Relationship between pre-partum body condition score changes, acute phase proteins and energy metabolism markers during the peripartum period in dairy cows

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ABSTRACT
This study evaluated the relationship of pre-partum BCS variations on the acute phase response around parturition and milk production of dairy cows. The animals were divided into two groups: cows that gained BCS from the third to the first week pre-partum (GBC = n 11) and those that lost BCS (LBC = n 9) in the same period. Blood samples were collected pre and post-partum to determine serum concentrations of glucose (GLU), non-esterified fatty acids (NEFA), insulin (INS), albumin (ALB), paraoxonase (PON), haptoglobin (HP) and blood cell counts. The GBC group showed higher PON concentration in the postpartum (p < .05) and higher ALB concentration both pre and postpartum periods (p < .05), whereas the LBC animals showed a higher HP concentration in both periods (p < .05). Milk yield increased 3 kg/cow/day in the group that increased their BCS (p < .03). The LBC cows showed increased monocyte counts (p < .03), in addition to having a greater number of animals with the neutrophil:lymphocyte ratio higher than 1 (p < .03). The remaining parameters did not differ between groups. In conclusion, animals that lost BCS during the pre-partum period demonstrated to have a higher inflammatory status around parturition and lower milk production.

Introduction
The transition period in dairy cows is characterised by intense metabolic changes and extends from three weeks pre to three weeks postpartum (Goff & Horst 1997). These changes are due to a decreased feed intake prior to calving, associated with a rapid increase in the demand of nutrients for foetal growth and lactogenesis, as well as endocrine changes in preparation for calving (Grummer 1995). This imbalance is exacerbated in the firstpostpartum weeks, as energy requirements for milk production which are greater than the those met by dry matter intake (DMI) (Ingvartsen & Andersen 2000), leading to a negative energy balance (NEB) (Bobé et al. 2004) which may impair the immune and reproductive functions of dairy cows.

In order to meet this heightened energy demand, triglycerides are mobilised from adipose tissue, increasing the blood concentration of free fatty acids (Herdt 2000; Douglas et al. 2007); which may lead to re-synthesis and accumulation of triglycerides in the liver (Bobé et al. 2004; Overton & Waldron 2004). This intense mobilisation of lipids during the transition period is associated with an increased susceptibility to disease, in dairy cows, such a way that 90% of metabolic diseases in dairy cattle occurs during the first four days of lactation (Ingvartsen 2006). Evidences suggest that altered immunocompetence around parturition may be partly explained by increased non-esterified fatty acids (NEFA) blood concentrations (Contreras et al. 2012), compromised liver function (Bertoni et al. 2008) and altered proliferation and synthesis of cytokines by blood mononuclear cells (Lacetera et al. 2004). Consequently, the liver alters the synthesis of some metabolites, including acute phase proteins (APP), classified as positive (+APP), such as haptoglobin (HP), or negative (−APP), such as paraoxonase (PON) and albumin (ALB) (Bionaz et al. 2007).

Fat mobilisation can be confirmed by evaluating the body condition score (BCS), which is a practical
tool to access energy reserves and estimate the nutritional status of the animal, with a reasonable accuracy ($r^2 = 0.75–0.93$) (Wildman et al. 1982; Roche et al. 2009). There is a positive correlation between the high BCS at calving and postpartum BCS loss (Dechow et al. 2002). Studies indicate that animals which lost BCS before calving had a greater chance of developing disorders during the first postpartum days (Domecq et al. 1997; Gillund et al. 2001). In addition, animals with altered APP profile, indicated by the increased in HP levels and decrease of PON and ALB levels, around parturition also have higher risk of developing disorders in the first days postpartum (Ametaj et al. 2011). Based on those, the aim of this study was to determine how the changes in BCS during the pre-partum period affect the APP profile (HP, PON and ALB) and the energy metabolism markers (insulin (INS), glucose (GLU) and NEFA) during the transition period.

### Materials and methods

#### Experimental design

Thirty-five Holstein cows, starting their third or greater lactation with $7891 \pm 1184$ kg/305days average of milk production in their previous lactation were selected for this study. This study was approved by the Ethics and Animal Experimentation Committee from the Federal University of Pelotas, under the registration number 5273. The cows were maintained in a semi-extensive management system, which was based on pasture and mineral supplementation to achieve an anionic diet and pasture plus concentrate supplementation after each milking (Table 1), in a commercial farm in southern Brazil ($32^\circ$ 16’ S, $52^\circ$ 32’ E, seven metres above sea level). Cows were monitored from day -21 pre-partum to 30 days postpartum. Disorders, such as hypocalcaemia, mastitis and retained placenta, were monitored by rectal temperature, heart rate and respiration rate. Fifteen cows were removed from the trial, and all the analysis were performed with the healthy 20 remaining animals. Cows were milked twice a day (at 3:00 a.m. and 3:00 p.m.) and the milk yield (kg/cow) was recorded daily (ALPRO Tetra Laval Group©, Sweden), and averages were generated every five days from day 16 to day 41 postpartum. Body weight was measured weekly using a weighing platform (EziWeigh5 - TRU TEST®, Farm Tech Group Ljutomer, Slovenia).

