).

The pasture sward mass (1650 ± 230 kg DM) was estimated with a comparative yield method adapted from Haydock and Shaw (1975), and an allowance of 15–18 kg of DM per cow per day was offered through weekly adjustments of the daily strip size. The cows had access to the grazing plot between the morning and the afternoon milkings. The corn silage was fed in the afternoon (after milking), and the concentrate was equally distributed during milking time (twice a day). Cows were milked twice a day, and milk production for the two daily milkings was measured every 15 days. BCS was determined every 15 days by the same person from 2 months before until the 3 months after parturition using a scale from 1 to 5 according to Edmonson et al. (1989), and BW was determined at the same time. Animals were classified according to BCS at calving in lean (<3, $n = 20$) or fat cows (≥ 3, $n = 22$). Blood samples were obtained every 2 weeks, from around 30 days prepartum to 60 days postpartum. Blood was collected from the jugular vein in heparinized vials approximately 1 h after the morning milking (e.g. 1 h after the morning concentrate) and centrifuged, and plasma was stored −20°C until assayed.

Blood biochemistry

Blood biochemistry was analysed according to the following colorimetric methodologies, total protein: Biuret reaction (Wiener Lab 864102502), albumin: Bromcresol green (Wiener Lab 861250000), urea: urease UV (Wiener Lab 861237004), cholesterol: CHOD-PAP (Wiener Lab 861231904), calcium: o-cresolphtaleine (Wiener Lab 861241502), magnesium: xylidyl blue-EGTA (Wiener Lab 861251501), phosphorus: phosphomolybdate UV (Wiener Lab 861232402), gamma-glutamyltransferase (GGT): Szasz (37°C) (Wiener Lab 861233502), and aspartate aminotransferase (AST): IFCC optimized (37°C) (Wiener Lab 861234302). For these determinations commercial kits from Weiner Laboratory (Rosario, Argentina) calibrated with A plus calibrator serum (Wiener Lab 861244507), and were used on a Vitalab Selectra 2 autoanalyser (Vital Scientific, Dieren, the Netherlands). Globulin was estimated as the difference between total protein minus albumin contents. Metabolites were determined in samples with an interval of 10 days. All samples were assayed in two assays. For quality controls, Lyotrol N (Ref. 62 373, lot no. 7490620), and P (Ref. 62 373, lot no. 7348842), and internal controls of the laboratory Miguel C. Rubino (DILAVE, Uruguay) were used.

β-Hydroxybutyrate (BHB) and non-esterified fatty acids (NEFAs) were determined by a D-3-hydroxybutyrate kit, catalogue no. RB 1007 and NEFA kit, catalogue no. FA 115 (Randox Laboratories Ltd, Ardmore, UK) in the laboratory of the Research Institute for Animal Breeding and Nutrition (Herceghalom, Hungary).

Statistical analyses

Data on milk production, BW, BCS and metabolite concentrations were analysed using the mixed procedure (Statistical Analysis System; SAS Institute Inc., Cary, NC, USA, 2000), and the statistical model included the effects of parity (primiparous = L1 or multiparous = L2), BCS at parturition (BCS <3 or ≥ 3), interactions between them, and interactions between them and postpartum days (linear and quadratic functions). The covariance structure was autoregressive order 1 and cow within category * BCS at parturition was set as random effect. Functions were calculated for each dependent variable and differences in the parameter of the curve were analysed according to parity and BCS at parturition. Postpartum days were categorized in intervals of 10 days during the experimental period (day 0 = day of parturition) and data are presented as least square means ± pooled standard error. Tukey–Kramer tests were conducted to analyse differences between mean values. The level of significance was $P < 0.05$, except where otherwise specified. Correlations between variables were also analysed using the Pearson correlation coefficient.

Results and Discussion

Milk production

Primiparous cows produced less milk than multiparous cows during the experimental period ($P < 0.001$, Fig. 1) and while the latter showed the expected lactation curve with peak levels at fortnights 3 and 4 PP, primiparous heifers showed a plateau during fortnights 2 to 4. Milk production was also affected by days postpartum (PP), but there were no main effects with BCS at parturition or parity or an interaction between them (Table 1). It was negatively correlated with BCS and with NEFA but it was positively correlated with protein and albumin (Table 3).

