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Subclinical mastitis in Jersey dairy cows and its effects on productivity and inflammatory markers

Mastite subclínica em vacas leiteiras da raça Jersey e seus efeitos sobre os marcadores produtivos e inflamatórios

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Highlights

Lactose is a good marker for subclinical mastitis in Jersey dairy cows.
A precise technique for detecting subclinical mastitis is nonexistent.
The somatic cell count is an indicator of mammary gland health.
Subclinical mastitis affects productivity and inflammatory markers in milk.

Abstract

This study assessed the effect of subclinical mastitis on the productivity and inflammatory markers in Jersey dairy cows. Blood, milk, and milk yield data were collected from 59 Jersey dairy cows reared under a semi-extensive system. Milk samples were collected from individual collectors and evaluated for their somatic cell count (SCC), lactose (Lact), protein, fat, total and defatted dry extracts (DDE), casein, freezing point, and milk urea nitrogen (MUN) levels. After milking, blood was collected by puncturing the coccygeal arteriovenous complex. In the serum samples, the inflammatory biomarkers paraoxonase-1, albumin, and total plasma protein levels were analyzed using colorimetric methods. Samples of the entire diet provided and pastures were submitted for bromatological analysis. Additionally, the body condition score, number of lactations, milk yield, days of lactation, calving interval, number of inseminations until conception, calving-conception interval, and days of pregnancy were analyzed. The

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cows were categorized based on their SCC into a subclinical mastitis group (SubG), with SCC levels greater than or equal to 200,000 cells/mL, and a control group (CG), with SCC levels below 200,000 cells/mL. Blood metabolic variables, milk quality, and milk production data were analyzed using multivariate regression, analysis of variance, and logistic regression using the SAS® program. The multivariate regression analysis revealed that several markers, such as Lact, total protein, and casein, influenced milk composition, predicting over 97% of the data. The SubG showed lower concentrations of Lact (4.37 vs. 4.47%; P = 0.0002), MUN (20.55 vs. 23.85 mg/dL; P = 0.02), and DDE (9.24 vs. 9.50%; P = 0.02) compared with the CG; moreover, animals in the SubG had a higher number of lactations compared with those in the CG (4.30 vs. 2.69; P = 0.0039). Cows with a Lact content lower than 4.265% (quartile 25%), compared with those with Lact content greater than 4.565% (quartile 100%), were 52 times more likely to have subclinical mastitis. Jersey dairy cows with subclinical mastitis have lower Lact, urea, and defatted dry extract levels in their milk as well as a higher number of lactations. Therefore, Lact levels in milk serve as a good diagnostic marker of subclinical mastitis in Jersey cows. Subclinical mastitis in Jersey dairy cows did not decrease milk yield nor effect reproductive performance.
Key words: Dairy cattle. Somatic cell count. Lactose. Paraoxonase 1.

Resumo

O objetivo deste estudo foi verificar a influência da mastite subclínica sobre os marcadores produtivos e inflamatórios em vacas leiteiras da raça Jersey. Foram coletadas amostras de sangue, leite e dados produtivos de 59 vacas Jersey mantidas em sistema semi-extensivo. As amostras de leite foram coletadas a partir do uso do copo coletor individual e avaliadas quanto a contagem de células somáticas (CCS), lactose (Lact), proteína, gordura, extrato seco total e desengordurado (ESD), caseína, ponto de congelamento e nitrogênio ureico (Nul). A colheita de sangue foi realizada após a ordenha, por punção do complexo arteriovenoso coccígeo. Nas amostras de soro foram analisados os biomarcadores inflamatórios Paraoxonase-1, albumina e proteínas plasmáticas totais, por métodos colorimétricos. Amostras da dieta total fornecida e das pastagens foram submetidas as análises bromatológicas. Além disso, foram analisados o escore de condição corporal, número de lactações, produção, dias em lactação, intervalo entre partos, número de inseminações até concepção, intervalo parto concepção e dias de gestação. As vacas foram categorizadas pela contagem de células somáticas (CCS) em grupo com mastite subclínica (Gsub) com resultados de CCS maiores ou iguais a 200.000 céls/ml e grupo controle (GC) com níveis de CCS abaixo de 200.000 céls/mL. Os dados das variáveis metabólicas sanguíneas, qualidade do leite e dados produtivos foram submetidos a regressão multivariada, análise de variância e regressão logística utilizando o programa SAS®. A análise de regressão multivariada demonstrou que vários marcadores influenciam na composição do leite, predizendo mais que 97% dos dados, como para os parâmetros lactose, proteína total e caseína. O GSub apresentou menores concentrações de Lact (4,37 vs 4,47%; P = 0,0002), Nul (20,55 vs 23,85 mg/dL; P = 0,02) e de ESD (9,24% vs 9,50%; P = 0,02) em comparação ao GC. Foi observado que os animais do Gsub possuíam maior número de lactações em relação ao GC (4,30 vs 2,69; P =0,0039). As vacas que apresentaram teores de lactose menores que 4,265% (Quartil 25) em comparação com as de percentuais maiores que 4,565% (Quartil 100) apresentaram 52 vezes mais chances de ter mastite subclínica. Vacas Jersey com mastite subclínica possuem menor teor de lactose, ureia e extrato seco desengordurado no leite,