BCS was evaluated on a weekly basis by three trained professionals, on a scale of 1 to 5 according to Wildman et al. (1982). Animals were divided into two groups based on a retrospective observation of BCS changes found from week -3 to week -1 related to calving. Thus, the group which lost BCS (LBC $=9$) showed a mean BCS decrease of $0.8 \pm 0.1$ (going from $3.7 \pm 0.1$ to $3.0 \pm 0.1$ points), and the group that gained BCS (GBC $n =11$) had a mean BCS increase of $0.25 \pm 0.2$ (going from $3.2 \pm 0.2$ to $3.4 \pm 0.2$ points).

#### Table 1. Ingredient and nutrient composition of prepartum and postpartum diets.

| Ingredients (feed) | Prepartum, kg/day | Postpartum, kg/day |
|-------------------|-------------------|-------------------|
| Native pasture    | Ad libitum        | –                 |
| Forage sorghum    | –                 | Ad libitum        |
| Hay-crop silage   | –                 | 15.00             |
| Wheat bran        | 0.50              | 1.50              |
| Soybean meal      | 1.00              | 2.40              |
| Rice bran         | 0.68              | 2.88              |
| Ground maize      | 1.05              | 3.00              |
| Sorghum grain     | 1.05              | 2.13              |
| Bicarbonate of soda| 0.40             | 0.11              |
| Urea              | –                 | 0.09              |
| Mineral supplement| 0.12              | 0.19              |
| Calctic limestone | 0.12              | 0.19              |
| Salt              | –                 | 0.002             |
| Protected fat     | 0.20              | –                 |

| Nutrient composition (dry matter basis) | Forage | Concentrate | Forage | Pre-dried | Concentrate |
|----------------------------------------|--------|-------------|--------|-----------|-------------|
| NDF                                    | 67.65  | 47.42       | 64.32  | 63.46     | 32.57       |
| ADF                                    | 51.37  | 13.56       | 41.74  | 45.75     | 13.14       |
| Crude protein                          | 16.51  | 15.61       | 9.84   | 8.88      | 14.92       |
| Fat                                    | 1.73   | 3.57        | 2.02   | 2.00      | 4.01        |
| Minerals                               | 9.23   | 8.90        | 9.99   | 8.84      | 9.02        |

NDF: neutral detergent fibre; ADF: acid detergent fibre. Analysis performed by methodology described by Van Soest and Wine (1967).
Blood sampling and analyses

Blood samples were taken from the coccygeal vein or artery on days -21, -14, -7 and -3, 0, 3, 6, 9, 23 and 30 relative to calving, collected after morning milking and before concentrate supplementation into vacuum tubes containing potassium fluoride (13 × 75 mm, 3 mL Vacuplast® - Zhejiang, China), no anticoagulant (16 × 100 mm, 1 mL Vacuplast® - Shandong, China) or EDTA (13 × 75 mm, 4 mL, BD Vacutainer® - Franklin Lakes, NJ).

Total leukocyte, haemoglobin, packed cell volume (PCV) and haemoglobin counts were performed in a Poch 100iVDiff automatic counter (Sysmex®, São Paulo, Brazil). Blood smears were stained with Romanowski Quick Panoptic (LaborClin®, Paraná, Brazil), and for the differential cell count 200 cells were identified through visualisation in an optical microscope (George et al. 2010). The neutrophil:lymphocyte ratio was calculated by dividing the neutrophil count by the lymphocyte count.

