DOI: 10.5433/1679-0359.2017v38n5p3371

Paraoxonase activity in the serum of peripartum dairy cows with different placental lactogen concentrations

Atividade sérica de Paraoxanase durante o periparto de vacas leiteiras com diferentes concentrações de Lactogênio Placentário

Marina Menoncin Weschenfelder Rohenkohl[1]; Matheus Gomes Lopes[1]*; Antônio Amaral Barbosa[2]; Ana Rita Tavares Krause[3]; Paula Montagner[2]; Elizabeth Schwegler[4]; Cássio Cassal Brauner[5]; Augusto Schneider[5]; Eduardo Schmitt[5]; Francisco Augusto Burkert Del Pino[6]; Marcio Nunes Corrêa[7]

Abstract

The action of the bovine placental lactogen (bPL) hormone on maternal metabolism is still poorly known. Some markers, such as the acute phase protein paraoxonase (PON1), are used as indicators of liver function and help to determine the metabolic condition during the transition period in dairy cows. The aim of this study was to evaluate the activity of paraoxonase (PON1) in the serum of peripartum dairy cows with different levels of bPL. Based on the plasma bPL concentration, 18 cows were divided equally into three groups: LOW (< 2,68 ng bPL mL^{-1}), MEDIUM (2,68–2,80 ng bPL mL^{-1}), and HIGH (> 2,80 ng bPL mL^{-1}). The experiment was conducted between 21 days prepartum and 28 days postpartum. Serum samples were collected during the experiment for the determination of bPL concentrations and PON1 activity. The bPL concentration was significantly different between the experimental groups ($P \leq 0,0001$) and the days of serum collection ($P \leq 0,0001$). In the prepartum dairy cows, the PON1 levels were different between the groups ($P \leq 0,05$) and the days of serum collection ($P \leq 0,05$). Cows with high bPL concentration had lower serum PON1 activity ($P \leq 0,05$), while cows with low hormone levels had higher enzyme activity ($P \leq 0,05$). In the postpartum period, there was a significant difference between the days of serum collection ($P \leq 0,0001$) and the interaction between groups and collections ($P \leq 0,01$). The group with high concentrations of bPL had lower levels of PON1 ($P \leq 0,01$), while the group with low bPL maintained higher concentrations of PON1 ($P \leq 0,01$). It was concluded that the cows with higher concentrations of bPL in the prepartum period present a reduction in the serum activity of the PON1 enzyme during the peripartum period.
Key words: Hormones. Inflammation. Proteins. Transition period.

Resumo

A ação do hormônio Lactogênio Placentário Bovino (bLP) no metabolismo materno ainda é pouco conhecida. Alguns marcadores, como a proteína de fase aguda Paraoxanase (PON1), são utilizados como indicadores da função hepática auxiliando na determinação da condição metabólica no período

[1] Discentes de Mestrado, Universidade Federal de Pelotas, UFPEL, Pelotas, RS, Brasil. E-mail: marinamwrohenkohl@gmail.com; matheus_p5@hotmail.com
[2] Discente de Doutorado, UFPEL, Pelotas, RS, Brasil. Email: antoniobarbosa.vet@hotmail.com; pmontagner@hotmail.com
[3] Discente de Doutorado, University of Saskatchewan, USASK, Saskatoon, SK, Canadá. E-mail: anaritatkrause@gmail.com
[4] Profª Adjunto, Instituto Federal Catarinense, IFC, Araquari, SC, Brasil. E-mail: bethveterinaria@gmail.com
[5] Profs. Adjunto, UFPEL, Pelotas, RS, Brasil. E-mail: cassiocb@gmail.com; augustoschneider@gmail.com; schmitt.edu@gmail.com
[6] Prof. Titular, UFPEL, Pelotas, RS, Brasil. E-mail: fabdelpino@gmail.com
[7] Prof. Associado, UFPEL, Pelotas, RS, Brasil. E-mail: marcio.nunescorrea@gmail.com
* Author for correspondence

