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Original Article

Leucogram, fibrinogen, plasmatic proteins and glucose evaluation in dairy cows before and after calving

Avaliação do leucograma, fibrinogênio, proteínas plasmáticas e glicose em vacas leiteiras no pré e pós-parto imediato

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A B S T R A C T

Immunological deficiency observed during the transition period in dairy cows promotes development of puerperal diseases. Thus, the research aimed to evaluate hematological (leukogram) and biochemical (glucose, fibrinogen, and total proteins) parameters in Holstein cows (n = 44) before (day -20) and after (day +20) parturition and the possibility of identifying initial signs of reproductive illness, thereby assisting the professional in decision making. There was a difference in the number of total leukocytes, which decreased postpartum (8,888.4 cells/mm^3). There was no difference in neutrophils at both time points. Females who presented high concentration of fibrinogen (5.2 g/L) and total plasmatic proteins (78.9 g/L) at postpartum presented retention of fetal appendages and later uterine disease. These results are consistent with the literature on antepartum leukocytosis and inflammation indicated by increased fibrinogen and plasmatic protein and differ in terms of neutrophil behavior. Thus, laboratory assays can be complementary tools to physical examination, aiding in the diagnosis and identification of changes even before the appearance of clinical signs in animals, which is necessary for the prevention of metabolic and infectious disorders.

Palavras-chave:
Bovinos
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Período de transição

R E S U M O

A deficiência imunológica observada no período de transição em vacas leiteiras suscetibiliza o desenvolvimento de enfermidades puerperais. Desta forma, a pesquisa objetivou a avaliação hematológica (leucograma) e bioquímica (glicose, fibrinogênio e proteínas totais) de vacas Holandesas (n=44) próximas (dia -20) e após (dia +20) o parto e a possibilidade de identificar sinais de enfermidades de ordem reprodutiva ao seu início, auxiliando o profissional na tomada de decisões. Houve diferença no número de leucócitos totais, que diminuíram no pós-parto (8.888,4 células/mm^3). Não houve diferença para neutrófilos nos dois momentos. Fêmeas que manifestaram concentração de fibrinogênio (5,2 g/L) e proteínas plasmáticas totais (78,9 g/L) elevados ao pós-parto apresentaram quadro de retenção dos anexos fetais e posteriormente enfermidade uterina. Esses resultados corroboram com o demonstrado na literatura para leucocitose pré-parto e inflamação indicada pelo aumento de fibrinogênio e proteína plasmática e não difere quanto ao comportamento dos neutrófilos. Desta forma, os exames laboratoriais surgem como ferramentas complementares ao exame físico, permitindo auxiliar no diagnóstico e identificar alterações antes mesmo do aparecimento dos sinais clínicos nos animais, sendo necessários para prevenção dos transtornos metabólicos e infecciosos.

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INTRODUCTION

Genetic improvement programs were implemented in dairy farms in recent years to increase milk production, and Brazil ranks fourth in the world in the production of milk products (IBGE, 2016). However, the intensification of production increases energy requirements, especially in the transition period (3 weeks before and 3 weeks after calving), for fetal nutrition, synthesis of colostrum, and lactation (INGVARTSEN; MOYES, 2013).

In the transition period, cows undergo the greatest challenge, particularly the highly productive cows. This period is characterized by a decrease in dry matter intake, leading to a negative energy balance, which reduces serum glucose and calcium and increases the mobilization of body fat, especially non-esterified fatty acids (VAZQUES-AÑON *et al.*, 1994). Moreover, the increased use of energy reserves for lactation and fetal development at the expense of homeostasis promotes metabolic imbalance and immunosuppression (GOFF *et al.*, 2002).

The degree of immunosuppression at the end of the gestational period, i.e., the speed with which cows resume immune function, is relevant to the development of diseases, including metabolic dysfunctions such as ketosis, acute rumen acidosis, and hepatic lipidosis (ESPOSITO *et al.*, 2014), and uterine diseases such as placental retention, metritis, and endometritis (LEBLANC, 2010). The reduced function of granulocytes and monocytes impairs the ability of dairy cows to fight bacterial infections, favoring the development of uterine diseases and inflammation, which directly affect profit and yield (SHELDON *et al.*, 2009).

Reduced immune function is accompanied by the decreased phagocytic capacity of leukocytes (e.g., neutrophils in cows with endometritis), lower glycogen concentration and higher expression of the tumor growth factor (KIM *et al.*, 2005; GALVÃO, 2012). Defense cells depend on extracellular glucose as well as intracellular glycogen and glycogenolysis to obtain energy for phagocytosis, chemotaxis, and elimination of pathogenic agents (KUEHL; EGAN, 1980).