apresentando também maior número de lactações. A lactose se mostrou um bom marcador no leite para diagnóstico de mastite subclínica em vacas Jersey. A mastite subclínica em vacas leiteiras da raça Jersey não diminuiu a produção de leite e não afetou o desempenho reprodutivo.
Palavras-chave: Bovinos de leite. Contagem de células somáticas. Lactose. Paraoxonase 1.

Introduction

Mastitis is one of the most important inflammatory diseases in dairy farming as it causes reduced milk production, premature discarding of animals, increased medication costs, and losses in the quality of milk and its derivatives, leading to significant economic losses within this industry (Carvalho-Sombra et al., 2021). There is a growing global concern regarding increasing health problems in dairy farming (Masia et al., 2022) and declining milk quality (Stocco et al., 2020), with the somatic cell count (SCC) being internationally recognized as a standard for monitoring mammary gland health and milk quality (Bobbo et al., 2017; Rienesl et al., 2022).

Breeding is an important factor that affects milk production and composition. Jersey cows generally have a higher concentration of milk fat than Holstein cows, indicating a different mechanism of fat regulation (Sears et al., 2020). Numerous studies have extensively investigated characteristics such as milk yield, body weight, fertility, and somatic cells in the Holstein breed (Gallo et al., 2023; Gibson & Dechow, 2018; Pegolo et al., 2021), whereas studies in the Jersey breed remain scarce (Roveglia et al., 2019).

Early detection of mastitis is important for preventing economic losses and ensuring prompt and effective treatment. However, delays in detecting subclinical mastitis and the absence of a suitable and accurate diagnostic technique contribute to its increased incidence (Sathiyabarathi et al., 2018). It is among the four main diseases affecting dairy cows worldwide (Shi et al., 2016). However, an effective solution for both preventing and treating this condition remains non-existent.

Due to the etiological complexity of mastitis (Sathiyabarathi et al., 2018) and the difficulty of its applicability across farms using different laboratory diagnoses (Chakraborty et al., 2019), analyzing the relationship among indicators of milk quality, serum levels of inflammatory biomarkers, and the productivity data of animals is fundamental (Rainard et al., 2018). The milk pH, levels of enzymes, proteins, peptides, milk components, and SCC are primary biomarkers, in addition to molecular tests, genomics, and proteomic analyses, which can be used alone or in combination (Chakraborty et al., 2019).

In addition to the SCC, lactose levels have been widely studied as an indicator of mastitis. Lactose is the central osmotic component of milk and majorly influences water secretion. Thus, a lower lactose content results in a lower volume of milk being produced (Boas et al., 2017). Negative metabolic conditions and poor udder health are associated with an increasing SCC and decreasing lactose level. In addition, reproductive success may be positively

correlated with milk lactose levels (Antanaitis et al., 2021). This study assessed the effect of subclinical mastitis on the productivity and inflammatory markers in Jersey dairy cows.

Material and Methods

All procedures performed during the experimental period were approved and conducted in accordance with the guidelines of the Animal Use Ethics Committee (CEUA) of the Instituto Federal Catarinense, under the number 385/2021.

Animals and diets

Data were collected in a commercial farm in Braço do Norte city (SC) (28°13'24.3" S, 28°13'24.3" S) in October 2021. A total of 59 healthy lactating Jersey cows were used, with a mean body weight of 478 ± 35 kg, body condition score (BCS) of 3.36 ± 0.36, and lactation days (DIM) of 193.95 ± 124. Cows were categorized via their SCC into the subclinical mastitis group (SubG), with SCC results ≥ 200,000 cells/mL, and control group (CG), with SCC levels < 200,000 cells/mL (International Dairy Federation [IDF], 2013).