Received: Apr. 24, 2016 Approved: July 05, 2017

de transição em vacas leiteiras. O objetivo deste trabalho foi avaliar a atividade sérica de PON1 durante o periparto em vacas leiteiras com diferentes níveis de bLP. As vacas foram divididas em três grupos de acordo com as concentrações plasmáticas de bLP, em que BAIXO < 2,68 ng mL^{-1} (n=6), MÉDIO > 2,68 ng mL^{-1} e < 2,80 ng mL^{-1} (n=6) e ALTO > 2,80 ng mL^{-1} (n=6). O período experimental ocorreu entre os 21 dias pré-parto e 28 dias pós-parto. Amostras de soro foram coletadas para a determinação das concentrações de bLP e atividade sérica de PON1. Houve diferença entre os três grupos ($P \leq 0{,}0001$) de acordo com as concentrações de bLP, assim como entre os dias coletados ($P \leq 0{,}0001$). No pré-parto, os níveis de PON1 apresentaram diferença entre grupos ($P \leq 0{,}05$) e coletas ($P \leq 0{,}05$). Vacas com alta concentração de bLP apresentaram menor atividade sérica de PON1 ($P \leq 0{,}05$), enquanto vacas com baixos níveis do hormônio obtiveram maior atividade da enzima ($P \leq 0{,}05$). No pós-parto não houve diferença entre grupos ($P \geq 0{,}10$), houve diferença entre os dias coletados ($P \leq 0{,}0001$) e interação entre grupos e coletas ($P \leq 0{,}01$). O grupo com altas concentrações de bLP apresentou menores níveis de PON1 ($P \leq 0{,}01$), enquanto vacas do grupo com baixo bLP mantiveram maiores concentrações de PON1 ($P \leq 0{,}01$). Conclui-se que, vacas com maiores concentrações de bLP no período pré-parto apresentam redução na atividade sérica da enzima PON1 durante o período periparto.

Palavras-chave: Hormônios. Inflamação. Período de transição. Proteínas.

The transition period, which comprises the three weeks that precede the partum and extends until the first three weeks into lactation, is associated with a significant variation in the presence and concentration of metabolites in the serum such as the acute phase proteins (APPs). Paraoxonase (PON1), a negative APP, is synthesized in the liver and released into the bloodstream in response to the cytokines produced during inflammation. PON1 acts as an index of liver function during the inflammatory process and assists in the precocious diagnosis of some diseases (BIONAZ et al., 2007). A higher rate of lipolysis, associated with the transition period, can downregulate PON1 synthesis. Providing a pro-inflammatory ambient causes a release of adipokines that have an influence in the enzymatic synthesis (KOSKINEN et al., 2011). Alvarez (2008) reported that the bPL concentrations can vary according to the corporal condition; however, this was not observed in this study because of the homogeneity of the selected cows (HIGH, BCS=2,9±0,05; MEDIUM, BCS=3,1±0,03, and LOW, BCS=2,9±0,05). In view of the diverse diseases that occur in dairy cows during the peripartum period, a precocious identification of the infection in its subclinical phase is important for adopting appropriate treatment measures.

The placental lactogen (PL) is a peptide hormone that is synthesized in the placental bi-nucleated trophoblastic cells of ruminants and belongs to the family of prolactin (PRL) and growth hormone (GH). PL plays a significant role in determining the physiological and morphological changes associated with the gestation period. The PL, in conjunction with other hormones, controls mammogenesis and placental histotrophic functions to facilitate the passage of nutrients of maternal origin across the placenta (ENDERS; WELSH, 1993). The bovine PL (bPL) begins to appear in the maternal circulation from the 40th day of gestation, and its concentration continues to increase until it reaches the maximum in the late gestational trimester, returning to undetectable levels soon after the partum (BYATT et al., 1992). In dairy cows, the bPL helps in lactation through the development of alveoli in the mammary glands. Despite the importance of bPL during transition, the mechanistic underpinnings of its role in the development of mammary glands, fetal growth, and maternal metabolism are poorly understood. Therefore, an understanding of the role of various serum metabolites and their interactions during the transition stage is essential to ensure effective disease intervention and lactation during this critical stage. To address the aforementioned knowledge gap, we evaluated the PON1 serum activity in transition cows that were grouped by their serum bPL concentration.

The experiment was conducted in a commercial dairy farm localized in soul of Brazil (32,8°16′S, 52,8°32′L) and approved by the Ethics Commission on Animal Use at the Federal University of Pelotas–RS (registry number 10017). The animals were selected on the basis of number of lactations, average milk yield in 305 days of the previous lactation, and negative history of diseases in the last productive year. Eighteen multiparous Holstein cows with three lactations, averaging a milk yield of 29,43±7,94 L for 46 days in milk (DIM), and 644,55±93,67 kg average weight were selected for the experiment.

