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Pathogen effects on milk yield and composition in chronic subclinical mastitis in dairy cows

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ABSTRACT

This study aimed to evaluate the effects of chronic subclinical mastitis (CSM) on milk production and component yields in dairy cows. A total of six herds located in the Midwest area of São Paulo State, Brazil were selected. Herds were visited once every 2 weeks to measure milk yield and to collect milk samples from lactating Holstein cows. Milk samples were collected at two stages (1 and 2), and each stage comprised three milk samplings. In stage 1, a total of 117 of 647 cows were diagnosed with CSM based on at least two of three repeated somatic cell counts (SCC) > 2000,000 cells/mL and positive bacterial milk culture results (BC). Cows with CSM were selected for the second stage. In stage 2, selected cows had quarter sampling aseptically collected for BC analyses prior to milking, and quarter milk yield was measured. Milk components (total protein, fat, lactose, and total solids) were measured using mid-infrared spectroscopy. Mammary quarters were considered healthy if all three repeated SCC results were ≤ 200,000 cells/mL and no bacterial growth was detected on BC. All quarters with positive bacterial growth were classified as having (non-chronic) subclinical mastitis when only one of three SCC results were > 200,000 cells/mL, and CSM when at least two of three SCC results were > 200,000 cells/mL. The effects of CSM by type of pathogen on milk and components yield were assessed using a linear mixed model.

Mammary quarters with CSM caused by major pathogens had milk loss of 1.1 kg/quarter milking in comparison to healthy quarters. Milk losses were 0.8 and 1.3 kg/quarter milking when CSM was caused by *Staphylococcus aureus* or environmental streptococci, respectively. In addition, healthy quarters produced more milk components than quarters with CSM caused by major pathogens. Minor pathogens causing CSM (non-*aureus* staphylococci and *Corynebacterium* spp.) had no effect on milk yield. Quarters with CSM had lower milk and component yields when compared with healthy quarters. Milk losses varied according to the type of pathogen and were higher when associated with major pathogens such as *S. aureus* and environmental streptococci compared with healthy quarters.

Introduction

Subclinical mastitis (SM) is the most prevalent production disease in dairy herds (Viguier et al., 2009; Bobbo et al., 2017). Although SM does not present signs of local or systemic inflammation, it does cause increased somatic cell count (SCC) in response to bacterial infections (Gonçalves et al., 2018b). The prevalence of SM varies with cow age, breed, immunological status and lactation stage (Gonçalves et al., 2018a). Subclinical mastitis negatively affects milk yield and composition, with both short- and long-term effects, sometimes overlapping to the next lactation (Seegers et al., 2003; Hogeveen et al., 2011).

It is generally thought that changes in milk yield associated with SM depend on the type of bacteria invading into milk secretory epithelia of the mammary gland (Coulon et al., 2002; Viguier et al., 2009). The major mastitis pathogens elicit a greater somatic cell response (cow level SCC > 200,000 cells/mL) than minor pathogens such as non-*aureus* staphylococci and

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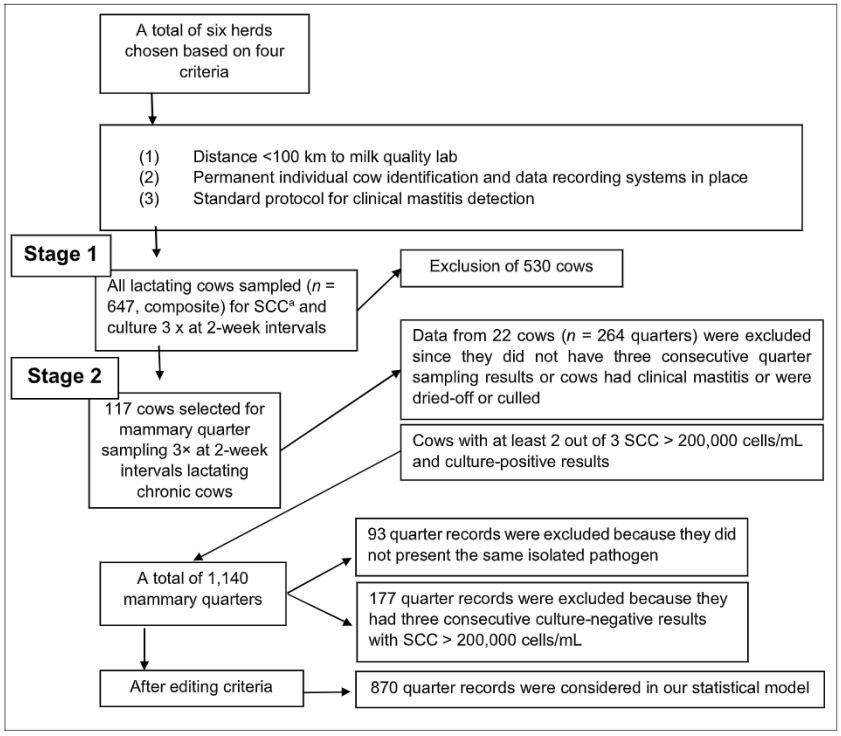