Plasma NEFA concentrations were obtained using a commercial kit (Wako NEFA-HR, WakoChemicals®, Richmond, VA), performed in accordance with the micro-method as described by Ballou et al. (2009) using a plate reader (Thermo Plate® TP-Reader, São Paulo, Brazil). The insulin concentrations were determined by a commercial ELISA kit (Ins-Easia®, DiaSource, Louvain-La-Neuve, Belgium), which presents 100% cross-reactivity in cattle (Beitinger et al. 2012) and a minimum detection limit of 1.13 µU/mL. PON activity was determined by an enzymatic technique using a commercial kit (ZeptoMetrix Corporation®, Buffalo, NY), which was coupled to a kinetic spectrophotometry system (T80 UV/VIS Spectrometer, PG Instruments Ltd., PTC-2 Peltier Temperature Controller, software UVWin80 v3.0.5, Wibtoft, England), HP concentrations were analysed by a colorimetric method as described by Jones and Mould (1984) and adapted by Schneider et al. (2013), absorbance was obtained using a plate reader (Thermo Plate® TP-Reader, São Paulo, Brazil). ALB and GLU concentrations were measured by a colorimetric method using a light visible spectrophotometer (Biospectro®, SP-220. Curitiba PR Brazil) through commercial kits (LabTest Diagnostica®, Lagoa Santa, MG, Brazil). The intra- and inter-assay coefficients of variation (CV) for NEFA, PON, HP, INS, ALB and GLU were lower than 12%.

Statistical analysis

All statistical analyses were performed using 9.0 SAS software (SAS® Institute Inc., Cary, NC, 2004). Plasma concentrations of metabolites (GLU, INS, HP, PON, ALB and NEFA), blood cell count (erythrocytes, haematocrit and haemoglobin levels, total leukocytes, neutrophils, lymphocytes, eosinophils and monocytes), live weight and BCS were evaluated through analysis of variance (ANOVA) with MIXED procedure to assess the main effect of group, time (in days) and their interaction (Littell et al. 1998). The chi-square test was used to calculate the neutrophil:lymphocyte ratio (Yazici et al. 2013), and the data were qualitatively categorised as >1 = 0 and <1 = 1 values. p < .05 values were considered significant and tendencies at p ≤ .10. Data analyses were performed separately for pre-partum and postpartum periods.

Results

The groups did not show differences in body condition in the overall pre-partum period, only at day -21 pre-partum, the animals showed different BCS (LBC 3.7 ± 0.1 vs. GBC 3.2 ± 0.2, p < .05). Also, no differences between groups were observed at calving day (LBC 2.6 ± 0.1 vs. GBC 2.8 ± 0.1, p > .05). However, during the postpartum period, the BCS was higher in the GBC group (p > .05, Figure 1A). Body weight did not show
differences between groups at day -21 (LBC 670 ± 30 vs. GBC 600 ± 26 kg), and had a tendency ($p = .09$) to be higher in the postpartum period for GBC animals (GBC 610 ± 18 vs. LBC 566 ± 18 kg) (Figure 1B).

The HP serum concentration was higher in the LBC group in the pre-partum ($p < .004$) and postpartum ($p < .004$) (Figure 2A). The PON activity showed no differences between groups in the pre-partum, however, it was lower ($p < .05$) in LBC animals in the postpartum (Figure 2B). In the LBC group, the concentration of ALB was lower in the pre-partum ($p = .03$) and post-partum ($p = .002$) periods (Figure 2C). No differences were observed between groups, either in the pre-partum or postpartum periods ($p > .05$), in the markers related to energy metabolism (NEFA, INS and GLU, INS). The LBC group also had lower milk production.
high NEFA concentration plays a crucial role impairing liver and immune function in animals (Ingvartsen et al. 2003). However, no differences were observed in NEFA concentrations between groups, suggesting that the APP alteration observed in the group that lost body condition may be more related to an increased

Discussion

The results of this study indicate that the group of cows that lost body condition in the pre-partum period showed an exacerbated inflammatory condition when compared to the group that gained body condition. This was evident by the reduction of ALB concentration, concomitantly with increased HP concentrations. During the early postpartum period, dairy cows experience a pro-inflammatory condition (Sordillo et al. 2009), as evidenced by a reduction in –APP (PON and ALB) and an increase in +APP (HP). These changes are enhanced in dairy cows with uterine infection (Schneider et al. 2013), metritis (Bionaz et al. 2007), impaired ovulatory function (Krause et al. 2014). Cows with experimental Escherichia coli mastitis also have increased serum amyloid A (+APP) (Khatun et al. 2013). In addition, this study also suggests that alteration of APP may be associated with changes in body condition during the pre-partum period, being indicative of some possible clinical or subclinical disease in the postpartum.

