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Milk yield and composition in dairy cows with post-partum disorders

[*Produção e composição do leite em vacas com desordens no pós-parto*]

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ABSTRACT

This study aimed to determine the impact of different post-partum disorders on milk yield and composition. One hundred and fifteen Holstein cows from a commercial dairy farm located in the state of Rio Grande do Sul in southern Brazil were monitored up to 62 days post-partum. During this period, body condition score evaluation and animal clinical examination were conducted. Percentages of fat, protein, and lactose, as well as somatic cells score, were determined in milk samples. The AST activity and concentrations of NEFA, calcium, and BHBA, were analyzed in blood samples. The occurrence of clinical disorders was identified in 30 (26%) cows. Subclinical disorders were identified in 64 (56%) cows. Only 21 (18%) cows did not suffer any kind of disorder within the studied period. In this study, no significant differences were found in milk production, protein, and somatic cell count in clinical, subclinical, and healthy cows. Milk fat and the fat: protein quotient (F:P) were higher in cows with clinical disorders and the 6 to 21 days in milk, and lactose were lower in cows with clinical disorders and the 22 to 42 days in milk ($P<0.05$).

Keywords: metabolic disorders, metabolic profile, milk quality

RESUMO

O objetivo deste estudo foi determinar o impacto de diferentes distúrbios após o parto na produção de leite e em sua composição. Cento e quinze vacas Holandesas de uma fazenda de gado leiteiro, localizada em estado da região Sul do Brasil, foram monitoradas até 62 dias após o parto. Durante esse período, foram realizadas avaliações do escore de condição corporal e exame clínico nos animais. As porcentagens de gordura, proteína e lactose, bem como o escore de células somáticas, foram determinadas nas amostras de leite. A atividade do AST e as concentrações de NEFA, cálcio e BHBA foram analisadas em amostras de sangue. A ocorrência de distúrbios clínicos foi identificada em 30 (26%) vacas, os distúrbios subclínicos foram identificados em 64 (56%) vacas. Apenas 21 (18%) vacas não sofreram nenhum tipo de distúrbio ao longo do período estudado. Neste estudo, não foram encontradas diferenças significativas na produção do leite, proteína e na contagem de células somáticas em vacas com doenças clínicas, subclínicas e saudáveis. No leite, a gordura e o quociente gordura e proteína (G:P) foram maiores em vacas com doença clínica no período de seis a 21 dias de lactação, e a lactose foi menor em vacas com doença clínica no período de 22 a 42 dias de lactação (P<0,05).

Palavras-chave: transtornos metabólicos, perfil metabólico, qualidade do leite

INTRODUCTION

High milk production imposes challenging metabolic demands on dairy cows to maintain their homeostatic balance (Zanella, 2016). These demands may result in metabolic changes that can directly affect milk yield, or the physicochemical characteristics of their milk (Arnould *et al.*,

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20013. Milk composition is affected by different factors including animal genetics, environmental influences, energy levels, and protein or mineral imbalances (Filipejová *et al*., 2011). The most critical period for dairy cows is the transition to lactation period, when immune functions may be impaired (Sordillo and Raphael, 2013). The beginning of lactation, together with calving stress, can dramatically affect dairy cow feed intake and metabolism (Hayirli *et al*., 2002). Thus, during the transition period, dairy cows may go through a negative energy balance (NEB), marked by a large mobilization of energy reserves, mainly fat and protein, to meet the demands of high milk production (Barletta *et al.*, 2017).

Thus, nutrition management is critical and requires the use of rapidly fermentable carbohydrates in adequate proportion with fiber to prevent ruminal and metabolic disorders (Chapinal *et al*., 2011). The occurrence of metabolic disorders in postpartum dairy cows can strongly impact the health of the cow during the production period. Generally, one disorder can predispose an animal to the occurrence of another, causing a domino effect that will reduce productive/reproductive performance (Allen and Piantoni, 2015). As an example, the occurrence of subclinical ketosis during the first weeks after calving will predispose animals to the occurrence of the displaced abomasum, metritis, and clinical ketosis. In cows, 75% of cases of milk fever, ketosis, displaced abomasum, retained placenta, and uterine infections occur during the first 30 days postpartum (Leblanc, 2010).