Fig. 1. Flow diagram of cow and quarter selection; selection criteria and number of records excluded or remaining for the next stage.

Corynebacterium spp. (Tomazi et al., 2015; Gonçalves et al., 2016). Another important aspect of some major mastitis pathogens is the capacity to cause intermittent infection (e.g. *S. aureus*), which may lead to chronic subclinical mastitis (CSM). In CSM, there can be the replacement of healthy secretory tissue by fibrotic tissue, damaging the capacity to synthesize milk (Benites et al., 2002, Botaro et al., 2015). The effects of CSM, by type of pathogen, on milk and milk components have not been well-described. To date, few studies have considered whether mastitis (clinical or subclinical) could lead to long-term milk production decreases (e.g., 40–160 kg per month) when the pathogen resists the immune defense and adapts to the mammary tissue (Seegers et al., 2003; Blum et al., 2014).

Most studies have evaluated the effects of SM using only a single milk sample (Leitner et al., 2006; Bezman et al., 2015; Gonçalves et al., 2018b). Bobbo et al. (2017) suggested that further studies would be required to evaluate the effect of SM on milk and components yield at the quarter level in order to avoid a dilution effect, which occurs when evaluating effects at the udder level. In this context, we questioned whether the use of repeated milk quarter sampling to evaluate milk losses would be a more accurate approach to estimate the effects caused by CSM compared with only one sampling. Furthermore, only a few studies have investigated chronic mastitis (Swinkels et al., 2005; Boutet et al., 2007; Steeneveld et al., 2007; Cardozo et al., 2015) and none of these have used repeated quarter milk sampling and milk production measurements. Therefore, the aim of this study was to evaluate the effect of CSM on milk and component yields in dairy cows using repeated quarter milk sampling, as proposed by Berglund et al. (2007), adding bacteriological culturing (BC) analyses with species confirmation by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS).

Material and methods

The study was approved by the Ethics Committee on Animal Use of the School of Veterinary Medicine and Animal Science of University of São Paulo (Approval number, 3020; Approval date, 26 June 2013).

Dairy herds, cow selection (stage 1) and quarter milking (stage 2)

Herds were selected based on (1) the proximity to the milk quality lab (<100 km); (2) having permanent individual cow identification and data recording systems in place; and (3) use of a mastitis control program consistent with those established by the National Mastitis Council (NMC[1]). This included consistent use of pre- and postmilking teat dipping, use of dry cow therapy, periodic milking machine maintenance, and standard milking and intramammary treatment procedures. These criteria led to the selection of six of 21 possible herds, all located in the Midwest area of São Paulo State, Brazil. Herds were visited every 2 weeks to measure milk production and to collect milk samples from lactating cows, as described in a companion study by Gonçalves et al. (2018b). All lactating cows were housed in free-stall barn facilities and milked in herringbone milking parlors twice daily. In all herds, cows were fed a total mixed ration (TMR) composed of corn silage, grain concentrate, and minerals. Water was available ad libitum.