The serum concentrations of liver acute-phase proteins are correlated with the severity of inflammatory processes and can be used as markers of inflammation. Serum markers whose levels are increased during inflammation include C-reactive protein, haptoglobin, ceruloplasmin, and fibrinogen, and markers whose levels are decreased during inflammation include albumin and transferrin (GARCIA; ISSY; SAKATA, 2002; CÉRON; ECKERSALL; MARTINEZ-SUBIELA, 2005). In this respect, serum biochemistry has been used as a tool for early diagnosis of peripartum diseases, and consequently, developing and implementing efficient measures to reduce economic losses (ROBERTS *et al.*, 2012).

The objective of this study was to evaluate the relationship between the total number of polymorphonuclear neutrophils, the concentration of fibrinogen, total plasma proteins, and glucose, and the development of puerperal diseases in dairy cows before and immediately after calving.

MATERIAL AND METHODS

The study was carried out in a dairy farm located in the municipality of Xanxerê (latitude, 26º 52' 37" S; longitude, 52º 24' 15" W; altitude, 800 m) Santa Catarina State from August to November 2018. Forty-four multiparous Holstein cows reared in a compost barn feedlot system were used. The animals were evaluated by physical and gynecological examination at 20 to 25 days before calving (GRUNERT, BIRGEL, and VALE, 2005) and classified according to body condition on a scale of 1 to 5 (DO LAGO *et al.*, 2001) and gestational age. The procedures involving the animals were performed in accordance with the guidelines established by the Animal Use Ethics Committee (Comitê de Ética de Utilização de Animais–CEUA) of University of Santa Catarina West (Protocol No. 046/2016).

Blood samples were collected at 20 days before and after calving (transition period). The samples were collected from the middle coccygeal vein in vacutainer vacuum tubes containing 10% EDTA (BD Vacutainer®) and sent to the laboratory for evaluation of the white blood cell count. The analyses were performed in the clinical pathology laboratory of the University of Santa Catarina West- Veterinary Hospital.

Total leukocytes were counted using the microdilution technique and a Neubauer chamber. The differential count was performed by analysis of blood smears stained with Quick Panoptic (Laborclin; Paraná, Brazil) and was read under an optical microscope (1000×). The results were expressed as percent and absolute values (100 cells). Leukocytes were classified according to morphology and color. Glucose concentration was measured using a portable glucometer (G_TECH free lite®, Infopia Co. Ltd.) (ALEIXO *et al.*, 2010). Plasma fibrinogen was determined using the heat precipitation method, and total plasma protein concentration was measured by refractometry (JAIN, 1993).

Approximately 30 days after calving, the cows were submitted to gynecological examination performed by a trained professional using vaginal speculum and ultrasound (Mindray-DP-10Vet) to assess the reproductive condition. Reproductive complications were diagnosed based on the time of calving, presence of systemic signs, and the type of vaginal secretion. Possible complications included metritis, endometritis, and retention of fetal membranes (SHELDON *et al.*, 2009).

Data obtained both before and after calving were analyzed using the Student's *t*-test (for comparison of the means) and the two-proportion test using Action Stat in Microsoft Excel. The level of significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

The total number of leukocytes was lower at 20 days after calving (8,888.4 ± 3,156.8 cells/mm^3) than before calving (10,400.0 ± 3,379.3 cells/mm^3) ($p < 0.05$). There were no significant differences in the number of neutrophils ($p > 0.05$) between before and after calving (Table 1). Saut and Birgel (2008) observed that in blood samples collected in the first four days after calving in cows with retention of fetal membranes, the number of circulating leukocytes was lower (by approximately 4,000 cells) than that in healthy animals. The white blood cell count was affected by the retention of fetal membranes until day 10 after calving. In contrast, it was previously shown that a decrease in leukocyte count in the first week after calving was followed by an increase in this parameter in the following three weeks (KIM *et al.*, 2005; SINGH *et al.*, 2008). Nonetheless, in the present study, total leukocytes decreased on day 20 after calving.

Table 1. Hematological and biochemical parameters of Holstein cows 20 days before and after calving.

