Journal of Dairy Research

cambridge.org/dar

Chronic subclinical mastitis reduces milk and components yield at the cow level

Larissa Martins[1], Melina Melo Barcelos[1], Roger I. Cue[2], Kevin L. Anderson[3], Marcos Veiga dos Santos[1] and Juliano Leonel Gonçalves[1]

[1]Department of Animal Nutrition and Production, School of Veterinary Medicine and Animal Sciences, University of São Paulo, 13635-900 Pirassununga Montreal, Quebec, SP, Brazil; [2]Department of Animal Science, Macdonald Campus, McGill University, H9X-3V9, Canada and [3]Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA

Research Article

Cite this article: Martins L, Barcelos MM, Cue RI, Anderson KL, Santos MV and Gonçalves JL (2020). Chronic subclinical mastitis reduces milk and components yield at the cow level. *Journal of Dairy Research* **87**, 298–305. https://doi.org/10.1017/S0022029920000321

Received: 25 September 2019
Revised: 9 March 2020
Accepted: 12 March 2020
First published online: 13 May 2020

Keywords:
Chronic; pathogen; subclinical mastitis

Author for correspondence:
Juliano Leonel Goncalves,
Email: julianolg@usp.br

Abstract

We evaluated the effects of chronic subclinical mastitis (CSM) caused by different types of pathogens on milk yield and milk components at the cow level. A total of 388 Holstein cows had milk yield measured and were milk sampled three times at intervals of two weeks for determination of SCC and milk composition, and microbiological culture was performed. Cows were considered healthy if all three samples of SCC were ≤200 000 cells/ml and were culture-negative at the third milk sampling. Cows with one result of SCC > 200 000 cells/ml were considered to suffer non-chronic subclinical mastitis whereas cows with at least 2 out of 3 results of SCC > 200 000 cells/ml had CSM. These latter cows were further sorted according to culture results into chronic negative-culture or chronic positive-culture. This resulted in four udder health statuses: healthy, non-chronic, chronicNC or chronicPC. The milk and components yields were evaluated according to the udder health status and by pathogen using a linear mixed effects model. A total of 134 out of 388 cows (34.5%) were chronicPC, 57 cows (14.7%) were chronicNC, 78 cows (20.1%) were non-chronic and 119 cows (30.7%) were considered healthy, which resulted in a grand total of 1164 cow records included in the statistical model. The healthy cows produced more milk than each of the other groups (+2.1 to +5.7 kg/cow/day) and produced higher milk component yields than the chronicPC cows. The healthy cows produced more milk than cows with chronicPC caused by minor (+5.2 kg/cow/day) and major pathogens (+7.1 kg/cow/day) and losses varied from 5.8 to 11.8 kg/cow/day depending on the pathogen causing chronicPC mastitis. Chronic positive-culture cows had a reduction of at least 24.5% of milk yield and 22.4% of total solids yield.

Subclinical mastitis (SM) is the most prevalent disease of dairy cows, and is characterized by an increased somatic cell count (SCC), altered milk composition and lower milk yield (Seegers *et al*., 2003; Halasa *et al*., 2007). The duration of SM may be short or long-lasting, the latter is usually considered chronic subclinical mastitis (CSM) (Viguier *et al*., 2009). Several studies have evaluated milk losses (ML) due to SM based on the assumption that a rise in SCC above a threshold of 200 000 cells/ml causes a concomitant reduction in milk yield (Schukken *et al*., 2003; Bradley and Green, 2005; Hagnestam-Nielsen *et al*., 2009; Hand *et al*., 2012; Archer *et al*., 2013). For example, ML evaluated from milk herd test records were estimated to be 0.3 to 0.7 kg/day for first-lactation cows and 0.6 to 1.9 kg/day for adult-lactation cows per unit increase of Ln SCC above the cutoff of 5.3 (200 000 cells/ml) (Dürr *et al*., 2008; Hagnestam-Nielsen *et al*., 2009; Halasa *et al*., 2009; Hand *et al*., 2012; Gonçalves *et al*., 2018*a*).

