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Serum Metabolic Markers Pre and Postpartum in Holstein Cows According to the Mastitis Occurrence

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***Background*:** Bovine mastitis causes major economic losses for milk producers by reducing the quantity and the quality of the milk or even leading to the complete loss of the mammary gland secretory capacity. During the transition period, dairy cows are susceptible to infectious diseases; therefore, markers that allow early identification of cows in higher risk of developing diseases are especially useful at this time. Therefore, the aim of this study was to evaluate serum markers in the pre and postpartum of multiparous dairy cows with clinical mastitis and with health condition in the postpartum period in a semi-extensive management system.

***Materials, Methods & Results*:** Thirty-Six Holstein cows were monitored daily during milking until 59 days postpartum and were categorized according to the pre-milking strip cup test into clinical mastitis (mastitis group (MG)) and absence of symptoms (control group (CG)) that were negative to the test, representing the health cows. All cows were reared as one group and maintained in a semi-extensive pasture-based system. Blood samples were collected weekly after morning milking via venipuncture of the coccinea vein into tubes without anticoagulant and grouped for prepartum (-21 to 0 days from calving), early postpartum (0 to 30 days from calving), and late postpartum (30 to 59 days from calving) periods. Milk production was recorded daily. The serum markers albumin, aspartate aminotransferase (AST), phosphorus, gamma-glutamyltransferase (GGT) and non-esterified fatty acids (NEFA) were measured. Statistical analyses were performed using SAS®. The cases of clinical mastitis occurred on average at 37.2 ± 4.9 days postpartum. Health cows (CG) had higher milk production compared to the mastitis group (MG) only in the late postpartum period ($P < 0.05$). There was no difference among groups for albumin and NEFA concentrations in all periods evaluated ($P > 0.05$). In the early postpartum period the AST activity was higher in CG than in MG ($P = 0.02$). The GGT enzyme tended to be more concentrated in the CG than in the MG during the early ($P = 0.06$) and late ($P = 0.08$) postpartum periods. Late postpartum phosphorus concentration was lower for MG than CG ($P = 0.04$). In the prepartum and early postpartum periods, there was no difference among groups for phosphorus concentration ($P > 0.05$).

***Discussion*:** A decrease in milk production in MG compared to CG observed in late postpartum period was due to the incidence of mastitis observed around 37 days postpartum. Cows that presented clinical mastitis in the postpartum period did not differ in the blood concentration of NEFA in the prepartum period. In the late postpartum period higher concentration of phosphorus was observed in the CG than in MG, indicating that animals affected by mastitis may be in the weakest energy status. Regarding liver health, the concentration of AST was higher in the recent postpartum period for CG, in disagreement with previous studies that related AST to tissue injury caused by mastitis. The GGT enzyme tended to had higher concentrations in CG than MG during the whole postpartum period and may be related to increased hepatic metabolism due to higher production. There were no changes in albumin levels among healthy and mastitis cows, indicating that this marker can not be used to predict clinical mastitis. There were no metabolic alterations in the prepartum period related to the occurrence of postpartum mastitis in multiparous cows in a semi-extensive management system.

Keywords: AST, dairy cows, NEFA.

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INTRODUCTION

Bovine mastitis is a dairy cattle disease that causes greater losses in milk production, both in quantity and quality [14], and may lead to total loss of the secretory capacity of the mammary gland [3]. A previous study carried out in a semi-extensive management system showed that primiparous Holstein dairy cows which had postpartum mastitis presented alterations in the energy metabolism already in the prepartum period, with high non-esterified fatty acids (NEFA) and lower glucose concentrations compared to healthy cows [15]. Also Melendez *et al*. [8] observed the relation of the high concentration of NEFA at calving and its negative effect in the immune system, consequently, increasing the incidence of diseases. These evidences indicated that adequate prepartum management is essential for good performance in the postpartum period.

High NEFA and beta-hydroxybutyrate (BHBA) and low glucose concentrations in the transition period were related to the severity of the negative energy balance that the cows undergo in this period, contributing to the suppression of the immune system [9], changing the synthesis of several acute phase proteins, including albumin [2].

Therefore, the objective of this study was to evaluate the pre and postpartum serum metabolic markers of multiparous dairy cows with clinical mastitis and with health condition in the postpartum period in a semi-extensive management system.

MATERIALS AND METHODS

Location and animals

The experiment was carried out in the period from December to June at a commercial farm in southern Brazil located at 32°16' S, 52°32' W with average altitude of 5 meters. Thirty-Six Holstein dairy cows were selected based on the number of lactations (≥ 3), milk production adjusted for 305 days of the previous lactation (≥ 7.000 kg), and negative history of diseases in the previous lactation. Two cows presented disease symptoms (metritis and placenta retention) and were removed from the experiment. The initial body weight was 629.8 ± 42.7 kg and body condition score was 3.1 ± 0.4.

The cows were monitored daily during milking by a trained technician until 59 days postpartum and were categorized into two groups: clinical mastitis group (MG = 9), according to the pre-milking strip cup test and control group (CG = 25) that were negative to the test, representing the health cows.