Cow selection (stage 1, three samplings; timeframe, 6 weeks) was designed to identify cows with evidence of CSM and to disregard cows with previous episodes of clinical mastitis. Cows with a previous history of clinical mastitis or with clinical mastitis during selection and the experiment were eliminated from the study. All Holstein lactating cows ($n = 647$) with four functional quarters and a milk yield $\geq$10 kg/day were evaluated during a 9-month period organized in two major stages, each with three milk samplings at a biweekly interval.

In stage 1, composite milk samples (pool of the four quarters) were collected from all lactating cows during three repeated samplings, once every 2 weeks, for SCC analyses. Additionally,

[1] See: National Mastitis Council — NMC. https://www.nmconline.org/nmc-protocols-guidelines-and-procedures/ (Accessed 8 May 2020).

aseptic composite foremilk[1] samples were collected for BC analyses. All composite foremilk samples submitted for BC analyses were collected using aseptic technique according to the National Mastitis Council guidelines (National Mastitis Council (NMC, 1999). A total of 117 of 647 cows (average milk yield of 22.3 kg/day ±0.2), had CSM, based on at least two of three repeated composite SCC results > 200,000 cells/mL (IDF, 2013, Schukken et al., 2003) and positive BC results. These cows were selected for stage 2 sampling.

Quarter milking (stage 2, three samplings; timeframe, 6 weeks) was designed to identify individual mammary quarters with evidence of CSM. This second major stage involved collection of quarter milk samples (n = 1,316) during three repeated samplings, once every 2 weeks, from all cows (n = 117) selected in stage 1; an interval of 2 weeks between samplings was adopted (Sears et al. 1990). After the sampling period, quarters from 22 cows were excluded from further analyses because they lacked the third sampling because they had clinical mastitis (n = 11); they had been dried-off (n = 7); or had been culled from the herd (n = 4). This left 1,140 quarter sampling results from 95 cows. Of these, 93 quarters yielded bacterial isolates, and 177 quarters had SCC > 200,000 cells/mL but were culture-negative. Excluding these quarters resulted in a total of 870 quarter records in our statistical model (Fig. 1).

At each sampling one quarter milk sample (14 mL) was collected aseptically for BC prior to milking and another milk sample was collected from the milk meter (40 mL) after milking. Milk meter samples were collected into plastic tubes containing Bronopol (2-bromo-2-nitropropane-1,3-diol) as a preservative (0.05 g/100 mL milk) for SCC and milk component analysis according to International Dairy Federation guidelines.[2] Milk yield was measured by milking mammary quarters individually using the bucket milking system previously described by Gonçalves et al. (2018b). Briefly, this milking equipment included a pulsator and a cluster of four liners connected to individual silicone tubes, equipped with valves for vacuum release. Each liner was fitted with a milk meter (MM6 DeLaval, Campinas, Brazil) to estimate milk yield at the quarter level. In short, samples were stored with ice in a cooler at 4–7 °C until they were transported to the laboratory for BC, milk composition and SCC analyses.

Microbiological and milk composition analyses

In short, 10 μL of milk was inoculated onto blood agar plates containing 5% defibrinated bovine blood and incubated at 37 °C for 48 h. Plates were observed every 24 h for colony morphology (format, size, number, and color) and hemolysis. Milk samples with more than one pathogen detected were not included in the model (n = 10); in four milk samples, two different major pathogens were isolated, and in six milk samples, isolation of minor and major pathogens was observed. Milk samples with more than two pathogens detected were considered contaminated (n = 11) and were eliminated from all subsequent analyses.

Bacterial colonies were submitted for species-specific identification using MALDI-TOF MS methodology (Barcelos et al., 2019). For bacterial species identification using MALDI-TOF MS, one colony was applied to the steel plate spot with the aid of a wooden stick. A volume of 1.0 μL of formic acid (70%) was applied to the spot and allowed to dry at room temperature. After drying, 1.0 μL of HCCA matrix solution was applied. A standard protein solution (bacterial test standard, BTS; Bruker) was used for calibration and the spectrum was obtained in FlexControl 3. The spectral data processing was performed using MALDI Biotyper 4.1.70 (Bruker Daltonik) computer software for microorganism identification (MBT version 7311 MPS library). A score of ≥ 2.0 indicated species-level identification.