Milk composition, particularly fat and protein, has been used to assess the nutritional status of dairy herds. The fat to protein quotient (F:P) in milk has been used as an indicator of lipomobilization (Duffield *et al*., 1997), and milk urea has been used to assess protein metabolism (Hristov and Ropp, 2003). Blood metabolic profiles can be used to assess protein, energy, and mineral metabolism, and to assess liver function (Wittwer, 1995). Blood biochemistry offers reliable tests to measure the status of nutrient metabolism in animal tissues, making it a useful tool for the diagnosis of metabolic disorders, especially in pre-clinical or subclinical stages (Cozzi *et al*., 2011). Milk chemical composition is a non-invasive and less expensive alternative compared to blood analyses to verify health parameters (Feltes *et al*., 2016). This study aimed to determine the occurrence of clinical and subclinical disorders in postpartum dairy cows and assess the possible impact of these disorders on milk production and composition.

MATERIALS AND METHODS

This project was approved by the Committees for the Ethical Use of Animals of the Federal University of Rio Grande do Sul, Porto Alegre, Brazil (Project nº 22666) and the University of Passo Fundo, Passo Fundo, Brazil (Project nº 025/2012). One hundred and fifteen Holstein cows were monitored from the date of calving up to 62 days postpartum. Animals were from a commercial dairy farm located in the southern region of Brazil (state of Rio Grande do Sul) at coordinates 28°10′S, 52°50′W, and 598 m above sea level. Data collection was performed from April to November (Autumn, Winter, and Spring in the southern hemisphere).

Animals were raised semi-confined on a diet formulated to meet a milk production target of 40kg/day, containing 3.5% fat, 3.1% protein, and 4.8% lactose during the first two months of lactation (Nutrient…, 2001). Cows received 26.1 kg DM/day, consisting of ryegrass in pastures (*Lolium multiflorum*), corn silage, Tifton pasture hay, ground corn, soybean hulls, soybean meal, and minerals. The chemical composition of the diet (total mixed ration) was as follows: 37.7% neutral detergent fiber, 1.6 Mcal/day metabolizable energy, and 20.6% crude protein. The concentrations of minerals and vitamins were 0.8% Ca, 0.3% P, 0.2% Mg, 1.2% K, 0.4% Na, 0.4% Cl, 0.2% S, 9.4 ppm Co, 9.4 ppm Cu, 14 ppm Fe, 0.5 ppm I, 27 ppm Mn, 0.3 ppm Se, 54 ppm Zn, 7.4 IU/kg vitamin A, 0.7 IU/kg vitamin D, and 22.9 IU/kg vitamin E on a dry matter (DM) basis. Part of the diet was prepared using a *Total-Mix Feeder Wagon*, delivered after each milking, with the cows constrained in yokes. The rest of the diet was obtained through ryegrass grazing. Water was available *ad libitum* in the barns, in grazing paddocks, and the milking waiting room and exit. Also, animals had shade in the corridors leading to the paddocks.

The farm had three daily milkings (06:00, 14:00, and 21:00) for a total of 315 milking cows. The herd was milked with automated equipment (Gea Farm Technologies, Germany) with the piped

system, low line, containment in a fishbone-shaped double 6, with vacuum cutting system and automatic extractor of the teat. The milking equipment incorporated individual milk sampling capability. Milking control data consists of health information obtained by an experienced veterinarian and, from day 6 to day 62 postpartum, data of milk production and milk composition. For compositional analysis, 40 mL of milk samples were collected. Samples were placed in vials containing bronopol as a preservative and maintained at a temperature of 8°C until analysis. The analysis was performed the day after collection, using a combined system (Delta Instruments, USA) that determines the somatic cells score (SCS) by flow cytometry (SomatoScop CA3A5), and the chemical composition of milk (fat, protein, and lactose) by near-infrared spectrophotometry (FT LactoScop 10).

Blood samples were collected on three occasions after calving, from 6 to 21, 22 to 42, and 43 to 62 days of lactation (DIM). Sampling was always performed after morning milking. Blood samples (8 mL) were taken from the coccygeal vein into vacuum tubes without anticoagulant (Vacutainer, BD, Brazil). Blood was stored in insulated boxes at 8°C. After arrival at the laboratory, samples were centrifuged at 1.400 *g* for 10 minutes. Serum samples were placed in 1.5 mL Eppendorf tubes, labeled, and frozen at -20°C until analysis. Serum samples were processed in automated equipment (Wiener Lab CM200, Argentina). Colorimetric methods (Labtest, Brazil and Randox, Ireland) were used for the analysis of aspartate aminotransferase (ultraviolet kinetic method), non-esterified fatty acids (aminoantipyrine colorimetric method), calcium (phthalein purple method), and beta-hydroxybutyrate (ultraviolet kinetic enzymatic method). Body condition scores (BCS) were determined on the same days as blood sampling using visual techniques, and inspection of the croup (pelvis and tail set), ribs, and loin, with a ranking scale from 1 to 5 as described by Edmonson *et al*., (1989).

