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Reduction of liver function delays resumption of postpartum ovarian activity and alters the synthesis of acute phase proteins in dairy cows

Paula Montagner *, Ana Rita Tavares Krause, Elizabeth Schwegler, Marina Menoncin Weschenfelder, Viviane Rohrig Rabassa, Augusto Schneider, Rubens Alves Pereira, Cássio Cassal Brauner, Francisco Augusto Burkert Del Pino, Fernanda Medeiros Gonçalves, Marcio Nunes Corrêa

Federal University of Pelotas, Brazil, CEP 96010-900 Pelotas, RS, Brazil
Núcleo de Pesquisa, Ensino e Extensão em Pecuária — Livestock Research, Education and Extension Center (NUPEEC – http://www.ufpel.edu.br/nupeec), CEP 96010-900 Pelotas, RS, Brazil

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ABSTRACT

The aim of this study was to evaluate the concentration of acute phase proteins, milk production, and resumption of postpartum ovarian activity of clinically healthy dairy cows in a semi-extensive system with different Liver Functionality Index (LFI) values. The animals were divided into two groups: Low LFI (LLFI: −7 to −12; n: 10) and High LFI (HLFI: −7 to −4; n: 10). Animals with LLFI had lower paraoxonase activity and lower albumin concentration in the pre- and postpartum periods ($P < 0.05$), higher non-esterified fatty acids prepartum ($P < 0.005$), and higher haptoglobin concentration postpartum ($P < 0.01$). The LLFI group showed lower resumption of ovarian activity until 44 days postpartum (29%; $P < 0.05$) than HLFI (86%). Milk production did not differ between groups. Therefore, this study suggests that the LFI is an important biomarker of synthesis of acute phase proteins and the first ovulation interval, and it can be used to improve the production and reproductive performance.

1. Introduction

The transition period from three weeks prepartum to three weeks after calving (Mulligan and Doherty, 2008) is a critical phase for dairy cows, since it is characterized by a high incidence of metabolic and infectious diseases, and reproductive problems (Goff and Horst, 1997). It is also marked by inflammatory conditions due to an increase in inflammatory cytokine synthesis as result of adipose tissue mobilization (Contreras and Sordillo, 2011).

The main effects of cytokines, during inflammation, are in the partitioning of nutrients (Elsasser et al., 2000), and a decrease in the dry matter intake (DMI) (Johnson and Finck, 2001) and reproductive activity (Butler, 2000). In the liver, cytokines are primarily responsible for promoting an inflammatory response and inducing an increase in positive acute phase proteins (APP), such as haptoglobin (HP), and for decreasing the production of negative APP, such as paraoxonase (PON) and albumin (ALB) (Fleck, 1989; Bionaz et al., 2007).

The search for accurate markers to define immunity, inflammatory, and metabolic conditions in dairy cows is a constant concern. One way to characterize the extent and severity of inflammation on protein synthesis is using the Liver Functionality Index (LFI), which takes into account changes in ALB synthesis, cholesterol (CHOL), and bilirubin (BIL) in the first month of lactation (Bertoni and Trevisi, 2013). In intensive systems, cows with low LFI show lower DMI, lower milk production, and higher incidence of disease (Trevisi et al., 2012). However, these results are limited to intensive systems, there is no evidence if cows in semi-extensive systems show the same performance, since animal challenges are different, such as heat stress, and walking to milking and grazing. Thus, the aim of this study was to evaluate changes in APP concentration, milk production, and resumption of ovarian activity in clinically healthy dairy cows in a semi-extensive system with LFI.

2. Materials and methods

2.1. Experimental design

Thirty-seven pluriparous dairy Holstein cows from a commercial farm in southern Brazil (32° 16′ S, 52° 32′ L) were used in the trial. This study was approved by the Ethics and Animal Experimentation Committee from the Federal University of Pelotas, under the registration number 5273. Animals were kept in a semi-extensive system, had three calves or more, 7891 ± 1184 kg/305 days of milk production average, and they were kept in the same nutritional conditions (Table 1) from 21 days prepartum to day 30 postpartum. Cows were monitored daily (rectal temperature, heart rate and respiration rate) during the experiment and were excluded in cases of disease (mastitis, hypocalcemia,

* Corresponding author.
E-mail address: paulamontagner@gmail.com (P. Montagner).

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

	Prepartum (kg/day)	Postpartum (kg/day)
Ingredients		
Native pasture	*Ad libitum*	–
Forage Sorghum	–	*Ad libitum*
Pre-dried	–	15
Wheat bran	0.5	1.5
Soybean meal	1.0	2.4
Rice bran	0.68	2.88
ground corn	1.05	3.0
Sorghum grain	1.05	2.13
Bicarbonate of soda	0.4	0.11
Urea	–	0.09
Mineral Supplement	–	0.19
Calcitic limestone	0.12	0.19
Salt	–	0.002
Protected fat	0.2	–

Nutrient composition (dry matter basis)

	Prepartum (%)		Postpartum (%)		
	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	9.16	15.61	9.84	8.88	14.92
Fat	1.73	3.57	2.02	2.00	4.01
Minerals	9.23	8.9	9.99	8.84	9.02

Estimated based on diet analyses in National Research Council (NRC) software (2001). Neutral detergent fiber (NDF) and acid detergent fiber (ADF).

retained placenta). Twenty cows that did not have a history of illness during the experiment were used for the laboratory analysis.

Cows were milked twice a day (3:00 AM and 3:00 PM) and milk yield (kg/cow) was recorded daily by software (ALPRO Tetra Laval Group®, Sweden). Every five days (16 days to 60 days in milk) has generated a mean of the total milk produced. Body weight was measured weekly using a weighing platform (EziWeigh5, TRU Test®, Farm Tech Group Ljutomer, Slovenia). Body condition score (BCS) was determined weekly by three evaluators on a scale from 1 to 5 according to Wildman et al. (1982).