The cows that presented clinical mastitis during the experiment were treated with antibiotics. All cows were reared as one group with the same nutritional and environmental conditions from 21 days prepartum until 59 days postpartum (Table 1). For the duration of the experiment, cows were maintained in a semi-extensive pasture-based system with access to the pasture during the day and also received concentrate in in the trough twice a day after milking.

Blood sampling and serum metabolic markers analysis

Blood samples were collected after morning milking via venipuncture of the coccinea vein into tubes without anticoagulant on days 21, 14, 7 and 3 prepartum, on the calving day (0), and on days 3, 6, 9, 16, 23, 30, 38, 45, 52 and 59 postpartum. Samples were grouped for prepartum (-21 to 0 days from calving), early postpartum (0 to 30 days from calving), and late postpartum (30 to 59 days from calving) periods.

In the postpartum period, cows were milked daily at 03:30 and 15:30 hours. and the milk production was recorded (ALPRO®)[1].

The serum markers albumin, aspartate aminotransferase (AST), phosphorus and gamma-glutamyltransferase (GGT) were measured colorimetrically using commercial kits[2] [16], and non-esterified fatty acids (NEFA) concentration (NEFA-HR)[3] was measured by the micro-method [1]. The coefficient of variation for this assay was less than 10 %.

Statistical Analysis

All statistical analyses were performed using SAS (SAS® Institute, Cary, NC, USA, version 9.3). Milk production, albumin, AST, GGT, phosphorus and NEFA data were analyzed as repeated measures over time (PROC MIXED) to evaluate the main effects of period (prepartum, early postpartum and late postpartum), treatment (CG and MG) and their interactions [7]. Statistical differences were considered significant when $P < 0.05$ and a tendency considered when P value was between 0.05 and ≤ 0.1.

Table 1. Ingredient (kg) and nutrient composition of the experimental diets.

Ingredient	Prepartum Up to -21 days to expected calving	Postpartum 0 to 59 days in milk
Native pasture	*Ad libitum*	-
Forage sorghum	-	*Ad libitum*
Pre-dried, kg	-	15
Wheat bran, kg	0.5	1.5
Soybean meal, kg	1	2.4
Rice bran, kg	0.68	2.88
Ground corn, kg	1.05	3
Ground sorghum, kg	1.05	2.13
Mineral supplement, kg	0.4	0.11
Urea, kg	-	0.09
Sodium bicarbonate, kg	-	0.19
Calcitic limestone, kg	0.12	0.19
Salt	-	0.002
Protected fat	0.2	-

Nutrient composition (dry matter basis)					
	Native pasture	Concentrate	Forage sorghum	Pre-dried	Concentrate
Dry matter, %	89.2	87.67	27.40	52.94	87.31
Neutral detergent fiber, %	67.65	47.42	64.32	63.46	32.57
Acid detergent fiber, %	51.37	13.56	41.74	45.75	13.14
Crude protein, %	9.16	15.61	9.84	8.88	14.92
Ether extract, %	1.73	3.57	2.02	2.00	4.01
Mineral matter, %	9.23	8.9	9.99	8.84	9.02

RESULTS

Nine cows presented clinical mastitis between 0 and 59 days postpartum, and 4 presented the disease twice in this period. The cases of clinical mastitis occurred on average at 37.2 ± 4.9 days postpartum. It was observed that health cows (CG) had higher milk production compared to the mastitis group (MG) only in the late postpartum period ($P < 0.05$; Figure 1).

There was no difference among groups for albumin and NEFA concentrations in all periods evaluated ($P > 0.05$) (Table 2). In the early postpartum period the AST activity was higher in CG (68.1 U/L) than in MG (62.1 U/L) ($P = 0.02$). However, in the prepartum and late postpartum periods, there was no difference among groups for AST concentration ($P > 0.05$).

The GGT enzyme tended to be more concentrated in the CG than in the MG during the early (CG = 49.5 mg/dL and MG = 41.9 mg/dL, $P = 0.06$) and late (CG = 73.5 mg/dL and MG = 65.5 mg/dL, $P = 0.08$) postpartum periods.

Late postpartum phosphorus concentration was lower for MG (5.56 mg/dL) than CG (6.37 mg/dL) ($P = 0.04$). In the prepartum and early postpartum periods, there was no difference among groups for phosphorus concentration ($P > 0.05$).

Table 2. Serum metabolic markers pre and postpartum in Holstein dairy cows in a semi-extensive based-system according to the mastitis incidence.