The changes observed in the APP profile in the group of animals that lost body condition may be related to an impaired liver function. The trend for higher HP concentrations observed in the pre-partum and the enhanced concentration in the postpartum, are consistent with impaired hepatic function, as demonstrated by Yoshino et al. (1992) and Ametaj et al. (2005), when evaluating cows with fatty liver. Bossaert et al. (2012) observed that animals with increased liver function had lower PON activity on the eighth day after calving, similarly to the results obtained in our study. Likewise, Farid et al. (2013) demonstrated that the evaluation of PON activity contributes with a more accurate hepatic steatosis diagnosis. The ALB data show differences between the groups, as it is also considered a nutritional status marker. This suggests a reduced dry matter intake, especially in the pre-partum period, by the animals that lost body condition (Bell et al. 2000). In addition, low-ALB concentration is also connected to an impairment of liver function (Trevisi et al. 2010) and may evidence an excessive NEFA infiltration into the liver (Bobè et al. 2004). The high NEFA concentration plays a crucial role impairing liver and immune function in animals (Ingvartsen et al. 2003). However, no differences were observed in NEFA concentrations between groups, suggesting that the APP alteration observed in the group that lost body condition may be more related to an increased

(3 kg/cow/day, \(p < .03\)) compared to the group that gained body condition before parturition (LBC 24.2 ± 0.8 vs. GBC 27.2 ± 1.9 kg/cow/day, Figure 3).

A lower erythrocyte count was observed in the pre-partum period for the LBC group (LBC 5.6 ± 0.1 vs. GBC 6.2 ± 0.1 \(×10^{6}\)/mL; \(p < .03\)). LBC animals had a higher monocyte count in the pre-partum (LBC 1121 ± 210 vs. GBC 708 ± 157; \(p < .05\)) and postpartum period (LBC 663 ± 93 vs. GBC 331 ± 103; \(p < .03\)). LBC group also had a greater number of animals with the N:L ratio > 1 in the pre-partum (LBC 41.6% vs. GBC 13.7%; \(p < .03\)) and postpartum periods (LBC 37% vs. GBC 13%; \(p < .02\)). Results of correlation analyses are summarised in Table 2. The LBC group showed a negative correlation between BCS and NEFA \((r = –.61, p = .05)\), as well as between PON and HP \((r = –.49, p = .05)\) and a positive correlation between NEFA and ALB \((r = .53, p = .05)\) in the pre-partum period. In the postpartum period, the LBC group showed a negative correlation between BCS and NEFA \((r = –.25, p = .06)\), between ALB and HP \((r = –.36, p = .05)\) and between PON and HP \((r = –.45, p = .05)\), and a positive correlation between HP and ALB \((r = .42, p = .05)\). The GBC group showed a negative correlation between BCS and INS \((r = –.46, p = .05)\), as well as between BCS and GLU \((r = –.43, p = .05)\) and a positive correlation between BCS and PON \((r = .62, p = .05)\), BCS and ALB \((r = .52, p = .05)\) and between ALB and PON \((r = .43, p = .05)\) in the pre-partum period. In the postpartum period, GBC group showed a negative correlation between INS and NEFA \((r = –.32, p = .05)\), BCS and milk production \((r = –.41, p = .05)\), milk production and HP \((r = –.31, p = .06)\) and a positive correlation between PON and ALB \((r = .25, p = .06)\).
production of inflammatory cytokines, which is in agreement to observations made by Bionaz et al. (2007).

Another finding, consistent with a greater inflammatory response in the group that lost body condition, is the higher number of monocytes. These cells are innate immunity mediators, they act mediating phagocytosis and are stimulated by pro-inflammatory cytokines secretion, as IL6 and IL1, during the inflammatory response (Sordillo et al. 1995). Boyle et al. (2012) observed an increased number of monocytes and macrophages compared with physiological parameter, during the transition period in dairy cows, which could contribute to a greater incidence of subclinical disease during this period, such as sub-acute ruminal acidosis (SARA), mastitis or hypocalcaemia. The evidence of higher pro-inflammatory status of the animals that lost body condition is reinforced by the greater number of animals with the N:L > 1, as this ratio is considered an indicative of inflammation in ruminants when it exceeds the value of 1 (George et al. 2010). Therefore, it is likely that the animals which lose body condition have a greater chance of developing diseases during the peripartum period. Nevertheless, in our study, animals with clinical signs of disease were excluded from the trial, providing evidence that this relation may be relevant even at a sub-clinical level.

A lower milk production (3 kg/cow/day) was observed in animals that lost body condition during the pre-partum period. Bertoni et al. (2008) reported the reduction of the fertility and milk production in cows with high genetic merit which seem to be more associated with inflammatory conditions than to the actual manifestation of clinical disease, which is in agreement with this study, as animals that demonstrated a greater pro-inflammatory state had lower milk production.