Body weight and body condition score

BCS was affected by parity and days postpartum, with an interaction between both effects (Table 1). Parity and BCS at

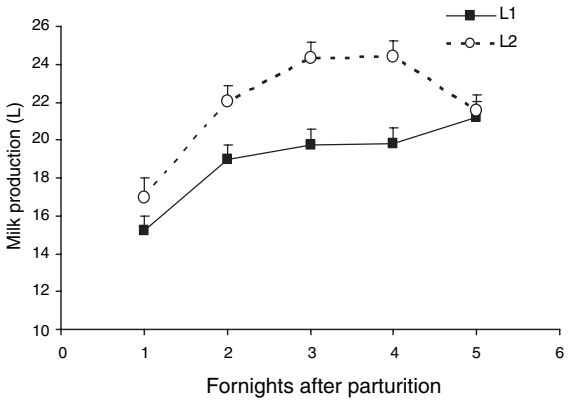

Fig. 1. Milk production (mean ± SEM) of primiparous (L1) and multiparous (L2) cows under grazing conditions.

Table 1. *F*-tests of fixed effects included in the model for body condition score, body weight and metabolite concentrations in cows under grazing conditions. Fixed effects are the effects of parity (P), body condition score (BCS) at parturition (C), postpartum days [linear (DPP) and quadratic (DPP^2) functions] and interactions between them. Only variables with significant differences according to parity are presented

Variable	P	C	DPP	DPP^2	P * C	DPP * P	DPP * C	DPP^2 * P	DPP^2 * C
BCS	0.07	***	***	***	0.11	***	0.11		0.08
BW	***	*	***	***		*	0.10		
Milk			***	***					
NEFA	0.11		***	***		***		**	
BHB			*	*		0.07		0.08	
Cholesterol			***	***		***			
Proteins	**		***	***		0.10			
Albumin	***	0.15	***	***		***		*	
Globulin	*		***	***		***			
Urea		0.14	***	***		*			0.10
AST[b]			***	***					
Calcium	***		***	NS		***			
Phosphorus			***	**	0.10			0.15	
Magnesium	0.09		***	***		*			

DPP, days postpartum; AST, aspartate aminotransferase.
$^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Table 2. Estimates of the functions in primiparous and multiparous cows. Only variables with significant differences according to parity are presented

	Primiparous cows			Multiparous cows		
Variable	Intercept	DPP	DPP^2	Intercept	DPP	DPP^2
BCS	2.61[a]	− 0.01861[a]	0.00013[a]	2.75[b]	−0.01284[b]	0.00008[b]
BW	521[a]	−1.6933[a]	0.009153[a]	583[b]	−1.3286[b]	0.005954[a]
NEFA	0.4197[a]	0.003901[a]	−0.00010[a]	0.3863[a]	0.001728[b]	−0.00006[b]
Cholesterol	2.6350[a]	0.01362[a]	0.000235[a]	2.6486[a]	0.02325[b]	0.000285[a]
Albumin	32.5[a]	0.04770[a]	−0.00043[a]	34.3[a]	0.08794[b]	−0.00085[b]
Urea	3.7884[a]	−0.00142[a]	0.000313[a]	4.1414[a]	−0.01241[b]	0.000201[a]
Calcium	1.9638[a]	0.001662[a]	0.000011[a]	2.0638[a]	0.003744[b]	−0.00001[a]
Magnesium	0.8632[a]	0.000856[a]	−0.00004[a]	0.901[a]	0.003180[b]	−0.00006[a]

Values with different superscripts within estimates (intercept, DPP and DPP^2) differ $P < 0.05$.

Table 3. Pearson correlation coefficients of the different variables studied

Variable	Milk	BW	NEFA	BHB	Chol	Proteins	Albumin	Globulin	AST	Urea	Ca	P
Milk			−0.24*			0.26**	0.35***					
BCS	−0.35*	0.75***	−0.35***	−0.17*	−0.46***	−0.18*		−0.22**	−0.29**			−0.33***
BW			−0.33***	−0.26**	−0.30***				−0.43***		0.30***	−0.30***
NEFA				0.53***			0.15*					0.14*
BHB						0.15*				0.18*	0.18*	
Chol						0.57***	0.51***	0.43***	0.39***		0.45***	0.27***
Proteins							0.47***	0.93***	0.39***		0.49***	0.28***
Albumin								0.13**	0.47***		0.70***	0.41***
Globulin										0.10*	0.27***	0.15*
AST											0.37***	0.36***
Urea												0.10*
Ca												0.27***
Mg							0.29***			−0.14*		