Milk components (total protein, fat, lactose, and total solids) were determined by mid-infrared spectroscopy using a milk analyzer (Bentley 2000, Bentley Instruments). The SCC were determined utilizing flow cytometry equipment (Somacount 300, Bentley Instruments).

Intramammary infection status

After BC and SCC determination, the quarters were categorized as 'healthy' if all three samples had a SCC ≤ 200,000 cells/mL and no bacterial growth. Quarters with positive bacterial growth were classified as having SM when only one of three results had SCC > 200,000 cells/mL. Lastly, quarters with positive bacterial growth of the same pathogen identification at the species level were classified as having CSM when at least two of three results had SCC > 200,000 cells/mL.

Statistical analyses

The effects of CSM on milk and components yield were assessed using a linear mixed effects model and a nested structure of the measurements (cow nested within herd and parity; quarter nested within cow, herd, parity and either UdderStatus or PathogenType). Intramammary infection status was considered in two ways: (1) quarter status, or (2) pathogen type. The following mixed model was used:

$$Y_{ijklmnop} = \mu + H_i + P_n + IMI_o + C_{inj} + Q_k \times C_l(H_i P_n IMI_o) + S_p + IMI_o \times S_p + b_1 \times DIM + b_2 \times e^{(-0.05 \times DIM)} + e_{ijklmnop}$$

in which $Y_{ijklmnop}$ was considered as the continuous dependent variable (milk, yield components and log SCC); μ represented the general average; H_i represented the random effect of the ith herd (i = 1–6); and P_n was considered the fixed classification effect of the nth parity (n = 1, 2 and 3+). For UdderStatus, intramammary infection (IMI_o; Quarter status) represented the udder quarter health status regarding presence or absence of mastitis during the three milk samplings (o = 1–3; healthy, SM and CSM, respectively), For PathogenType, IMI_o (PathogenType) represented the udder quarter health status regarding the type of pathogen during the three milk samplings (o = 1–4; 1, minor: non-*aureus* staphylococci and *Corynebacterium* spp.; 2, major: all other isolated pathogens with exception of minor; 3, genus; and 4, species level identification). C_{inj} was the random effect of jth cow nested within ith herd and nth parity (j = 1–95); $Q_k \times C_l(H_i P_n IMI_o)$ represented the random effect of the quarter, nested within cow, herd, parity and IMI category; S_p represented the fixed effect of time of mammary quarter sampling (p = 1–3); $IMI_o \times S_p$ was the interaction between udder quarter health status and sampling period, and $e_{ijklmno}$ represented the random residual. The effect of days in milk was modeled using a Wilmink function ($b_1 \times DIM + b_2 \times e^{(-0.05 \times DIM)}$; Wilmink, 1987). An unstructured variance-covariance matrix was used to estimate the random effects and an autoregressive correlation (AR1) was used to model the correlation among the consecutive timepoints (S_p) at the quarter level. To achieve data normality, somatic cell counts ($\times 10^3$) were (natural) log transformed for all statistical analyses and were back transformed for presentation in tables and figures. Statistical models were assessed using the SAS MIXED procedure (version 9.4, SAS). The goodness of fit and appropriateness of the various random effects (herd, cow, quarter and AR1) were assessed using BIC fit statistics. The AR(1)

[2] See: International Dairy Federation guidelines — IDF. https://store.fil-idf.org/product/guidelines-for-the-use-and-interpretation-of-bovine-milk-somatic-cell-counts-scc-in-the-dairy-industry/ (Accessed 11 May 2020).

and cow variances were substantial (as demonstrated by the large differences in BIC; Supplementary Table S1). Estimates of variance components and AR(1) correlations are shown in Supplementary Table S2.These estimates were provided for use by other researchers for planning and experimental design purposes. $P \leq 0.05$ was considered statistically significant.

Results

A total of 257 of 870 quarter milk samples (29.5%) had positive BC results. Minor pathogens (*non-aureus* staphylococci and *Corynebacterium* spp.) accounted for 15% (131 isolates) of these, while 14.5% ($n = 126$ isolates) were major pathogens (environmental streptococci, *S. aureus*, *Streptococcus agalactiae*, *Streptococcus* like-bacteria, Gram-negative bacteria and yeast spp.; Table 1).