Clinical diseases were diagnosed by clinical signs indicating the disease (Radostits *et al*., 2000). Retained placenta (RP) was diagnosed by the presence of outstanding wraps on the vulva, metritis by gynecological examination with evidence of content in the vaginal and uterine canal. Displaced abomasum (DA) was identified by a sharp reduction in milk production and appetite associated with auscultation and percussion, and mastitis was identified by edema and pain in the mammary gland and visual alterations in milk, with the presence of lumps. Clinical ketosis was considered when serum BHBA was > 2.6 mmol/L (Duffield, 2000), subclinical ketosis was diagnosed when serum BHBA was ≥1.4 mmol/L (Carrier *et al*., 2004) and subclinical hypocalcemia was considered when serum calcium was less than 2.0 mmol/L (Goff, 2008). NEFA concentrations > 720 μmol/L were considered to indicate a high degree of lipomobilization with the risk of ketosis (Cucunubo, *et al*., 2013). Cows not classified with a clinical or subclinical disorder were considered healthy.

Data were analyzed using SPSS program v.20.0. Variables were analyzed for normality and homoscedasticity. Data were analyzed by linear model analysis of variance considering the postpartum periods 6-21 days, 22-42 days, and 43-62 days separately for milk, blood, and body condition parameters, using the following model:

$$Y(ij)= \mu + Di + Pj + DPij + \varepsilon ij$$

Where μ is the overall mean, Di is the effect of the i^{th} disorder event, P_j is the effect of the j^{th} number of parturitions, DPij is the interaction effect between a disorder event and the number of parturitions, and εij is random error. Significant differences between means were compared with the Tukey test ($P < 0.05$).

RESULTS

Out of the 115 cows, 30 cows (26%) were found with clinical disorders, including displaced abomasum, ketosis, mastitis, metritis, RP, and two disorders combined. Subclinical disorders were diagnosed in 64 cows (56%), including hypocalcemia, ketosis, and a combination of two disorders. During the studied period, 21 (18%) cows showed no sign of clinical or subclinical disorder and were considered healthy cows (Table 1).

Table 1. Number and percentage of Holstein cows with clinical and subclinical disorders up to 62 days in milk in southern Brazil

Type of disorder	N (%)
Clinical disorders	30 (26)
Displaced abomasum	5 (4.3)
Ketosis	4 (3.4)
Mastitis	6 (5.2)
Metritis	5 (4.3)
Retained placenta	5 (4.3)
Two disorders	5 (4.3)
Subclinical disorders	64 (55.6)
Hypocalcemia	31 (26.9)
Ketosis	19 (16.5)
Hypocalcemia + ketosis	14 (12.1)
Healthy cows	21 (18.2)

No statistical differences were found in milk production, protein, and somatic cell score, among the three groups of cows (with clinical disorders, with subclinical disorders, and healthy cows) (Table 2). The fat content was higher (P<0,05) in cows with clinical disorders during the period of 6 to 21 DIM. The lactose content was higher (P<0.05) in healthy cows during the period of 22 to 42 DIM. The Fat: Protein (F:P) quotient was higher (P<0.05) in cows with clinical disorders in the period 6 to 21 DIM, and in cows with subclinical disorders in the period of 43 to 62 DIM. The AST and BHBA values were higher (P<0.05) in cows with clinical disorders in the period of 6 to 21 DIM (Table 3), whereas the values of NEFA were higher in cows with clinical disorders in the period of 6 to 21 DIM and 22 to 42 DIM. The BCS values were lower (P<0.05) in cows with clinical disorders along the studied period (Table 3). No statistical differences were found in calcium content.

DISCUSSION

In this study, 26% of the animals showed clinical disorders including displaced abomasum (4.3%), ketosis (3.4%), mastitis (5.2%), metritis (4.3), RP (4.3%), and two or more combined disorders (4.3%) during the postpartum period. Subclinical disorders were identified in 55.6% of the animals, represented by hypocalcemia (27.1%), ketosis (16.6%), and hypocalcemia associated with ketosis (12.3%). Only 21 (18%) cows did not show any disorder within the studied period. Metabolic disorders in dairy cows may occur by the increasing selection pressure for higher production. In a retrospective study of 26 years in Canada, using information from five databases (Agricola, Medline, CAB Abstracts, Life Sciences, Focus On), Kelton *et al*. (1998) reported the following occurrences of clinical disorders in dairy cows: 8.6% retained placenta, 14.2% mastitis, 10.1% metritis, 4.8% ketosis, and 1.7% displaced abomasum.