The cows were milked twice daily using an automatic milking system (at 6:00 am and 5:00 pm). A concentrate fraction of the diet (CFD) (Table 1) was supplied in individual troughs twice daily after milking, considering each cow's milk production (1 kg of concentrate was supplied for every 3 kg of milk). Subsequently, the cows were moved to a "compost barn," where the roughage fraction of the diet was offered ad libitum (VFD) (Table 1), with free access to water. Every day after morning feeding, the animals were maintained on Cynodon spp. and Brachiaria mutica pastures (Table 1). The chemical compositions of the concentrate, roughage fraction, and pasture were determined following the analytical methods described by Prates (2007).

Data collection

Data and sample collection were conducted on a single day after the afternoon milking.

Milk

Milk samples were collected from individual collectors in the milking system and stored in two sterile tubes containing bronopol preservative. One tube was used for the SCC analysis and chemical composition analysis of fat content, total protein, lactose, total dry extract, defatted dry extract (DDE), casein, and milk urea nitrogen (MUN), whereas the other tube was used for the freezing point analysis. Subsequently, the samples were sent to the centralized milk analysis laboratory of the Associação Paranaense dos Criadores de Bovinos da Raça Holandês, Curitiba - PR (PARLPR). Analyses of fat content, total protein, lactose, total dry extract, defatted dry extract, somatic cell count, milk urea nitrogen, freezing point, and casein concentrations were performed via infrared spectrophotometry (Bentley 2000, Bentley Instruments® Inc., Chaska MN, USA), and SCC analysis was performed using flow cytometry (Somacount 300, Bentley Instruments Inc.) (Schwegler et al., 2013).

Table 1
Ingredients, percentage composition, and chemical composition of the concentrate, roughage fraction of the diet, and *Cynodon* spp + *Brachiaria mutica* pasture fed to Jersey dairy cows with or without subclinical mastitis on the day of sample collection

	Concentrate	Roughage	Pasture
Ingredients (%)			
Concentrate			
Ground corn - 04 mm	52.0		
Soybean meal 46% of PB	35.0		
Soybean hulls	6.00		
Mineral supplement*	5.00		
Sodium bicarbonate	2.00		
Roughage fraction			
Cottonseed		4.30	
Corn silage		94.41	
Rice straw		1.29	
Cynodon spp + *Brachiaria mutica*			*Ad libitum*
Chemical composition (% of DM)			
Neutral detergent fiber	16.74	51.69	57.39
Acid detergent fiber	8.63	34.80	34.28
Starch	33.48	16.73	2.56
Crude protein	26.40	9.92	20.92
Total digestible nutrients	77.27	57.68	65.21
Energy (Mcal/kg)			
Digestible energy	3.41	2.54	2.88
Net energy	1.77	1.29	1.26
Net energy of gain	1.37	0.69	0.97
Net energy of maintenance	2.02	1.26	1.57

* 28% Ca, 6.5% Na, 6% P, 1.5% Mg, 1.5% S, 0.0035% Co, 0.2% Zn, 0.12% Mn, 0.05% Cu, 0.2% Fe, 0.006% I, 0.0035% Se, 250.000 UI/kg of vitamin A, 80.000 UI/kg of vitamin D, and 1.500 UI/kg of vitamin E.

Blood

Blood samples were collected by puncturing the coccygeal arteriovenous complex and were stored in Vacutainer® blood collection tubes (10 mL). The tubes were centrifuged at 1800 G for 15 min to obtain the serum and stored at -20 ºC in Eppendorf® tubes for further biochemical analysis.

Serological samples from the cows were analyzed to determine the acute phase protein concentrations of paraoxonase 1 (PON-1), albumin (ALB), and total plasma protein (TPP) at the laboratory of the Research, Teaching, and Extension Nucleus (Nupeec of the Universidade Federal de Pelotas). PON-1 analysis was performed by the kinetic method using a previously

established protocol (Browne et al., 2007), and the reading was performed using an ultraviolet light spectrophotometer (FEMTO Cirrus 80MB, FEMTO Indústria e Comércio de Instrumentos, São Paulo, Brazil). ALB and TPP analyses were performed using commercial kits (Labtest Diagnostica, Lagoa Santa, SP, Brazil), and heir readings were measured using an automatic biochemical analyzer (Labmax plena®).