The experiment was conducted between 21 days prepartum and 28 days postpartum. The cows were housed in a semi-extensive system, allowed ad libitum pasture feeding, and given concentrated supplements after each milking. The complete nutritional management of the cows was conducted as described by Montagner et al. (2016). The plasma bPL levels were estimated, and the cows were categorized into three groups based on their serum bPL levels: LOW (<2,68 ng bPL mL^{-1}), MEDIUM (2,68–2,80 ng bPL mL^{-1}), and HIGH (>2,80 ng bPL mL^{-1}).

The arteriovenous-coccygeal complex was punctured using a vacutainer system with 25×0,8 mm needles for collecting blood samples in blood collection tubes with clot activator. During the prepartum period, the samples were collected on 21, 14, 7, 5, 3, 1, and 0 days. During the postpartum period, the samples were collected on 3, 6, 9, 14, 21, and 28 days. The blood samples were centrifuged at 400 × *g* for 15 min, and the serum was collected and stored in 1,5 mL microtubes at -80°C until analysis.

The serum bPL concentrations during the prepartum period were determined using ELISA (PL ELISA Kit, Novate In Biosciences, Cambridge, MA, USA) at the Mammalian Nutriphysiogenomics Laboratory of the Animal Science Department in Illinois University (Urbana, IL, USA). The determination of PON1 serum activity was determined according to the method described by Browne et al. (2007) at the Clinical Biochemical Laboratory of Nucleus of Research, Education and Extension in Livestock of the Federal University of Pelotas (NUPEEC-UFPEL, Pelotas, RS, BR).

Statistical analysis was conducted using the SAS® software (SAS Institute Inc., Cary, NC, USA). The values for bPL were subjected to $\log_{10}$ transformation and normalized across non-linear regression by using Prism® 5.0 software (GraphPad Software Inc., San Diego, CA, USA). A mixed model analysis of variance was conducted to compare the difference between groups, days, and the interaction among groups and days. Differences were considered statistically significant for P-values ≤0,05.

The concentration of bPL was significantly different between the three experimental groups (LOW=2,42±0,24 ng mL^{-1}; MEDIUM=2,72±0,03 ng mL^{-1}; HIGH=3,26±0,27 ng mL^{-1}; P≤0,0001) and diurnally (P≤0,0001). Hossner et al. (1997) reported that the bPL concentrations in the maternal serum was less than 5 ng mL^{-1} and can be detected after the first gestation month. At the end of gestational period, the increase in the fetal cortisol reduces the serum bPL levels drastically by inhibiting the release of hormone from the storage granules of binucleated cells (ALVAREZ-OXILEY et al., 2008). In previous studies, the maximum concentration of LP did not exceed 3 ng mL^{-1} in the last three weeks that preceded parturition; this concentration was much lower than the levels of LP in other animals such as sheep, goats, humans, and small rodents (WALLACE, 1993).

To our knowledge, this is the first study to show the relationship between various levels of bPL and the activity of PON1 in the serum. During the peripartum, the serum levels of PON1 was different between groups (P≤0,05) and among days (P≤0,05); however, there was no statistical interaction between groups and days (P≥0,10). The cows with high bPL concentration had lower PON1 serum activity (P≤0,05), while the cows with low bPL concentration showed higher activity (P≤0,05). Previous studies on rodents have shown that higher GH levels can downregulate the *PON1* gene expression, resulting in its reduced serum activity, indicating a compensatory action between these metabolites (CHENG; KLAASSEN, 2012). The bPL was the first hormone recognized to have biological effects similar to those of PRL, but in some species bPL can demonstrate biological activities analogous to both PRL and GH (BYATT et al., 1992). The bPL in bovines show interaction with the PRL (PRL-R) and GH receptors (GH-R). Through a double agonist action for PRL-R and GH-R, participates in the same signaling pathways, suggesting that both have the same receptors system (SOARES et al., 2004). The homology of the molecular structure of bPL with that of GH (23%) and PROL (50%) (BYATT et al., 1992) suggests that the actuation of bPL in the somatotropic axis can influence the hepatic synthesis of APPs, such as PON1.