Table 2. Milk yield and composition in Holstein cows with clinical and subclinical disorders, and healthy cows in southern Brazil during three stages of lactation

Days in lactation	Cows with clinical disorders	Cows with subclinical disorders	Healthy cows	Cows with clinical disorders	Cows with subclinical disorders	Healthy cows
N	30	64	21	30	64	21
	Milk production (L/cow/day)			Lactose (g/100 g)		
6 to 21	30.5 ± 1.7	34.2 ± 1.0	34.6 ± 4.6	4.5 ± 0.1	4.7 ± 0.7	4.7 ± 0.1
22 to 42	33.7 ± 1.9	37.1 ± 1.2	37.4 ± 1.8	4.6 ±0.1^{b}	4.8 ± 0.1^{a}	4.9 ± 0.1^{a}
43 to 62	34.7 ± 2.8	37.7 ± 1.3	37.3 ± 2.0	4.8 ± 0.1	4.8 ± 0.1	4.9 ± 0.1
	Fat (g/100 g)			Protein (g/100 g)		
6 to 21	4.6 ± 0.2^{a}	3.8 ± 0.1^{b}	3.9 ± 0.2^{b}	3,0 ± 0.2	3.3 ± 0.1	3.1 ± 0.2
22 to 42	3.4 ± 0.2	3.4 ± 0.1	3.4 ± 0.1	2.8 ± 0.1	2.9 ± 0.1	3.0 ± 0.1
43 to 62	3.1 ± 0.2	3.5 ± 0.1	3.1 ± 0.2	2.9 ± 0.1	2.9 ± 0.1	2.9 ± 0.1
	F:P			SCS		
6 to 21	1.5 ± 0.1^{a}	1.3 ± 0.4^{b}	1.2 ± 0.7^{b}	4.9 ± 0.3	4.7 ± 0.2	4.7 ± 0.3
22 to 42	1.2 ± 0.1	1.3 ± 0.0	1.2 ± 0.0	4.8 ± 0.4	4.6 ± 0.2	4.6 ± 0.3
43 to 62	1.1 ± 0.1^{ab}	1.2 ± 0.0^{a}	1.1 ± 0.0^{b}	4.5 ± 0.5	4.3 ±0.3	4.2 ± 0.4

Different letters in a row indicate significant differences (P< 0.05) among groups of cows. F:P fat: protein ratio; SCS: somatic cell scores.

Table 3. Mean and standard deviation of serum indicators and body condition scores in Holstein cows with clinical and subclinical disorders, and healthy cows in southern Brazil during three stages of lactation

Days in lactation	Cows with clinical disorders	Cows with subclinical disorders	Healthy cows
N	30	64	21
		AST (U/L)	
6 to 21	110.8 ± 6.8[a]	98.3 ± 3.8[b]	94.3 ± 6.7[b]
22 to 42	95.5 ± 6.2	85.8 ± 3.6	79.9 ± 5.7
43 to 62	82.5 ± 8.3	79.9 ± 3.5	95.3 ± 5.1
		NEFA (µmol/L)	
6 to 21	1059.8 ± 121.8[a]	794.5 ± 73.1[b]	695.1 ± 136.5[b]
22 to 42	626.4 ± 74.7[a]	495.3 ± 68.5[ab]	420.4 ± 73.4[b]
43 to 62	237.0 ± 60.5	363.8 ± 43,0	370.0 ± 52.7
		BCS (1 – 5)	
6 to 21	2.6 ± 0.1[b]	2.8 ± 0.1[a]	3.0 ± 0.1[a]
22 to 42	2.3 ± 0.1[b]	2.6 ± 0.1[a]	2.8 ± 0.1[a]
43 to 62	2.0 ± 0.2[b]	2.5 ± 0.1[a]	2.7 ± 0.1[a]
		Calcium (mmol/L)	
6 to 21	2,0 ± 0.1	2.0 ± 0.0	2.1 ± 0.1
22 to 42	2.1 ± 0.1	2.0 ± 0.0	2.1 ± 0.0
43 to 62	2.1 ± 0.1	2.0 ± 0.0	2.1 ± 0.0
		BHBA (mmol/L)	
6 to 21	1.4 ± 0.2[a]	1.2 ± 0.8[b]	1.0 ± 0.1[b]
22 to 42	1.0 ± 0.2	1.3 ± 0.1	1.0 ± 0.2
43 to 62	0.9 ± 0.2	1.0 ± 0.7	1.0 ± 0.1

Different letters in the lines indicate significant differences ($P < 0.05$) between the disorder groups. AST: aspartate transaminase; NEFA: non-esterified fatty acids; BCS: body condition score; BHBA: beta-hydroxybutyrate.