Chol, cholesterol; AST, aspartate aminotransferase.
$^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

parturition affected BW changes during the experimental period, and statistical differences were found on the drop in BW (kg/day: −1.7, primiparous, versus −1.3, multiparous, $P < 0.05$) but not on its recuperation (Table 2). A strong correlation between body weight and BCS was found ($r = 0.75$, $P < 0.0001$). BCS decreased from 30 days before calving, and this trend was steeper during the first 4 weeks after parturition (Fig. 2). Cows with better BCS at parturition (≥3) lost more body condition, mainly in the first month of lactation, and it was not recovered to initial prepartum values during the experimental period. Animals with lower BCS at calving had lower BCS during the experimental period, but tended ($P = 0.11$) to lose less body condition during the PP period as previously described by Flamenbaum et al. (1995). Heifers with BCS ≥3 at calving tend to lose more BCS, had a steeper decay in BCS than multiparous cows but they recuperated faster (Table 2). The lower BCS of primiparous than multiparous cows is consistent with a higher disequilibrium observed in the metabolic profiles (see below). This is probably related to increased needs for growth in primiparous cows occurring simultaneously with the demands of lactation and their lower feed intake capacity as described previously

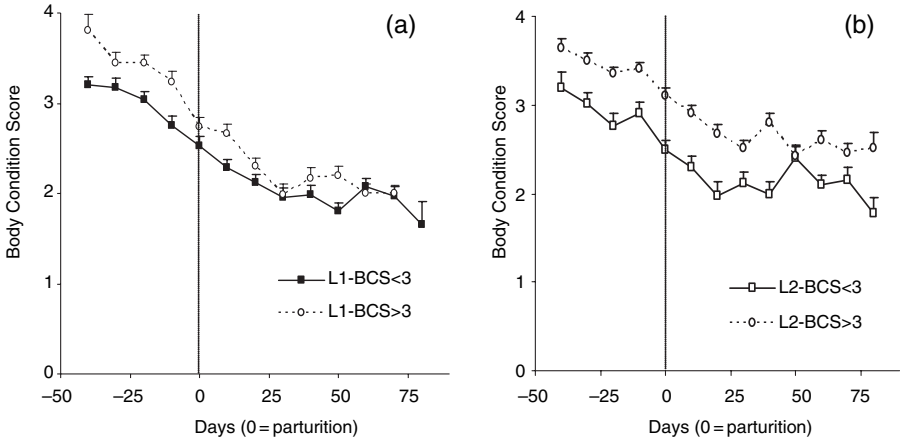

Fig. 2. Evolution of body condition score (mean ± SEM) in primiparous (L1) or multiparous (L2) cows with BCS at calving of <3 or ≥ 3.

(Remond et al., 1991). Similar results have also been reported in lactating grazing cows (Cavestany et al., 2001).

BCS was negatively correlated with protein, globulins, NEFA, BHB, cholesterol and phosphorus (Table 3).

Metabolic profiles

NEFA concentrations increased at calving, reached peak concentrations on day 14 PP and 20 PP for multiparous and primiparous cows and started to decrease thereafter. This increase is a result of a decrease in dry matter intake prior to parturition and of hormonal changes before and at parturition that stimulate mobilization of NEFA from adipose tissue to provide energy for parturition and lactogenesis (Vazquez-Añon et al., 1994; Grum et al., 1996). NEFA curves in primiparous and multiparous cows differed along the PP period; the increase was higher for primiparous cows and remained high for a longer period (Table 2). The high PP levels of NEFA would indicate that primiparous cows were mobilizing more long chain fatty acids from adipose tissue than multiparous cows (Belyea et al., 1975), which would agree with the results of Drackley et al. (2003). Postpartum NEFA levels were higher during a longer period than reported by others (Holtenius et al., 2003), probably because of a longer period of negative energy balance, and coincident with a continuous decline in BCS. However, the decline in NEFA concentrations started earlier in the PP period than the pattern reported by Chapa et al. (2001). NEFA was positively correlated with albumin (Table 3).