Mammary quarters chronically infected by major pathogens had lower milk and components yield when compared with healthy quarters (Table 2). Quarters chronically infected by major pathogens had higher SCC (678.57×10^3 cells/mL SE 216.48, $n = 96$) compared with quarters with SM (157.59×10^3 cells/mL SE 132.24, $n = 126$) and healthy quarters (64.07×10^3 cells/mL SE 2.75, $n = 363$). Healthy quarters produced 1.13 kg/quarter milking more than quarters chronically infected by major pathogens ($P = 0.0014$, Table 2). Additionally, healthy quarters produced greater milk component yields (total solids +251.88 g/quarter day; fat +66.03; total protein +58.27; lactose +108.73 and solids nonfat +186.66) than quarters chronically infected by major pathogens. Chronic quarters infected by minor pathogens had similar milk and component yields when compared with healthy quarters; with the exception of SCC (Table 3).

Milk and components yields did not differ significantly from healthy quarters when compared with quarters subclinically infected by *S. agalactiae* ($P = 0.4573$; $n = 7$), environmental streptococci ($P = 0.2641$; $n = 5$), *Streptococcus* like-bacteria ($P = 0.2179$; $n = 16$), Gram-negative bacteria ($P = 0.2638$; $n = 10$), *S. aureus* ($P = 0.8823$; $n = 2$), non-*aureus* staphylococci ($P = 0.1541$; $n = 23$) and *Corynebacterium* spp. ($P = 0.2050$; $n = 43$), data not shown.

Quarters with CSM and infected by *S. aureus* and environmental streptococci had lower milk and component yields in comparison with healthy quarters (Table 4). Quarters with CSM and infected by *S. aureus* had higher SCC (314.19×10^3 cells/mL SE 93.39, $n = 48$) than healthy quarters (56.82×10^3 cells/mL SE 2.75; $n = 363$). In addition, healthy quarters produced more milk (0.80 kg/quarter milking) and component yields (total solids +224.53 g/cow day; fat +57.96; total protein +58.76; lactose +92.50 and solids nonfat +189.83) than quarters chronically infected by *S. aureus*. Similarly, quarters chronically infected by environmental streptococci pathogens had higher SCC ($1{,}187.96 \times 10^3$ cells/mL SE 391.61; $n = 48$) than healthy quarters (56.82×10^3 cells/mL SE 2.75; $n = 363$). Thus, healthy quarters had higher milk (1.33 kg/quarter milking) and component yields (total solids +257.57 g/cow day; fat +68.50; total protein +54.81; lactose +115.19 and solids nonfat +168.87) than quarters chronically infected by environmental streptococci.

Table 1
Bacteriological culture results from quarters with subclinical mastitis (SM) and chronic subclinical mastitis (CSM).

Culture results	Udder health status[a]	
	Subclinical	Chronic
Negative	216 (24.8%)	34 (3.9%)
Non-*aureus* staphylococci[b]	23 (2.6%)	43 (4.9%)
Corynebacterium spp.	43 (4.9%)	22 (2.5%)
Environmental streptococci[c]	5 (0.6%)	36 (4.1%)
Staphylococcus aureus	2 (0.2%)	41 (4.7%)
Streptococcus agalactiae	7 (0.8%)	2 (0.2%)
Streptococcus like-bacteria[d]	16 (1.8%)	5 (0.6%)
Gram-negative bactéria[e]	10 (1.1%)	0
Yeast spp.	2 (0.2%)	0
Mammary gland status, Total	324 (37.2%)	183 (21.0%)

[a] A total of 363 mammary quarter (41.7%) had no bacterial growth and SCC < 200,000.
[b] *S. chromogenes* ($n = 63$), *S. epidermidis* ($n = 1$), *S. haemolyticus* ($n = 1$) and *S. hyicus* ($n = 1$).
[c] *S. uberis* ($n = 40$) and *S. dysgalactiae* ($n = 1$).
[d] *Aerococcus viridans* ($n = 10$), *Enterococcus faecalis* ($n = 3$), *Enterococcus faecium* ($n = 3$), *Enterococcus gallinarum* ($n = 3$), *Lactococcus garvieae* ($n = 1$) and *Lactococcus lactis* ($n = 1$).
[e] *Klebsiella* spp. ($n = 4$), *Citrobacter* spp. ($n = 2$), *Acinetobacter* spp. ($n = 1$), *Enterobacter* spp. ($n = 1$), *Proteus* spp. ($n = 1$) and *Pseudomonas* spp. ($n = 1$).