In the present study, the evaluation of PON1 during the postpartum period did not differ between groups (P≥0,10); however, there existed a significant difference in the levels of PON1 between the days of serum collection (P≤0,0001) and a statistical interaction between groups and days (P≤0,01). Low concentrations of PON1 remained in the serum of the group with high bPL concentrations after parturition (P≤0,01). While the intermediary group presented a peak in the PON1 levels out of standard in the second week postpartum (Day 9), the low bPL group maintained increasing concentrations of PON1 (P≤0,01) (Figure 1). The first days of milking are characterized by challenges because of physiological changes associated with the transition of a cow from pregnant to lactating stages. The histotrophic activity of bPL favoring the deviation of maternal nutrients for fetal growth (ENDERS; WELSH, 1993) over the obtained results seems to reflect in the recent postpartum. Despite the release of adipokines and lipomobilization during postpartum, the cows with high bPL levels demonstrated an intense decline in their energy reserves to meet the requirements of fetal metabolism. Lack of energetic reserves can be detrimental to a cow when facing adversities during the transition period. Previous studies have shown that reduced activities of PON1 in the prepartum period of dairy cows could later result in the development of uterine infections in the early postpartum period (SCHNEIDER et al., 2013).

Figure 1. Serum activity of PON1 in the different groups evaluated during the experimental period.

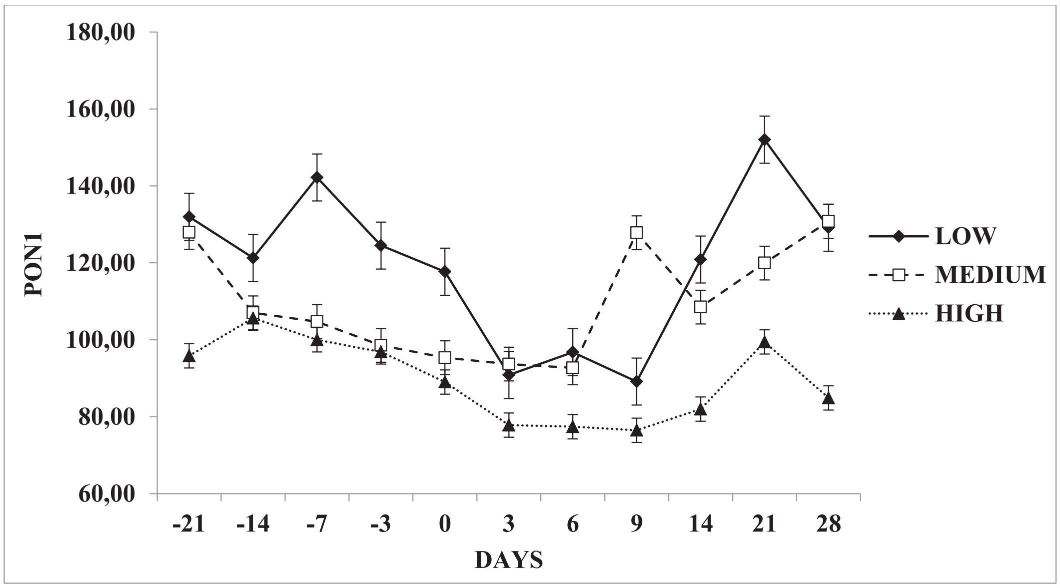

Table 1. Serum activity of PON1 (U mL^{-1}) during the experimental period.

				bPL		P value
PON1 (U mL^{-1})	**LOW**	**MEDIUM**	**HIGH**	**Cat_bPL**	**Day**	**Day×Cat_bPL**
Prepartum	127,53	106,70	97,46	0,05	0,03	0,44
Postpartum	113,13	112,23	82,99	0,10	<0,0001	0,009
Peripartum	119,68	109,72	89,57	0,08	<0,0001	0,006

We did not find any significant difference in the activity of PON1 between the experimental groups (P>0,10) during the prepartum period. However, a significant difference in the activity of the enzyme was noted during the prepartum period in the samples collected on different days (P≤0,0001) and a significant interaction also existed between the experimental groups and days (P≤0,01) (Table 1). The observed changes of the prepartum hormone levels in the postpartum period appear to be related to the interactions of hepatic receptors of PRL and GH. Because of the novelty of the relationship shown in this study, additional research is required to clarify the mechanisms of bPL action on the somatotropic axis, as well as the hepatic functions that influence the synthesis of APPs.