The animals were divided into two groups based on the LFI (Trevisi et al., 2012; Bertoni and Trevisi, 2013), where animals with Low LFI (−7 to −12; $n = 10$, LLFI) and animals with High LFI (−4 to −7; $n = 10$, HLFI). The LFI includes concentrations of albumin, lipoproteins (indirectly measured as total cholesterol), and bilirubin (as indirect measure of the enzymes synthesized by the liver, which also coordinate bilirubin clearance). LFI measures the relevant changes in concentrations between 3 and 28 DIM.

2.2. Blood sampling and analyses

Blood was were collected on days −21, −14, −7 and −3 prepartum, 0, 3, 6, 9, 23 and 30 postpartum, after milking through the coccygeal complex in 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, USA).

PON activity was determined by an enzymatic technique using a commercial kit (ZeptoMetrix Corporation®, Buffalo, NY, USA). The HP concentration was analyzed by a colorimetric method described by Jones and Mould (1984) and adapted by Schneider et al. (2013). Absorbance was obtained using a plate reader (Thermo Plate® TP-Reader, Sao Paulo, Brazil). Plasma non-esterified fatty acids (NEFA) concentration was obtained using a commercial kit (Wako NEFA-HR, WakoChemicals®, Richmond, USA), 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 concentration was determined by a commercial ELISA kit (Ins-Easia®, DiaSource, Louvain-La-Neuve, Belgium), which presents 100% cross-reactivity in cattle (Beitinger at al., 2012) and a minimum detection limit of 1.13 μlU/mL. Albumin (ALB), aspartate aminotransferase (AST) gamma-glutaryl transferase (GGT), glucose (GLU), bilirubin (BIL), and cholesterol (CHOL) concentrations were measured in plasma using a light-visible spectrophotometer (Biospectro®, SP-220, Curitiba PR Brazil) using commercial kits (LabTest Diagnostica®, Lagoa Santa, MG, Brazil). All these analyses was realized in the days −21, −14, −7 and −3 prepartum, 0, 3, 6, 9, 23 and 30 postpartum.

Serum progesterone (P4) was analyzed on days 16, 23, 30, 37, and 44 postpartum using a commercial radioimmunoassay kit (Coat-A Count®, Diagnostic Products Corporation, Los Angeles, USA) previously described by Burke et al. (2003). Cows that had P4 concentration higher than 1 ng/mL in two consecutive samples were considered ovulatory, and cows that did not resume ovarian activity (no increase in P4 above 1 ng/mL during same period) were considered anovulatory (Stevenson and Britt, 1979).

The intra- and inter-assay coefficients of variation (CV) for NEFA, PON, HP, INS, ALB, AST, GGT, PG, and GLU were lower than 12%.

2.3. Statistical analysis

All statistical analyses were performed using SAS 9.0 software (SAS® Institute Inc., Cary, NC, USA, 2004). Metabolite concentrations (NEFA, PON, HP, INS, ALB, AST, GGT, CHOL, BIL, and GLU), body weight, and BCS were evaluated through analysis of variance (ANOVA) with a MIXED procedure to assess the main effect of group, time (in days), and their interaction (Littell et al., 1998). The statistical model CATMOD (Categorical Data Analysis Procedures) from SAS was used for analysis of categorical data ovulation. $P < 0.05$ was considered significant, and data analyses were done separately for pre- and postpartum periods.

3. Results

In the first 7 weeks of lactation a lower proportion of 29% (3/10) in the LLFI group had normal ovarian activity resumption in comparison to 86% (9/10; $P < 0.05$) of HLFI.

The HP concentration prepartum did not differ between groups ($P > 0.05$). The LLFI group had higher HP concentrations in the postpartum period ($P < 0.01$; Fig. 1A). In LLFI group, PON activity showed a trend towards a decrease ($P = 0.07$) in the prepartum period, in postpartum period both groups showed a decrease in PON levels. However, in LLFI group this decrease was more significant (86.38 ± 3.84 KU/l, $P < 0.001$) compared to group HLFI (113.91 ± 3.87 KU/L; Fig. 1B) and showed a delayed increase compared to group HLFI. The levels of ALB were lower in LLFI pre- ($P < 0.02$) and postpartum ($P < 0.001$; Fig. 1C) periods than HLLFI. NEFA concentration was higher in the LLFI group during the prepartum period ($P < 0.005$; Fig. 2A) than HLFI, but no difference was observed in the postpartum period ($P > 0.05$). The INS shown a trend towards reduction ($P = 0.08$) in the LLFI treatment in the prepartum period (9.93 ± 1.52 U/mL LLFI vs. 13.76 ± 1.58 U/mL HLFI), but there was no effect in the postpartum period ($P = 0.05$; Fig. 2B). Blood levels of liver enzymes did not differ between the groups during the prepartum period, but postpartum animals with LLFI had higher levels of GGT ($P < 0.03$) and tended to have higher levels of AST ($P < 0.07$).

Bilirubin values analyzed in the days 3 and 28 to compose the LFI calculus, the results showed a difference ($P > 0.05$) in the interaction group * time in both moments. The LLIF groups (7.49 ± 0.8 mg/dL) showed lower values compared to HLFI (9.51 ± 0.8 mg/dL) on the day 3 postpartum; but on the 28 day postpartum the LLFI the group (8.87 ± 0.8 mg/dL) had higher values compared to HLFI (6.91 ± 0.8 mg/dL, $P > 0.05$). Cholesterol values also analyzed on 3 and 28 showed differences between groups ($P > 0.05$) and a tendency in group * time ($P < 0.07$). The LLFI group (day 3, 1.75 ± 00.15 mg/dL