Discussion

Our study determined whether there were differences in milk and component yields when healthy quarters were compared with those with CSM. In previous studies, SM was associated with increased SCC (per definition) and decreased milk production (Gonçalves et al., 2018a; Gonçalves et al., 2018b). However, few studies have investigated these effects in cows with CSM (Swinkels et al., 2005; Boutet et al., 2007; Steeneveld et al., 2007; Cardozo et al., 2015). To the best of our knowledge, there are very few published studies investigating the extent to which CSM influences SCC and milk production using repeated milk quarter sampling. Repeated milk quarter sampling would be expected to increase the accuracy of the estimation since mammary quarters are anatomically independent milk producing units (Schukken et al., 2003; Berglund et al., 2007; Viguier et al., 2009). Our study was unique because measurements of total milk yield using individual mammary quarter milkings were compared according to pathogen group. In general, quarters with CSM had lower milk and component yields compared with healthy quarters. Milk losses varied according to the type of pathogen causing CSM, and were higher when associated with the major pathogens *S. aureus* and environmental streptococci, when compared with healthy quarters.

Previous studies have suggested that milk losses were associated with damage caused by the intramammary infection and the inflammatory response of the mammary gland, which could result in the permanent loss of milk synthesis capacity (Coulon et al., 2002; Leitner et al., 2006; Blum et al., 2014; Bobbo et al., 2017). Berglund et al. (2007) compared milk production of mammary quarters with SM (six samplings, SCC > 200,000 cells/mL) with healthy quarters, but found no effect of SM on milk yield, which differed from our results. Surprisingly, milk yield was similar when we compared subclinical vs. healthy quarters, and was different from those reported by Forsback et al. (2009), who reported a milk loss of 0.3 kg/quarter milking when comparing healthy quarters with those affected by SM. It is possible that we would have observed a similar milk loss (0.3 kg/quarter milking), when we compared healthy quarters with those affected by SM, if we had not pre-selected all cows for chronic infection.

Major pathogens causing CSM were associated with milk losses of 1.13 kg/quarter milking (0.80 kg/quarter milking caused by *S. aureus*; 1.33 kg/quarter milking by environmental streptococci). However, we found no milk yield differences when quarters with SM were compared with healthy quarters. It is possible that significant effects would have been demonstrated if we had evaluated a larger number of quarters with SM. In contrast to our results, Tesfaye et al. (2010) reported that quarters subclinically infected by *S. aureus* had milk losses ranging from 0.4 to 0.8 kg/quarter milking when compared with healthy quarters. In addition, lower milk losses (0.1 kg/quarter milking) were described by Botaro et al. (2015), when milk yield of quarters infected by *S. aureus* were compared with contralateral healthy quarters in the

Table 2
Effect of subclinical mastitis and chronic subclinical mastitis caused by major pathogens on milk and components yield compared with healthy quarters. Losses were measured by differences in milk yield or components between 'Healthy - subclinical mastitis (SM)' and 'Healthy - chronic subclinical mastitis (CSM)'.