Productive parameters

BCS data (scale from 1 to 5) were obtained based on visual inspection and data on the number of lactations. BCS data, number of lactations, daily milk yield (hereafter referred to as yield), DIM, calving interval, number of inseminations until conception (NofAI), calving-conception interval (CCI), and days of pregnancy were obtained from the farm's database.

Statistical analysis

Data on the metabolic and productive variables of the cows were tested for normal distribution and homogeneity of the residues using the Shapiro-Wilk and Levene tests, respectively. To further evaluate these variables, a regression analysis (PROC REG) was performed.

Cows were categorized into two groups according to SCC: the SubG with SCC results ≥200,000 cells/mL and CG with SCC levels <200,000 cells/mL (IDF, 2013). Comparison between the SubG and CG was performed by analysis of variance using the MIXED procedure, and group means were compared using the Tukey's test at a significance level of 5%. Logistic regression analysis (PROC LOGISTIC) and odds ratio calculation were performed to identify the lactose interval (LI) that demonstrated the highest odds ratio for dairy cows to present subclinical mastitis. LIs were defined as LI-1: ≤4.265%; LI-2: >4.265 and ≤4.425%; LI-3: >4.425 and ≤4.565%; and LI-4: >4.565% according to 25%, 50%, 75%, and 100% quartiles. Data were analyzed using the SAS program (Analysis System Institute, Cary, NC, USA, version 9.3).

Results and Discussion

The herd had a mean SCC of 401.33 ± 621.54 (× 1000 cells/mL) and a median of 133.50 (× 1000 cells/mL). The SubG (n=23) presented SCC > 200,000 cells/mL, with a mean value of 898.69 ± 629.51 (× 1000 cells/mL), whereas the CG (n= 36) had a mean SCC of 83.57 ± 49.28 (× 1000 cells/mL) (Table 2). The multivariate regression analysis revealed that several markers, such as lactose, total protein, and casein, influenced milk composition and predicted over 97% of the data (Table 3).

The SubG had reduced concentrations of lactose (4.21 vs. 4.47%; P = 0.0002), MUN (20.55 vs. 23.85 mg/dL; P = 0.02), and DDE (9.24% vs. 9.50%; P = 0.02). Furthermore, animals in the SubG had a higher number of lactations than those in the CG (4.30 vs. 2.69; P = 0.0039). Other parameters were unaffected by the grouping (Table 4). Logistic regression analysis performed to calculate the LI demonstrated the highest odds ratio (OR) of dairy cows presenting subclinical mastitis (Table 5). Comparison between

the lowest (≤ 4.265%, LI-1) and highest (> 4.565%, LI-4) lactose concentration intervals demonstrated that LI-1 animals were 52 times more likely to have subclinical mastitis than LI-4 animals.

Table 2
Summary analysis of productive and inflammatory parameters in Jersey dairy cows with or without subclinical mastitis reared in a semi-extensive system

Parameters	Mean	Minimum	Maximum	Standard error	Standard deviation
Body condition score, scale from 1 to 5	3.36	2.50	4.00	0.04	0.35
Lactations number (n)	3.32	1.00	9.00	0.27	2.13
Milk yield (kg/day)	22.74	13.60	36.20	0.66	5.13
Lactation days (days)	193.94	17.00	596.00	16.14	124.02
Calving interval (days)	426.60	57.00	776.00	17.57	117.87
Inseminations number (n)	1.79	1.00	5.00	0.15	1.07
Calving-conception interval (days)	152.14	39	551.00	14.08	98.56
Days of pregnancy (days)	65.44	4.00	211.00	7.91	55.39
Somatic cell count (×1000 cell/mL)	401.33	16.00	3480.00	80.91	621.54
Protein (%)	3.95	3.12	4.54	0.04	0.34
Fat (%)	5.29	2.13	8.41	0.13	1.03
Lactose (%)	4.37	3.59	4.87	0.03	0.27
Total dry extract (%)	14.69	12.09	18.23	0.15	1.20
Defatted dry extract (%)	9.40	8.29	10.12	0.05	0.40
Casein (g/L)	3.13	2.38	3.76	0.04	0.31
Freezing point (°C)	-0.56	-0.60	-0.53	0	0.01
Milk urea nitrogen (mg/dL)	22.56	6.20	32.35	0.68	5.25
Albumin (g/dL)	3.06	2.03	3.56	0.03	0.28
Total plasmatic proteins (g/dL)	7.35	6.44	8.38	0.05	0.41
Paraoxonase 1(U/mL)	116.31	45.45	189.75	4.12	31.69