We conclude that cows with superior concentrations of bPL in the prepartum period show low serum activity of PON1 enzyme during the transition period.

References

ALVAREZ-OXILEY, A. V.; DE SOUSA, N. M.; BECKERS, J. F. Native and recombinant bovine placental lactogens. *Reproductive Biology and Endocrinology*, Ramat Gan, v. 8, n. 2, p. 85-106, 2008.

BIONAZ, M.; TREVISI, E.; CALAMARI, L.; LIBRANDI, F.; FERRARI, A.; BERTONI, G. Plasma paraoxonase, health, inflammatory conditions, and liver function in transition dairy cows. *Journal Dairy Science*, Champaign, v. 90, n. 4, p. 1740-1750, 2007.

BROWNE, R. W.; KOURY, S. T.; MARION, S.; WILDING, G.; MUTI, P.; TREVISAN, M. Accuracy and biological variation of human serum Paraoxonase 1 activity and polymorphism (Q192R) by kinetic enzyme assay. *Clinical Chemistry*, Washington, v. 53, n. 2, p. 310-317, 2007.

BYATT, J. C.; EPPARD, P. J.; VEENHUIZEN, J. J.; SORBET, R. H.; BUONOMO, F. C.; CURRAN, D. F.; COLLIER, R. J. Serum half-life and in-vivo actions of recombinant bovine placental lactogen in the dairy cow. *Journal Endocrinology*, Bristol, v. 132, n. 2, p. 185-193, 1992.

CHENG, X.; KLAASSEN, C. D. Hormonal and chemical regulation of paraoxonases in mice. *Journal Pharmacology and Experimental Therapeutics*, Bethesda, v. 342, n. 3, p. 688-695, 2012.

ENDERS, A. C.; WELSH, A. O. Structural interactions of trophoblast and uterus during hemochorial placenta formation. *Journal Experimental Zoology*, Medford, v. 266, n. 6, p. 578-587, 1993.

HOSSNER, K. L.; HOLLAND, M. D.; WILLIAMS, S. E.; WALLACE, C. R.; NISWENDER, G. D.; ODDE, K. G. Serum concentrations of insulin-like growth factors and placental lactogen during gestation in cattle. *Domestical Animal Endocrinology*, Beeville, v. 14, n. 4, p. 316-324, 1997.

KOSKINEN, A.; JUSLIN, S.; NIEMINEN, R.; MOILANEN, T.; VUOLTEENAHO, K.; MOILANEN, E. Adiponectin associates with markers of cartilage degradation in osteoarthritis and induces production of proinflammatory and catabolic factors through mitogen-activated protein kinase pathways. *Arthritis Research and Therapy*, Birmingham, v. 13, n. 6, p. 184-194, 2011.

MONTAGNER, P.; KRAUSE, A. R. T.; SCHWEGLER, E.; WESCHENFELDER, M. M.; MAFFI, A. S.; XAVIER, E. G.; SCHNEIDER, A.; PEREIRA, R. A.; JACOMETO, C. B.; SCHMITT, E.; BRAUNER, C. C.; DEL PINO. F. A. B.; CORRÊA, M. N. Relationship between pre-partum body condition score changes, acute phase proteins and energy metabolism markers during the peripartum period in dairy cows. *Italian Journal of Animal Science,* Pisa, v. 16, n. 2, p. 329-336, 2016.

SCHNEIDER, A.; CORRÊA, M. N.; BUTLER, W. R. Short communication: acute phase proteins in Holstein cows diagnosed with uterine infection. *Research in Veterinary Science*, Rome, v. 95, n. 1, p. 269-291, 2013.

SOARES. M. The prolactin and growth hormone families: pregnancy-specific hormone/cytokines at the maternal fetal interface. *Reproductive Biology and Endocrinology*, Ramat Gan, v. 2, n. 1, p. 51, 2004.

WALLACE, C. R. Concentration of bovine placental lactogen in dairy and beef cows across gestation. *Domestical Animal Endocrinology*, Beeville, v. 10, n. 1, p. 67-70, 1993.