Despite previous studies determining ML based on SCC, none have used our approach of evaluating the effects of CSM caused by type of pathogen at the cow-level. How much of the increase of SCC in a cow affected by CSM is reflected in milk yield and quality at the cow-level? We questioned whether CSM could accentuate the effects on milk yield and composition when compared to non-chronic cases since there are results emphasizing that *S. aureus*-CSM produces virulence factors (cytolytic toxins, exfoliative toxin (TSST-1) and hyaluronidases which cause epithelial damage and mammary gland parenchyma necrosis) that may gradually lead to the replacement of the secretory tissue by a fibrotic one (Gudding *et al*., 1984; Barkema *et al*., 2009). Changes in milk yield and quality strongly depend on the pathogen (Coulon *et al*., 2002). Thus, we suggest major economic losses might be expected from CSM cases because mammary tissue from non-chronic cases can presumably return to some level of functionality. To our knowledge, few reports consider our comparison (CSM vs. non-chronic) using repeated milk sampling, and only Halasa *et al*. (2009) proposed an evaluation of the SM effects on milk composition.

Our hypothesis is that ML and any effects on components yield caused by CSM is higher than for SM, and is differently affected according to pathogen. Therefore, the study aimed to

Hannah Dairy Research Foundation

CAMBRIDGE UNIVERSITY PRESS

evaluate the effects of CSM caused by type of pathogens on milk and components yield at the cow level.

Materials and methods

Ethics approval was obtained from the Ethical Committee on the Use of Animals of the School of Veterinary Medicine and Animal Science, University of São Paulo (CEUA, FMVZ, USP – Brazil/SP, protocol number 3020/2013).

Data collection: selection of herds and cows, sampling procedures and laboratory procedures

Herds and cow selection

Herds were selected based on (i) the proximity to the milk quality lab (<100 km), (ii) having permanent individual cow identification and data recording systems in place and, (iii) use of a mastitis control program consistent with those established by the National Mastitis Council (NMC; http://www.nmconline.org). This included consistent use of pre- and postmilking teat dipping, application of dry cow therapy, periodic milking machine maintenance, and proper milking and intramammary treatment procedures. For instance, a cow with a previous history of clinical mastitis or a cow that had an episode of clinical mastitis during the experiment had to be eliminated from the study. These criteria led to the selection of eight commercial dairy herds (*freestall* facilities and herringbone-type milking parlor) located in the Midwest area of São Paulo State, Brazil which were enrolled for a nine-month sampling period. All farms were considered small or medium-sized, with an average of 82 Holstein dairy cows in lactation (range 23 to 165) and a milk yield average of 22.3 kg/cow/day (range 10 to 62.5 kg/cow/day) or approximately 6,792 kg/cow/year.

A total of 790 cows were initially sampled; the intention was to collect three samples from each cow, once every two weeks producing a total of 2087 records. Cow were excluded for clinical mastitis, being dried-off (and hence not having all three samplings) in the study period and excessive days in milk as detailed in Table 1. After all editing there were 388 cows (from six herds) which had provided all three samples. Each milk sample was analyzed for SCC and milk composition (fat, protein, lactose, solids non-fat, and total solids). At the third sampling, composite milk samples were collected aseptically for microbiological culture.

Bacterial species identification using MALDI-TOF MS

Microbiological analyses of milk samples were performed in accordance with the National Mastitis Council guidelines (NMC, 2017). For bacterial species identification using MALDI-TOF MS, one colony was applied to the steel plate spot with the aid of a wooden stick (Barcelos *et al*., 2019). 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 α-cyano-4-hydroxycinnamic acid (HCCA) matrix solution was applied, and again left to dry at room temperature for 5 to 10 min. A standard protein solution (Bacterial Test Standard, BTS; Bruker) was used for calibration. The analysis employing the MALDI-TOF mass spectrometry methodology was performed in FlexControl 3.4 software (Bruker Daltonik, Bremen, Germany). The spectral data processing was done using the MALDI Biotyper 4.1.70 (Bruker Daltonik, Bremen, Germany) computer software for microorganism identification (MBT version 7311 MPS library) and a score of ≥ 2.0 indicated a species-level identification.

Milk composition analysis

Milk samples (40 ml) were collected into plastic tubes containing the antimicrobial bronopol (2-bromo-2-nitropropane-1,3-diol) as preservative (0.05 g/100 ml milk) according to the International Dairy Federation guidelines (IDF, 2013). Samples were kept refrigerated (4–7°C) until analysis of composition and SCC. Concentrations of milk fat, protein, lactose, total solids and solids non-fat were determined by infrared absorption system using a milk analyzer (Bentley 2000®, Bentley Instruments Inc., Chasca, MN USA). The SCC was determined by flow cytometry using a high capacity somatic cell counter (Somacount 300®, Bentley Instruments Inc., Chasca, MN, USA).