The incidence of clinical disorders in our study was below those found in the literature, except for DA which had the highest rate (4.3%) compared with the data reported by Kelton *et al*. (1998) who found frequencies of DA in 22 studies varying from 0.3% to 6.3%. The frequencies of subclinical disorders in dairy cows were described by Reinhardt *et al*. (2011) which have found plasma calcium levels below 2.0 mmol/L in 25% to 54% of the parturient cows, while Dohoo and Martin (1984) reported up to 33.9% of subclinical ketosis in cows up to 65 days postpartum. Garcia *et al*. (2011) observed a 24% occurrence of subclinical ketosis in high-yielding dairy cows in the Rio Grande do Sul (southern Brazil). The differences between the incidence of clinical and subclinical disorders in the literature can be explained by the different number of cows and the management practices adopted by the husbandry systems used in our study.

Early-onset of clinical or subclinical postpartum disorders did not impact milk yield, milk protein, or somatic cell scores, which may be attributed in the present work to the low number of cows presenting postpartum disorders. Fat, F:P and lactose in milk showed statistical differences when comparing the three categories of cows (clinical disorders, subclinical disorders, and healthy cows). The fat content and the F:P quotient of milk was higher in cows with clinical disorders during the period of 6 to 21 DIM and were associated with higher values of serum AST, NEFA, and BHBA and lower BCS in cows with clinical disorders possibly because of decreased food intake which led to a loss in the BCS up to 62 days postpartum. BCS loss is probably due to the greater negative energy balance and lipomobilization that also caused excessive NEFA levels in the liver, potentially contributing to hepatic lipidosis increasing BHBA levels (Gonzalez *et al*., 2011).

Arnould *et al*. (2013) analyzed the use of milk composition data as a herd management tool and an indicator of the metabolic status of the cows and concluded that milk fat composition is the most useful indicator as a management tool, mainly for monitoring and preventing various disorders in dairy cattle. The F:P quotient has been used to assess the nutritional status of herds (Duffield *et al*., 1997) as an indicator of lipomobilization (Mulligan *et al*., 2006). Milk analyses will have diagnostic value only if samples are collected individually from each cow.

The cows in the present study showed no evidence of hepatic lipidosis, as AST activity did not significantly increase, although cows with clinical diseases in the 6 to 21 DIM period had the highest AST values, which were statistically different from those in healthy cows and cows with subclinical disorders during the same period. Herdt (2000) used the values of NEFA and BHBA to define the severity of negative energy balance. Excessive weight loss and severe lipid mobilization that occur in clinical ketosis led to an increase of circulating NEFA in the blood that is incorporated in the mammary gland for the fat synthesis in milk. Bauman and Griinari (2003) suggest that cows in negative energy balance have lipolysis as the greatest source of milk fat. Thus, milk fat can be used as an indicator for the diagnosis of lipomobilization and for detecting the risk of ketosis (Duffield *et al*., 1997). The risk of ketosis increases when the quotient F:P is greater than 1.5 (Duffield *et al*., 1997) and Seifi *et al*. (2010) attributed a cut-off point of 1.2 in the quotient F:P as an indicator of increased risk of ketosis.

In this study, it was observed that the percentage of milk protein was not influenced by the clinical disorders during de period of 6 to 62 DIM. The literature mentions that diet, lactation stage, milk yield, SCS, season and thermal comfort can influence the concentration of milk protein (Bondan *et al*., 2018). However, those factors were controlled in the experimental model and SCS did not show a difference among studied groups. The lactose concentration in milk was lower in cows with clinical disorders in the period of 22 to 42 DIM, when they presented weight loss, evidenced by the lower BCS and by the rise in NEFA serum levels. The high lipomobilization attributed to the severe negative energy balance could be related to the decreasing levels of milk lactose (Muhlbach, 2003).

CONCLUSIONS

The present study shows that milk yield, milk protein, and somatic cell scores are unaffected by clinical or subclinical disorders in the studied dairy herd. However, cows with clinical disorders show higher values of milk fat and F:P quotient, as well as lower values of milk lactose, in the early postpartum period, which may be attributed to the higher lipomobilization and body condition loss in those animals.

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