The correlation results demonstrated a close relationship between metabolites during the pre-partum period in the group that gained body condition. As demonstrated by the positive correlation between ALB and BCS, and BCS and PON, could indicate a better immune and nutritional status in the animals that gained compared to animals that lost body condition. In the postpartum period, this relation is

### Table 2. Correlation between metabolites and milk production in cows that gain body condition in prepartum (GBC) and in cows that lost body condition (LBC).

|                | BCS | NEFA | INS | ALB | HAP | PON | MILK | GLU |
|----------------|-----|------|-----|-----|-----|-----|------|-----|
| **Prepartum LBC** |     |      |     |     |     |     |      |     |
| BCS            |     |      |     |     |     |     |      |     |
| NEFA           |     |      |     |     |     |     |      |     |
| INS            |     |      |     |     |     |     |      |     |
| ALB            |     |      |     |     |     |     |      |     |
| HAP            |     |      |     |     |     |     |      |     |
| PON            |     |      |     |     |     |     |      |     |
| GLU            |     |      |     |     |     |     |      |     |
| **Prepartum GBC** |     |      |     |     |     |     |      |     |
| BCS            |     |      |     |     |     |     |      |     |
| NEFA           |     |      |     |     |     |     |      |     |
| INS            |     |      |     |     |     |     |      |     |
| ALB            |     |      |     |     |     |     |      |     |
| HAP            |     |      |     |     |     |     |      |     |
| PON            |     |      |     |     |     |     |      |     |
| GLU            |     |      |     |     |     |     |      |     |
| **Postpartum LBC** |     |      |     |     |     |     |      |     |
| BCS            |     |      |     |     |     |     |      |     |
| NEFA           |     |      |     |     |     |     |      |     |
| INS            |     |      |     |     |     |     |      |     |
| ALB            |     |      |     |     |     |     |      |     |
| HAP            |     |      |     |     |     |     |      |     |
| PON            |     |      |     |     |     |     |      |     |
| MILK           |     |      |     |     |     |     |      |     |
| GLU            |     |      |     |     |     |     |      |     |
| **Postpartum GBC** |     |      |     |     |     |     |      |     |
| BCS            |     |      |     |     |     |     |      |     |
| NEFA           |     |      |     |     |     |     |      |     |
| INS            |     |      |     |     |     |     |      |     |
| ALB            |     |      |     |     |     |     |      |     |
| HAP            |     |      |     |     |     |     |      |     |
| PON            |     |      |     |     |     |     |      |     |
| MILK           |     |      |     |     |     |     |      |     |
| GLU            |     |      |     |     |     |     |      |     |

Milk production was only included in the postpartum model. Correlation is significant indicated *p* = .05, "p" = .06, ns (not significant) =p <.10. BCS: body condition score; GLU: glucose; NEFA: non-esterified fatty acids; INS: insulin; ALB: albumin; PON: paraoxonase; HP: haptoglobin; MILK: production of milk.
highlighted in animals that lost body condition, this revealed an imbalance both in the energy and immune systems, observed by greater variance between positive and negative acute phase proteins and possible changes in the health status of these animals. Huzzey et al. (2011) and Tóthová et al. (2008) observed a positive correlation between NEFA and HP, demonstrating an association between the mobilisation of body fat and inflammation. This correlation was not be observed in this study, probably due lower metabolic demands for a moderate milk production level, which did not increase NEFA concentration.

The negative correlations between NEFA and BCS in the pre-partum and postpartum in the group that lost body condition, is an indication that these animals experienced a more intense negative energy balance compared to the animals that gained body condition, thus likely to probable occurrence of subclinical and clinical diseases. Regarding the negative correlation between BCS, NEFA and globulin, our findings show similarities to which were obtained by Cavestany et al. (2005), developed under similar management conditions to the ones in this study. In the group that lost body condition, the negative correlation between HP and PON and the positive correlation between PON and ALB were similar to those observed by Schneider et al. (2013). Thus, animals with more severe inflammatory conditions (indicated by greater N:L ratio in the LBC group) have a strong correlation between +APP or –APP.

Therefore, the importance of adequate BCS management during the pre-partum is evidenced. Several strategies can be used in order to prevent pre-partum BCS decrease, as monitoring the changes in BCS and assessment of acute phase proteins production. Cows can benefit of these management of BCS control by increasing milk production, and reducing the risk of developing metabolic diseases.

Conclusions

The pre-partum loss of body condition altered the acute phase proteins profile in the postpartum, decreasing paraoxonase activity and albumin concentration, in addition to increasing the lymphocyte:neutrophil ratio and reducing milk production by 3 kg/cow/day. Those assessed energy markers did not demonstrate to be affected by body condition score changes in this study. These findings demonstrate the importance monitoring BCS changes and the relevance of the acute phase proteins concentrations during the transition period of dairy cow.

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Disclosure statement

All authors declare that they have no conflicts of interest.

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