BHB concentrations were low at calving, rose sharply up to approximately 10 days PP and slowly decreased thereafter, but they remained higher than the prepartum levels during the experimental period (Fig. 3a), reflecting the longer negative energy balance in these animals and the consequently mobilization of body reserves (Ingvartsen and Andersen, 2000; Moorby et al., 2000) associated with the onset of lactation (Vazquez-Añon et al., 1994). BHB levels in this study remained higher than those reported elsewhere (Vazquez-Añon et al., 1994). When BHB values of >1 mM/l were considered (cows with subnormal levels according to Whitaker et al., 1999) primiparous cows had more samples with these levels, which might suggest a subclinical ketosis in this category (Whitaker et al., 1999). The increase in BHB, although not so closely correlated to BCS as reported by Reist et al. (2002), might be also a reflection of a low energy postpartum diet. The BHB levels remained higher for a longer period after calving than reported by Moorby et al. (2000), probably because of different diet composition or lower dry matter intake.

NEFA and BHB levels were highly correlated (0.53, $P < 0.0001$; Table 3).

Cholesterol concentration increased during the PP period in both categories, although multiparous cows had higher cholesterol around day 60 PP than primiparous (Fig. 3b, Tables 1

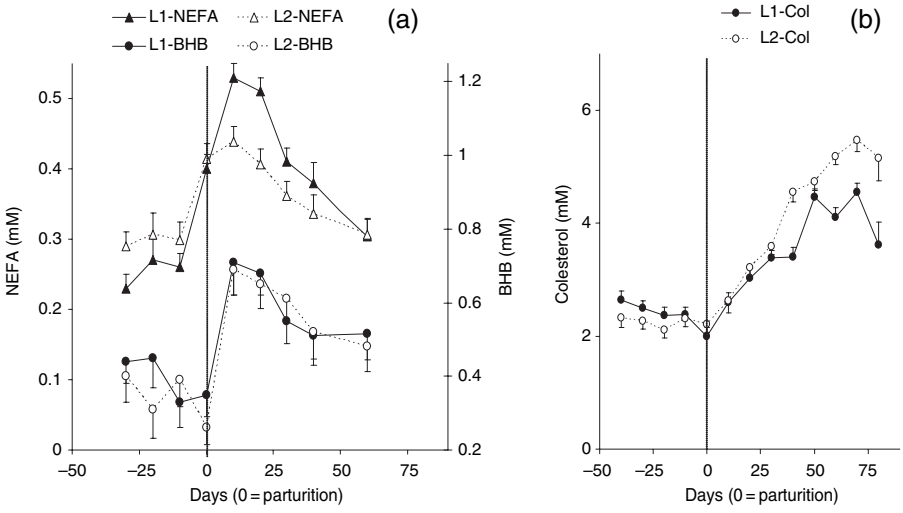

Fig. 3. Levels (least square means ± SEM) of (a) non-esterified acids (NEFA), β-hydroxybutyrate (BHB) and (b) cholesterol (Col) in primiparous (L1) and multiparous (L2) cows.

and 2), reflecting an increased lipid uptake by the liver (Belyea et al., 1975). This increase, greater than that reported by Bruss (1997), remained higher than the prepartum concentrations during the experimental period and followed a pattern similar to the one reported by Schwalm and Schultz (1976). According to Margolles (1983) hypercholesterolaemia can be considered physiological during lactation, either as a result of lipid mobilization caused by glucagon or to an increase in the synthesis of plasmatic lipoproteins. However, the increase in cholesterol concentrations may be due to a greater energy demand than that supplied by the offered diet. Taking into account that more severe losses of BCS were observed around parturition when cholesterol levels were low, our results suggest that the rise in cholesterol is associated with an improvement in energy balance.

Albumin and total protein concentrations were within the normal ranges (Kaneko, 1997), but primiparous cows had lower levels than multiparous cows, which may reflect a more negative protein balance. Multiparous cows had higher levels of total protein, globulin and albumin (Fig. 4a) than primiparous cows, but there was no effect of BCS at calving. Significant interactions were found between parity and days postpartum in albumin and globulin patterns (Table 1). The decrease in globulin concentration around parturition was reflected in the protein pattern, and was coincident with an increase in albumin concentration. Albumin concentrations are known to be higher during the prepartum period than after parturition (Whitaker et al., 1999) although we are not aware of reports related to an increase just prior to calving as found in this study. Total proteins and albumins were closely correlated with AST. Total protein, albumin and globulin were correlated with cholesterol, Ca, and P (Table 3).