Data preparation and mastitis definitions

Cows were considered healthy if all three samples of SCC were ≤200 000 cells/ml and were culture-negative. Cows with one SCC > 200 000 cells/ml and a culture-positive result were considered non-chronic (SM). Cows with at least two of the three results with a SCC > 200 000 cells/ml were considered CSM. These CSM were then further sorted on the bacteriology to be chronic negative (chronicNC) or chronic positive (chronicPC) cows. Culture-positive results were considered for mastitis definition when milk samples showed an isolation of >10 colonies (1000 cfu/ml) of minor pathogens (*Corynebacterium* spp. or non-*aureus Staphylococci*); and ≥1 colony (100 cfu/ml) of other pathogens mastitis-causing. There were therefore four udder health categories: healthy, non-chronic, chronicNC or chronicPC.

The milk yield and compositions were evaluated according to the udder health status (healthy, non-chronic, chronicNC or chronicPC), and to pathogen groups (1 - major pathogens and 2 -minor) and by type of pathogen (*Streptococcus uberis*, *Streptococcus dysgalactiae*, *Staphylococcus aureus*, *Streptococcus agalactiae*, non-*aureus Staphylococci* and *Corynebacterium* spp.). Milk samples with more than one pathogen type detected were not included in the model ($n = 10$). From four milk samples, two different major pathogens were isolated, and from six milk samples, minor and major pathogens were observed. Milk samples with more than two pathogens detected were considered contaminated ($n = 11$) and were eliminated from all subsequent analyses (Table 1).

Editing criteria and statistical analysis

The number of records excluded and retained at each step of the editing and the reasons for the exclusions are shown in Table 1. After editing, there were 1164 records retained for the initial analyses of the effects of CSM on milk and components yields. The effects of CSM on milk and components yields were evaluated (Table 2). A linear mixed effects model was used for the analysis; for each of the dependent variables there were three measurements (at days 1, 15 and 30 of sampling, considered as sampling 1, 2 or 3 respectively) on each cow, and cow (random effect) was nested within herd, udder health status and parity. Statistical models were assessed using the SAS MIXED procedure (version 9.4, SAS Institute, Cary, NC, USA). With regard to all of the statistical analyses, $P \leq 0.05$ was considered for significance. Thus, the following statistical models were applied:

https://doi.org/10.1017/S0022029920000321 Published online by Cambridge University Press

Table 1. Editing criteria and number of records retained and excluded

Excluded cows-records based on the following criteria	Initial number of records at each edit step	Number of records retained	Number of records excluded
Two herds without parity information	2087	1725	362
Two cows removed due to inconsistent data	1725	1719	6
Contaminated milk samples with more than two pathogens	1719	1698	21
Missing production information (milk yield, parity, DIM)	1698	1542	156
Days in milk ≤500 days	1542	1491	51
SCC ≤ 200×10^3 cells/ml but positive-culture results	1491	1400	91
Cows with less than 3 biweekly consecutive records	1400	1164	236

(1) $Y_{ijkmpqr} = \mu + H_i + \text{Status}_j(\text{UdderHealth}) + \text{Parity}_m + \text{Cow}_{ijkm} + \text{wDIM}_p + \text{Sampling}_q + \text{Sampling} \times \text{Status}_{jq} + e_{ijkmpqr}$

(2) $Y_{ijkmpqr} = \mu + H_i + \text{Status}_j(\text{PathogenType}) + \text{Parity}_m + \text{Cow}_{ijkm} + \text{wDIM}_p + \text{Sampling}_q + \text{Sampling} \times \text{Status}_{jq} + e_{ijkmpqr}$