Markers	Prepartum						Early postpartum						Late postpartum					
	Group		SEM	Pr>F			Group		SEM	Pr>F			Group		SEM	Pr>F		
	CG	MG		G	W	G*W	CG	MG		G	W	G*W	CG	MG		G	W	G*W
Albumin (g/L)	2.89	2.86	0.04	0.76	0.06	0.42	2.42	2.39	0.04	0.67	<0.001	0.57	2.16	2.11	0.05	0.58	0.76	0.99
AST (U/L)	67.28	66.86	1.98	0.89	<0.001	0.45	68.12[a]	62.18[b]	1.68	0.02	<0.001	0.59	57.76	58.10	1.79	0.90	<0.001	0.69
GGT (mg/dL)	47.35	43.81	3.00	0.43	<0.001	0.64	49.52	41.94	2.66	0.06	<0.001	0.57	73.51	65.55	2.99	0.08	<0.001	0.20
Phosphorus (mg/dL)	7.37	6.90	0.33	0.34	0.02	0.85	6.31	6.04	0.17	0.28	0.06	0.80	6.37[a]	5.56[b]	0.26	0.04	0.83	0.81
NEFA (mmol/L)	0.39	0.39	0.03	0.97	<0.001	0.07	0.51	0.45	0.05	0.41	<0.001	0.99	---	---	---	---	---	---

CG: control group; MG: mastitis group; SEM: standard error of the mean; G: effect of the group; W: effect of the week; G*W: group x W interaction effect; AST: aspartate aminotransferase; GGT: gamma-glutamyltransferase; NEFA: non-esterified fatty acids.

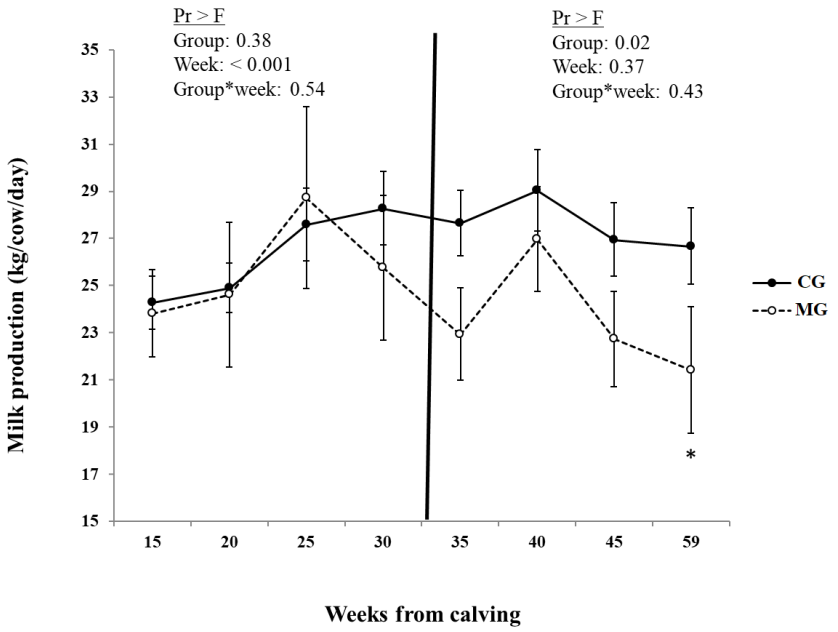

Figure 1. Milk production of multiparous Holstein dairy cows with (MG) and without clinical mastitis (CG) up to 59 days postpartum.

DISCUSSION

A decrease in milk production in MG compared to CG observed in late postpartum was due to the incidence of mastitis observed around 37 days postpartum. The decrease in milk production due to clinical mastitis has been elucidated by several studies [4,15], which can severely affect the secretory capacity of the mammary gland [3].

In the present study cows that presented clinical mastitis in the postpartum period did not differ in the blood concentration of NEFA in the prepartum period, in disagreement with previous studies with primiparous [3] and multiparous cows [8,9]. Thus, it can be inferred that the primiparous cows can be more affected by peripartum management than the multiparous cows in a semi-extensive management system. Also, multiparous had greater challenges than primiparous cows due to their higher milk production.

In the late postpartum period higher concentration of phosphorus was observed in the CG than in MG, indicating that animals affected by mastitis may be in the weakest energy status, because phosphorus is an intermediary of the energy metabolism and participates in important metabolic pathways involved in growth, differentiation, and cell integrity [10].

Regarding liver health, the concentration of AST was higher in the recent postpartum period for CG, in disagreement with previous studies that related AST to tissue injury caused by mastitis [12,15]. In primiparous cows, Schwegler *et al*. [15] observed higher AST concentration in MG than in CG in postpartum period. However Jánosi *et al*. [5] found no change in AST concentration among mastitis and healthy cows.

The GGT enzyme tended to have higher concentrations in CG than MG during the whole postpartum period and may be related to increased hepatic metabolism due to higher production, agreeing with Roos *et al*. [11] who verified that lactation cows presented higher concentrations of this enzyme than dry cows.

There were no changes in albumin levels among healthy and mastitis cows, indicating that this marker can not be used to predict clinical mastitis, as previously observed for endometritis and metritis [6,13].

CONCLUSIONS

Multiparous Holstein dairy cows in a semi-extensive management system did not present metabolic alterations in the prepartum period related to the occurrence of postpartum mastitis. This indicates that markers observed in other management systems can not be applied in this production system and other factors may be more important in determining the occurrence mastitis.

MANUFACTURERS

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***Ethical approval*.** All procedures performed in this experiment were approved by the Committee for Ethics in Animal Experimentation from the Federal University of Pelotas, number 4551.

***Declaration of interest*.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of paper.

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