* 28% Ca, 6.5% Na, 6% P, 1.5% Mg, 1.5% S, 0.0035% Co, 0.2% Zn, 0.12% Mn, 0.05% Cu, 0.2% Fe, 0.006% I, 0.0035% Se, 250.000 UI/kg of vitamin A, 80.000 UI/kg of vitamin D, and 1.500 UI/kg of vitamin E.

Table 3
Multivariate regression of productive and inflammatory parameters in Jersey dairy cows with or without subclinical mastitis reared in a semi-extensive system

Parameters	Equation	P value	R^2
Body condition score, scale from 1 to 5	2.13954 - 0.02940 yield + 0.26342 TPP	0.0428	0.20
Lactations number (n)	38.32293 - 0.07480 yield + 0.00074639 SCC - 2.68044 DDE- 1.03689 TPP	0.0005	0.52
Milk yield (kg)	14.41410 - 0.02009 Dayspreg + 8.14446 lact - 3.46443 DDE + 0.31862 MUN	0.0004	0.52
Lactation days (days)	- 3.63917 + 0.97036 CCI + 1.26289 Dayspreg	0.0002	0.73
Calving interval (days)	1236.84369 + 7.60842 yield + 26.39246 NofAI - 140.68870 TPP	0.0230	0.27
Inseminations number (n)	- 3.77102 + 0.00787 CCI + 0.59817 TPP	<.0001	0.50
Calving-conception interval (days)	104.42682 + 0.55545 DIM + 21.46303 NofAI - 0.80239 Dayspreg- 3.10393 MUN	<.0001	0.79
Days of pregnancy (days)	- 177.39713 + 0.41063 DIM - 0.48469 CCI - 57.46751 lact - 1139.83154 Freepoint - 30.62162 TPP +0.47446 PON1	0.0098	0.76
Somatic cell count (×1000 cell/mL)	5054.55022 + 159.80041 n° lact + 40.12612 Yield - 54.61375 MUN -1295.58356 Alb - 8.26125 PON1	0.0016	0.52
Protein (%)	- 0.92610 - 0.00001594 DIM + 0.00006807 Dayspreg- 0.00000320 SCC - 0.84080 lact - 0.00664 TDE + 0.92114 DDE - 0.00010747 PON1	<.0001	0.99
Fat (%)	-	-	-
Defatted dry extract (%)	-	-	-
Total dry extract (%)	-	-	-
Lactose (%)	- 1.10898 + 0.00061760 yield + 0.00006617 Dayspreg - 0.00000429 SCC - 1.13041 prot - 0.00976 fat + 1.08121 DDE - 0.05577 Casein - 0.00009748 PON1	0.0008	0.99
Casein (%)	- 0.11001 + 0.01349 NofAI + 0.96322 prot - 0.04760 TDE + 0.00566 MUN	<.0001	0.98
Freezing point (ºC)	– 0.33562 - 0.00113 NofAI - 0.00740 TDE - 0.01564 DDE + 0.00909 Casein	<.0001	0.88
Milk urea nitrogen (mg/dL)	40.97604 - 2.96264 BCS + 0.18449 yield - 0.00191 SCC - 20.19104 prot + 3.49725 fat + 15.73372 Casein	0.0004	0.73
Albumin (g/dL)	3.47677 - 0.00013961 SCC - 0.05446 fat	0.0031	0.28
Total plasmatic proteins (g/dL)	7.94289 + 0.25686 BCS - 0.07049 n° lact - 0.00105 CI + 0.09282 NofAI - 0.00234 Dayspreg- 0.41227 lact + 0.04675 MUN	0.0212	0.60
Paraoxonase 1 (U/mL)	120.03109 - 0.07679 CCI	0.0451	0.11

Casein = casein; prot = protein; DIM = lactation days; Dayspreg = days of pregnancy; CCI = calving-conception interval; BCS = body condition score; Fat = fat; Alb = albumin; Nº lact = lactation number; Lact = lactose; PON-1 = paraoxonase 1; TPP = total plasmatic proteins; DDE= defatted dry extract; TDE= total dry extract; NofAI = number of artificial inseminations; SCC= somatic cell count; CI= calving interval; n° lact = number of lactations; freepoint = freezing point; MUN= milk urea nitrogen; R^2: coefficient of determination.