Serum urea levels decreased in the last month of gestation, and rose after calving (Fig. 4b). No differences in urea concentrations were found according to parity, but there was an interaction between parity and days postpartum (Table 1). The simultaneous decrease in total protein, globulin and urea concentrations in the last days before parturition is associated with the decrease in feed intake (Bauchart, 1993), and to an uptake of globulin by the udder as mentioned by Kehrli et al. (1989), as the production of colostrum increases. Recovery of total protein, globulin and urea during the PP period can be related to the increase in dry matter intake occurring at the same time (Manston et al., 1975). The urea patterns for primiparous and multiparous were different during the postpartum period and we have no clear explanation for this fact. Kappel et al. (1984), Ruegg et al. (1992) and Shaffer et al. (1981) obtained similar results, which they could explain. However, Cisse et al. (1991) found no differences because of parity at weeks 14 and 20, but the experiment was not designed to observe differences along the postpartum period. Most of the values were within the reference range, even taking into account that the samples were collected after feeding and, therefore, they could result in higher levels of this metabolite (Gustafsson and Palmquist, 1993). The decrease in prepartum urea concentration could be related to lower feed intake. Postpartum urea levels, in relation to nutrition, vary according to protein content, protein degradability, non-protein nitrogen and energy of the diet (Park et al., 2002). As the samples were taken after feeding the concentrate, that relationship will be reflected more evidently and more individual variations can be expected.

Activity of aspartate aminotransferase (AST) was within the normal range, except in some isolated samples that also had high levels of gamma-glutamyltransferase. Plasma patterns of this enzyme showed an increase from day −10 to 25 and remained steady until day 60 (Fig. 5). It has been reported that an increase in AST activity with an increase in cholesterol levels reflects the existence of fatty liver (Cornelius, 1989; Ropstad et al., 1989). We found a positive correlation between AST and cholesterol, although the levels of this enzyme were not high enough to suggest that pathology.

AST activity was negatively correlated to BW and BCS, and presented a positive correlation with cholesterol, albumin, Ca and P (Table 3).

Primiparous cows had lower blood plasma levels of calcium and magnesium (Fig. 6a). Lower levels of calcium were found before parturition, but they remained fairly stable during the PP period. Around day 50 PP a diminished concentration in magnesium concentration was observed in primiparous cows. Postpartum phosphorus levels almost doubled the prepartum concentration (Fig. 6b).

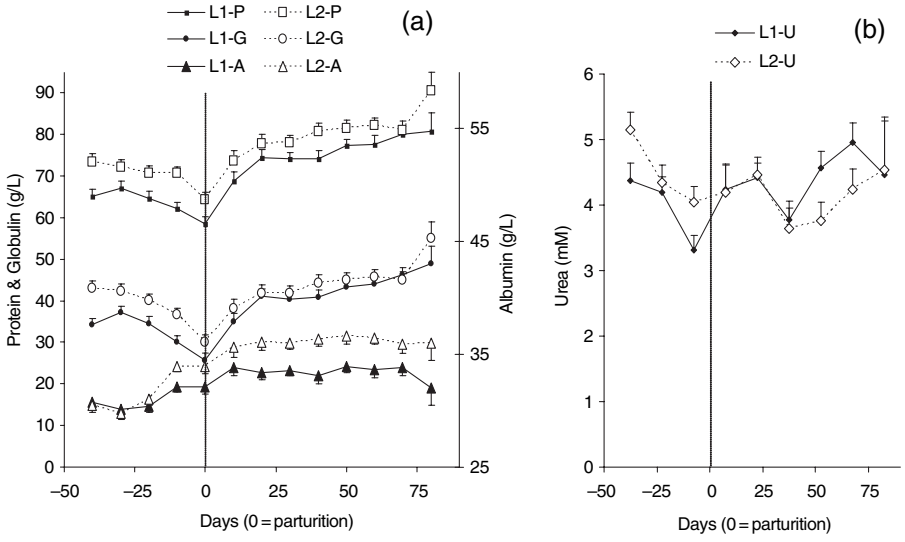

Fig. 4. Levels (least square means ± SEM) of (a) Total plasmatic protein (P), globulin (G) and albumin (A); and (b) urea (U) in primiparous (L1, $n = 21$) and multiparous (L2, $n = 21$) cows under grazing conditions.