Trait	Udder health status						*P*
	Healthy [a] (least squared mean) [standard error]		SM (least squared mean) [standard error]		CSM (least squared mean) [standard error]		
No.	363		126		96		–
LnSCC ($\times 10^3$cells/mL)	4.16 [64.07]	(0.17)^{C}	5.06 [157.59]	(0.19)^{B}	6.52 [678.57]	(0.22)^{A}	<0.0001
Milk yield (kg/quarter milking)	3.78	(0.27)^{A}	3.59	(0.30)^{A}	2.65	(0.33)^{B}	<0.0001
Milk losses (kg/quarter milking)	Reference		ns		1.13		<0.0001
Milk Components (g/quarter day)							
Fat	229.99	(19.90)^{A}	207.40	(20.93)^{B}	163.96	(22.61)^{C}	<0.0001
Fat losses	Reference		ns		66.03		<0.0001
Total protein	220.64	(20.91)^{A}	204.38	(21.98)^{A}	162.36	(23.45)^{B}	<0.0001
Total protein losses	Reference		ns		58.27		<0.0001
Lactose	316.90	(35.71)^{A}	287.14	(37.26)^{A}	208.17	(39.59)^{B}	<0.0001
Lactose losses	Reference		ns		108.73		<0.0001
Total solids	835.00	(82.45)^{A}	761.85	(86.19)^{A}	583.12	(91.78)^{B}	<0.0001
Total solids losses	Reference		ns		251.88		<0.0001
Solids non-fat	605.37	(63.87)^{A}	554.20	(66.79)^{A}	418.71	(71.05)^{B}	<0.0001
Solids non-fat losses	Reference		ns		186.66		<0.0001

LnSCC, Back transformed somatic cell count; ns, not significant.
Upper case letters within row indicate statistically significant differences ($P < 0.05$).

Table 3
Effect of subclinical mastitis (SM) and chronic subclinical mastitis (CSM) caused by minor pathogens on milk and components yield compared with healthy quarters. Losses were measured by the differences of milk yield or components between 'Healthy - SM' and 'Healthy - CSM'.

Trait	Udder health status						*P*
	Healthy[a] (least squared mean) [standard error]		SM (least squared mean) [standard error]		CSM (least squared mean) [standard error]		
No.	363		198		84		–
LnSCC ($\times 10^3$cells/mL)	4.14 [62.80]	(0.16)^{C}	5.43 [228.14]	(0.17)^{A}	5.09 [162.38]	(0.21)^{A}	<0.0001
Milk yield (kg/quarter milking)	3.63	(0.30)	3.30	(0.32)	3.60	(0.37)	0.1781
Milk losses (kg/quarter milking)	Reference		ns		ns		0.9172
Milk Components (g/quarter day)							
Fat	219.53	(18.34)	199.44	(19.54)	215.41	(22.96)	0.2063
Fat losses	Reference		ns		ns		0.8076
Total protein	212.40	(20.37)	194.58	(21.53)	208.12	(24.49)	0.2757
Total protein losses	Reference		ns		ns		0.7944
Lactose	302.62	(36.48)	275.42	(37.18)	293.25	(41.43)	0.2923
Lactose losses	Reference		ns		ns		0.7147
Total solids	800.05	(80.26)	728.00	(84.41)	779.93	(95.37)	0.2320
Total solids losses	Reference		ns		ns		0.7469
Solids non-fat	579.90	(63.21)	529.65	(66.26)	566.17	(74.30)	0.2888
Solids non-fat losses	Reference		ns		ns		0.7710

LnSCC, Back transformed somatic cell count; ns, not significant.
Upper case letters within row indicate statistically significant differences ($P < 0.05$).

same cow. Milk losses caused by environmental streptococci were 0.6 kg/quarter milking, when healthy vs. infected quarters were compared within cow (Bezman et al., 2015).

We observed lower milk component yields, but higher milk component concentrations, when quarters affected by CSM caused by major pathogens were compared with healthy quarters. Higher concentrations of fat and protein in cows with CSM caused by major pathogens (data not shown) could be contributed to by compensation for lower milk yield. Quarters chronically infected with major pathogens had higher total protein concentrations (%) but lower lactose concentrations compared with healthy quarters. Milk protein concentrations could be increased in quarters with IMI because of increased permeability of the blood-milk barrier, leading to an increased concentration of milk Na^+ and Cl^- and a concurrent efflux of lactose and K^+ into the circulation (Bansal et al., 2005; Gonçalves et al., 2018b). Lactose has a major osmotic regulatory function in milk synthesis and is a very stable component in milk (Forsback et al., 2010). Additionally, when SCC is increased, there is an influx of whey proteins such as bovine serum albumin and immunoglobulins from the blood to the milk; there may also be increases in proteolytic activity associated with indigenous milk enzymes (Fox and Kelly, 2006).The fat yield was lower in quarters with CSM in comparison with healthy quarters. Previous studies reported that increased SCC in milk was associated with higher lipolytic enzyme activities in response to the IMI (Santos et al., 2003). Lipolytic enzymes cause damage to the membrane of milk fat globules, exposing them to degradation by lipoprotein lipase in the milk, which leads to higher levels of free fatty acids in milk (Forsback et al., 2010).