Table 4
Productivity and inflammatory parameters of Jersey dairy cows with subclinical mastitis, categorized by somatic cell count in the control group (CG; n= 36; < 200,000 cells/mL) and subclinical mastitis group (SubG; n= 23; ≥ 200,000 cells/mL)

Parameters	Groups		Mean	Standard error	P Value
	CG	SubG			
Body condition score, scale from 1 to 5	3.38	3.32	3.36	0.04	0.5153
Lactation number (n)	2.69a	4.30b	3.32	0.27	0.0039
Milk yield (kg)	23.00	22.34	22.74	0.66	0.6371
Lactation days (days)	208.97	170.43	193.94	16.14	0.2478
Calving interval (days)	424.04	429.80	426.60	17.57	0.8728
Inseminations number (n)	1.87	1.66	1.79	0.15	0.8734
Calving-conception interval (days)	161.64	135.77	152.14	14.07	0.6481
Days of pregnancy (days)	61.70	71.88	65.44	7.91	0.5408
Protein (%)	3.95	3.94	3.95	0.04	0.8683
Fat (%)	5.32	5.24	5.29	0.13	0.7926
Lactose (%)	4.47a	4.21b	4.37	0.03	0.0002
Total dry extract (%)	14.82	14.49	14.69	0.15	0.3079
Defatted dry extract (%)	9.50a	9.24b	9.40	0.05	0.0174
Casein (g/L)	3.14	3.13	3.13	0.04	0.9121
Freezing point (ºC)	-0.57	-0.56	-0.57	0.001	0.0999
Milk urea nitrogen (mg/dL)	23.85a	20.55b	22.56	0.68	0.0174
Albumin (g/dL)	3.09	3.01	3.06	0.03	0.2539
Total plasmatic proteins (g/dL)	7.38	7.31	7.35	0.05	0.5052
Paraoxonase 1 (U/mL)	115.56	117.48	116.30	4.12	0.8229

Table 5
Odds ratio and confidence intervals for lactose levels (%) and the occurrence of subclinical mastitis (somatic cell count ≥ 200,000 cells/mL) in Jersey dairy cows reared in a semi-extensive system

Lactose level interval (%)	Estimated	95% confidence limit		Pr> Chisq	ROC curve
≤ 4.265 (LI-1) vs > 4.265 to ≤ 4.425 (LI-2)	11.000	1.998	60.572		
≤ 4.265 (LI-1) vs > 4.425 to ≤ 4.565 (LI-3)	6.000	1.172	30.725		
≤ 4.265 (LI-1) vs > 4.565 (LI-4)	51.995	4.739	570.439		
> 4.265 a ≤ 4.425 (LI-2) vs > 4.425 to ≤ 4.565 (LI-3)	0.545	0.117	2.549		
> 4.265 to ≤ 4.425 (LI-2) vs > 4.565 (LI-4)	4.727	0.458	48.763	0.0045	0.8037
> 4.425 to ≤ 4.565 (LI-3) vs > 4.565 (LI-4)	8.666	0.885	84.822		

LI, lactose interval; ROC, receiver operating characteristic.

The decrease in milk yield in clinical mastitis cases is easily observed, whereas in the subclinical form, the results are controversial, and the decline in milk production may not be detectable (Bobbo et al., 2017). This statement corroborates the results of the present study, in which milk production did not differ between the groups. Gonçalves et al. (2020) and Bonestroo et al. (2022) reported that in Holstein cows, milk yield is not influenced up to a certain SCC level; however, a negative effect is observed beyond 277,000 cells/mL, showing an increasing rate of reduction in milk yield with higher SCC levels.

The highest number of lactations was observed in the SubG. Repeated lactation, recurrent cases of mastitis, stage of lactation, and aging can affect the integrity of the mammary epithelium (Herve et al., 2018), consequently increasing SCC during a cow's productive life. Therefore, mastitis is more prevalent in herds of multiparous cows due to primiparous cows' greater resistance to inflammation (Costa et al., 2019). Notably, the percentage of lactose decreases with an increase in the number of lactations (Haile-Mariam & Pryce, 2017).