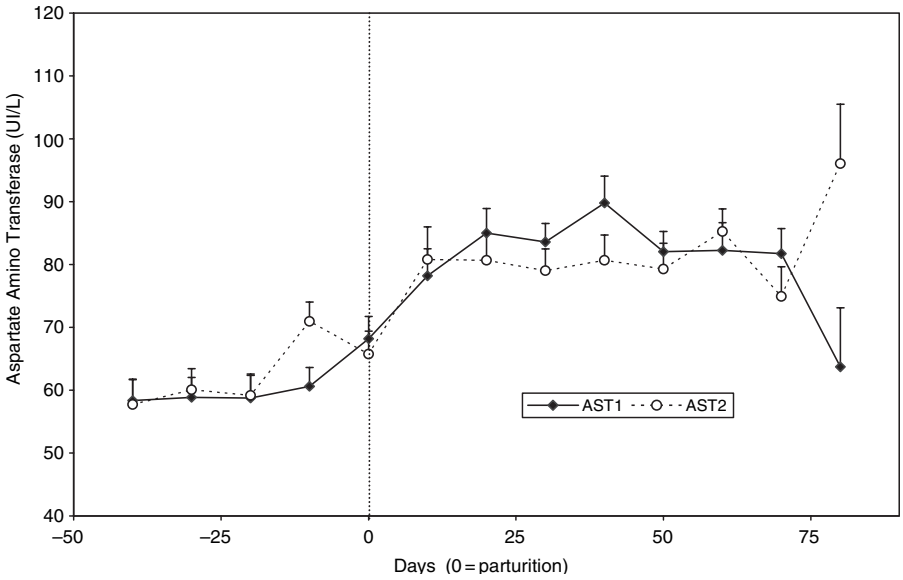

Fig. 5. Levels (least square means ± SEM) of aspartate amino transferase (AST) in primiparous (L1) and multiparous (L2) cows.

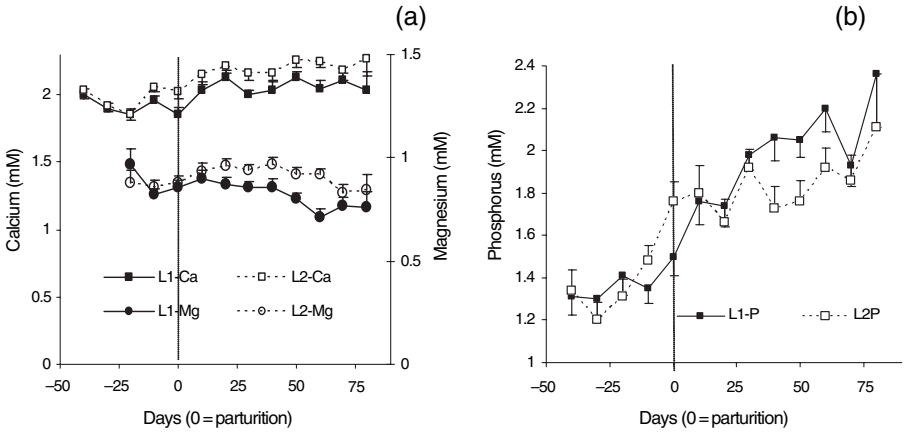

Fig. 6. Levels (least square means ± SEM) of (a) calcium (Ca) and Magnesium (Mg) in primiparous (L1) and multiparous (L2) cows and (b) Phosphorus (P) in primiparous (L1) or multiparous (L2).

Calcium, phosphorus and magnesium concentrations in primiparous cows showed more marked variations when compared with multiparous cows. Magnesium levels decreased during the week before parturition, which may be related to the decrease in calcium observed at parturition (Capen and Rosol, 1989). In multiparous cows, there was a significant decrease in Mg levels at around 60 days of lactation, consistent with a smaller decrease in Ca levels, although we don't have an explanation for this. Ca levels rose 10 days prior to calving, and this rise was associated with the increase in albumin concentrations, and these two were strongly correlated. This could be because one fraction of the total pool of calcium is linked to this protein and thus depends partly on its concentration (Goff, 2000). Lower P levels during the prepartum could be related to lower concentrate intake during this period. The further P increase observed during the PP may reflect the bone resorption of calcium and phosphorus to achieve the demands of milk production; the strict hormonal control in calcium levels results in maintenance of its level.

Calcium and phosphorous were positively correlated (Table 3).

In summary, primiparous cows showed a more unbalanced metabolic profile than multiparous cows, reflecting that they are recovering from the loss of BCS after calving with less success.

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