Parameter	Before calving	After calving	*p*-value
Leukocytes (cells/mm^3)	10,400.0 ± 3,379.3	8,888.4 ± 3,156.8	0.0014
Neutrophils (cells/mm^3)	4,750.4 ± 2,033.7	4,477.9 ± 2,499.4	0.5624
Plasma protein (g/dL)	75.8 ± 6.4	78.8 ± 6.0	0.0435
Fibrinogen (g/L)	4.2 ± 1.4	5.2 ± 1.7	0.0019
Glucose (mg/dL)	63.9±9.8	69.4±10.4	0.0039

The higher number of leukocytes (10,400 cells/mm^3) at 20 days before calving is consistent with the results reported in the study by Ferreira *et al.* (2009), wherein white blood cell count was reduced after calving, and leukocytosis owing to neutrophilia and lymphocytosis occurring during the last days of gestation. Birgel Jr. *et al.* (2001) determined reference values for the white blood cell count in Jersey cows in the state of São Paulo, Brazil, and found that the average count was approximately 11,847 cells/mm^3, whereas the total neutrophil count was approximately 2,537 cells/mm^3. However, it is worth highlighting that, in ruminants, the leukocyte response differs from that in other production animals, and cases of acute bacterial septicemia are accompanied by neutropenia instead of neutrophilia, demonstrating a lower predisposition of ruminants to develop significant neutrophilia (WEISS; PERMAN, 1992).

In this respect, it is noteworthy that 25% (11/44) of the cows showed retention of fetal membranes, which may be due to dietary imbalance or calving assistance. In the present study, of the 11 cows with placental retention, five (45%) presented dystocia. It is well-known that deliveries with some degree of dystocia are followed by the retention of fetal membranes (LE BLANC, 2010).

For this reason, serum calcium status should be evaluated. The decrease in calcium at the onset of lactation may lead to lower uterine contractility, contributing to the retention of fetal membranes. Furthermore, cows with serum calcium below 2.15 mmol/L in the first three days of lactation had a lower number of circulating neutrophils and lower phagocytic and oxidative capacity, consequently increasing susceptibility to disease (MARTINEZ *et al.*, 2014).

White blood cells depend on extracellular glucose as well as intracellular glycogen and glycogenolysis to obtain energy for phagocytosis, chemotaxis, and antigen elimination (KUEH; EGAN, 1980). Serum glucose concentrations before and after calving were 63.9 ± 9.8 mg/dL and 69.4 ± 10.4 mg/dL ($p < 0.05$), respectively. Cows in late gestation and early lactation have a negative energy balance, and lower dry matter intake at the end of the gestational period contributes to the reduction in serum glucose levels. Furthermore, the mammary gland requires a higher concentration of glucose for lactose synthesis. However, the glucose levels remained within the reference values for Holstein cows in southern Brazil (65.4 ± 5.3 mg/dL) (GONZÁLEZ *et al.*, 1996).

In addition to the leukocyte count, fibrinogen can also be measured when examining inflammatory diseases in cattle. Fibrinogen is an indicator of acute inflammation and is more sensitive than changes in leukocyte counts. The plasma concentration of this marker increases under conditions of inflammation, stress, and trauma (COLE *et al.*, 1997; SILVA *et al.*, 2008). The reference values for fibrinogen in cattle are 3 to 7 g/L (MORDAK *et al.*, 2017). In our study, the levels of this protein were 4.2 ± 1.4 g/L before calving and 5.2 ± 1.7 g/L after calving ($p < 0.05$). In animals with retention of fetal membranes (11/44), fibrinogen levels were higher after calving (5.5 ± 1.8 g/L) than before calving (3.8 ± 2.1 g/L) ($p < 0.05$) (Table 2). Even with this increase of 44.7%, the values were within the variability of the reference values but might be relevant in the early diagnosis of post-puerperal disorders.

In contrast, total plasma protein levels were different before and after calving (75.8 ± 6.4 vs. 78.8 ± 6.0 g/dL). It is worth noting that these levels were increased in animals with retention of fetal membranes. The levels before and after calving in these animals were 71.8 ± 7 g/dL and 81.8 ± 6.2 g/dL ($p < 0.05$), respectively (Table 2). González *et al.* (1996) found that the average total serum protein concentration in Holstein cattle in southern Brazil was 84.5 ± 18.8 g/dL.

Table 2. Comparison of hematological and biochemical parameters in Holstein cows with placental retention at 20 days before and after calving.

Parameter	20 days before calving	20 days after calving (cows with placental retention)	*p*-value
Leukocytes (cells/mm^3)	10,327.3 ± 2,649.2	8,227.3 ± 2,185.4	0.0230
Neutrophils (cells/mm^3)	5,629.5 ± 2,460.0	4,596.4 ± 2,061.3	0.2524
Plasma protein (g/dL)	71.8 ± 7.0	81.8 ± 6.2	0.0019
Fibrinogen (g/L)	3.8 ± 2.1	5.5 ± 1.8	0.0306
Glucose (mg/dL)	59.9 ± 9.7	67.9 ± 12.0	0.0954

CONCLUSIONS

Plasma fibrinogen and total protein levels were the main indicators of reproductive changes during the transition period, especially retention of fetal membranes, and further analysis of these parameters may improve the early diagnosis of post-puerperal disorders.

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