The reduction in lactose levels observed in the SubG and greater chance of subclinical mastitis occurrence in cows with lower levels of lactose can be explained by the fact that during subclinical mastitis, pathogenic agents use milk as a growth substrate (Costa et al., 2019). The infection-induced inflammatory response damages the secretory cells and compromises epithelial permeability (Pegolo et al., 2021). Through the alveoli, lactose is lost in the blood and excreted through the urine, reducing lactose levels in the milk. This is because SCC has three mechanisms of action: reduction in lactose secretion, loss of lactose into the bloodstream, and the use of lactose and glucose by pathogens as energy sources in the mammary glands (Alessio et al., 2021). Several studies have demonstrated that lactose serves as a health marker in cows and is associated with SCC. serving as an indicator of subclinical mastitis (Antanaitis et al., 2021; Bobbo et al., 2017).

Lactose levels vary less than fat and protein levels in the milk of healthy mammary glands. Thus, any change in lactose levels may be associated with inflammatory states, negative energy balance, and changes in mammary gland homeostasis (Costa et al., 2020; Ptak et al., 2012). The relationships between lactose content and other milk constituents (fat and protein), SCC (an indicator of mammary gland health), and total bacterial count (an indicator of microbiological quality) have been used to monitor milk quality standards. However, lactose content is associated with variations in fat and protein content (Alessio et al., 2021). Studying the relationship between lactose levels and reproductive success, Televičius et al. (2021) demonstrated that low lactose levels in milk were associated with lower corpus luteum activity and lower pregnancy rates.

In this study, the low DDE values, as observed in previous studies on Holstein animals, were due to lactose directly influencing DDEs as part of its composition, along with fats, proteins, and minerals (Liu et al., 2022). Total solids represent all milk constituents and can thus be used as an evaluation parameter for quality (Alessio et al., 2021).

The decrease in MUN values in the SubG can be explained by the variable level of urea in the milk of the same herd of cows, for which the predisposing factors are age at first calving, number of lactations, stage of lactation, breed (Bittante, 2022), and energy and mineral imbalance caused by inflammation (Martins et al., 2021). Cows with lower metabolic conditions may have elevated milk urea levels, which are closely related to blood urea concentrations (DePeters et al., 1992; Gustafsson & Palmquist, 1993), suggesting that the elimination of urea, which requires energy, is impaired in cows with a low glycemic index (Gross et al., 2020). Furthermore, high concentrations of plasma urea can induce stress in polymorphonuclear cells (Tsunoda et al., 2017), negatively affecting the immunological condition of animals (Gross et al., 2020).

No changes in the inflammatory markers were observed in the present study, possibly owing to less severe inflammatory processes in subclinical mastitis. PON-1 is a mammalian antioxidant and anti-inflammatory enzyme synthesized in the liver and secreted into the blood (Silveira et al., 2019). Our findings revealed no change in serum PON-1 levels in Jersey cows with subclinical mastitis. Conversely, Nedić et al. (2019) observed reduced PON-1 activity in the serum of Holstein cows with subclinical mastitis. PON-1 is sensitive to inflammatory conditions and is regarded by some authors as a negative acute-phase protein, which serum levels are reduced during infection (Silveira et al., 2019). This could explain our result of a lower inflammatory response and, consequently, an attenuated systemic reaction in less severe cases of subclinical mastitis. In previous studies on Holstein cows, no changes in ALB levels were observed in healthy cows or in cows with subclinical and clinical mastitis, indicating that ALB is not sensitive to this disease (Schwegler et al., 2013, 2018).

Subclinical mastitis failed to influence the NofAI and CCI, indicating that subclinical mastitis does not trigger a systemic inflammatory response sufficient to affect follicular growth and oocyte quality (Dahl et al., 2018) in Jersey cows. According to Fernandes et al. (2021), the chance of pregnancy during the first 300 days of lactation is not affected in dairy cows of different breeds presenting with subclinical mastitis during the first month of lactation. However, according to Masia et al. (2022), clinical mastitis in cows with more than two lactations reduces fertility and prolongs the estrous cycle by up to two-fold compared with healthy cows, thereby increasing the NofAI and CCI.

Conclusion

Jersey dairy cows with subclinical mastitis had lower levels of lactose, urea, and DDE in their milk and a higher number of lactations than the control group. Lactose in milk serves as a good marker for the diagnosis of subclinical mastitis in Jersey cows. Furthermore, subclinical mastitis does not decrease milk production or affect reproductive performance in Jersey dairy cows.

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