Our intent was to use repeated milk samplings to gain insight into the associations between CSM and milk yield, and to advance previous studies of SM which were based on a single milk sampling (e.g., Leitner et al., 2006; Bezman et al., 2015; Gonçalves et al., 2018b). Cows were initially selected on the basis of three bi-weekly composite samples in combination with positive milk bacteriological culture. Selected cows were subjected to further study using

Table 4
Effect of chronic subclinical mastitis caused by Staphylococcus aureus and environmental streptococci pathogens on milk and components yield in comparison with healthy quarters.

Trait	Healthy (least squared mean) [standard error]		*Staphylococcus aureus* (least squared mean) [standard error]		Environmental streptococci (least squared mean) [standard error]		*P*
No.	363		48		48		–
LnSCC ($\times 10^3$cells/mL) [a]	4.04 [56.82]	$(0.15)^C$	5.75 [314.19]	$(0.21)^B$	7.08 [1,187.96]	$(0.26)^A$	0.0043
Milk yield (kg/quarter milking)	3.85	$(0.28)^A$	3.05	$(0.41)^B$	2.47	$(0.39)^B$	0.0194
Milk losses (kg/quarter milking)	Reference		0.80		1.33		0.0176
Milk Components (g/quarter day)							
Fat	232.91	$(19.66)^A$	174.95	$(26.97)^B$	163.94	$(26.60)^B$	0.0204
Fat losses	Reference		57.96		68.50		0.0057
Total protein	223.19	$(20.66)^A$	164.43	$(27.18)^B$	167.53	$(26.94)^B$	0.0114
Total protein losses	Reference		58.76		54.81		0.0033
Lactose	322.23	$(35.80)^A$	229.72	$(45.53)^B$	204.98	$(45.25)^B$	0.0126
Lactose losses	Reference		92.50		115.19		0.0036
Total solids	846.63	$(81.71)^A$	622.10	$(105.80)^B$	585.26	$(105.01)^B$	0.0113
Total solids losses	Reference		224.53		257.57		0.0032
Solids non-fat	614.07	$(63.74)^A$	445.20	$(81.89)^B$	420.99	$(81.39)^B$	0.0128
Solids non-fat losses	Reference		168.87		189.83		0.0037

LnSCC, Back transformed somatic cell count.
Upper case letters within row indicate statistically significant differences ($P < 0.05$).

bi-weekly quarter milk sample testing for SCC, bacteriological culture, milk production and milk component yields. The recommended SCC cut-off of >200,000/mL for SM was used (Schukken et al., 2003; IDF, 2013). Our study design had a number of limitations. Firstly, the prior SCC history of the cow was not considered when selecting all lactating cows for composite milk sampling. Moreover, there is always the possibility of false negative culture results from quarters that, in reality, were infected with chronic pathogens. Another consideration is that the relatively brief 2-week duration between samplings might have made the distinction between chronic and non-chronic subclinical mastitis less distinct. However, longer sampling intervals could have allowed time for new infections to occur, and potentially reduced our ability to determine whether consecutive sampling was a more accurate estimate of the effect of CSM on milk yield. Despite these limitations, our results should be an improvement on studies based on single sampling methods. Finally, findings reported here mainly substantiate milk losses associated with subclinical mastitis under Brazilian conditions.

Conclusions

Chronically infected mammary quarters had lower milk and components yield when compared with healthy quarters. Milk losses varied according to the type of pathogen, and were higher than in healthy quarters when infections were caused by major pathogens such as *S. aureus* and environmental streptococci.

Conflict of interest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.tvjl.2020.105473.

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