in which $Y_{ijkmpqr}$ was considered as the continuous dependent variable, in turn log SCC, milk yield, fat yield and fat %, total protein yield and %, lactose yield and %, total solids yield and %, and solids not fat yield and % (see Table 3). μ represented the general average. H_i represented the herd ($i = 1$ to 6), considered as a fixed effect since herds were selected based on their willingness to collaborate in this study. For model 1, Status_j(UdderHealth) represented the presence or absence of infection during the three milk samplings ($j = 1$ to 4; healthy, non-chronic, chronicNC or chronicPC). For model 2, Status_j(PathogenType) represented the status regarding the type of pathogen during the three milk samplings ($o = 1$ to 8; major, minor, *S. uberis, S. dysgalactiae, S. aureus, S. agalactiae,* Non-*aureus Staphylococci* and, *Corynebacterium* spp.). Cow_{ijkm} represented the cow (within herd, status and parity) which was considered as a random effect ($k = 1$ to 388). Parity_m was considered as the fixed classification effect of the number of calvings ($m = 1$, 2 and ≥3). wDIM_p represents the weeks in milk, included as a fixed classification effect ($P = 1$ to 69). Sampling_q was the effect of time, milk samplings once every two weeks as interval ($q = 1$ to 3, sampling at 1st, 15th and 30th days of the study). $\text{Sampling} \times \text{Status}_{jq}$ was the interaction between sampling period and udder health status, and $e_{ijkmpqr}$ represented the random residual. Herd was considered fixed, as the selection of the herds cannot completely be considered random; in addition, with only six herds the reliability of any herd variance estimate would be low. Our interest was not to assess the variability amongst herds, rather the focus was on comparing the four cow udder health statuses. To achieve a normal distribution, somatic cell counts ($\times 10^3$) were (natural) log transformed for all statistical analyses, and were back transformed for presentation in tables and figures. Initial analyses, for both Model 1 and 2, included a herd × status interaction, to test whether the effects were the same/similar across herds, or not. The herd interactions were not statistically significant, this term was dropped from the model and is not shown.

Results

A total of 134 out of 388 cows (34.5%) had chronicPC episodes, 57 cows (14.7%) had chronicNC, 78 cows (20.1%) had non-chronic episodes and 119 cows (30.7%) were considered healthy, which resulted in a grand total of 1,164 cow records included in the statistical model (Tables 1 and 2). The culture-positive cows ($n = 330$) comprised 47.3% with minor pathogen infections ($n = 156/330$; Non-*aureus Staphylococci*, $n = 126$; and *Corynebacterium* spp., $n = 30$), 44.5% with major pathogen infections ($n = 147/330$; *S. uberis,* $n = 42$; *S. dysgalactiae,* $n = 36$; *S. agalactiae* $n = 39$; and *S. aureus,* $n = 30$) and the remainder with *Streptococcus* like-bacteria (6.4%, $n = 21/330$) or *Klebsiella* spp. (1.8%, $n = 6/330$) infections (Table 2).

Effects of udder health status on milk and components yield

Cows with chronicNC and chronicPC had lower milk and components yields when compared with non-chronic and healthy cows ($P < 0.05$; Table 3). ChronicPC cows had a higher SCC (756.2×10^3 cells ml, $n = 402$) in comparison with chronicNC cows (437.1×10^3 cells/ml, $n = 171$), non-chronic cows (168.8×10^3 cells ml, $n = 234$), and healthy cows (62.2×10^3 cells/ml, $n = 357$). The healthy cows produced more milk than non-chronic chronic cows (+2.1 kg/cow/day), chronicNC (+4.1 kg/cow/day) and chronicPC (+5.7 kg/cow/day). Non-chronic mastitis caused a 9% of milk reduction, 17.3% of reduction when cows had chronicNC and 24.1% when cows had chronicPC, but milk reduction was even higher in cows where chronicPC was caused by major pathogens (29.5%).

The milk component yields of the healthy cows were similar to those observed for non-chronic cows, except for lactose (healthy, 108.5 g/cow/day *vs.* non-chronic, 97.5 g/cow/day: Table 3). ChronicNC cows' component yields were lower than those from healthy cows except for fat (healthy, 93.78 g/cow/day *vs.* chronicNC, 87.0 g/cow/day). In addition, healthy cows produced more milk components than chronicPC cows (total solids, +69.6; fat +19.4; total protein +15.1; lactose +29.9 and solids non-fat +50.3 g/cow/day)

Effects of type of pathogens causing CSM on milk and components yield

ChronicPC cows infected by both major and minor pathogens cows had lower milk and component yields when compared with healthy cows ($P < 0.05$; Table 4). The SCC of chronicPC cows caused by major pathogens (792.7×10^3 cells, $n = 129$) and minor pathogens (644.3×10^3 cells, $n = 111$) were much higher than for healthy cows (about 60×10^3 cells, $n = 357$). Consequently, healthy cows produced more milk than cows with chronicPC caused by minor

https://doi.org/10.1017/S0022029920000321 Published online by Cambridge University Press

Table 2. Frequency of cow records according to udder health status (healthy, non-chronic, chronicPC and chronicNC) and culture results (N, negative; or P, pathogen isolation) based on three milk samplings

	Cows status categorization			
Udder health status, culture results and pathogens sub-datasets	Healthy	Non-chronic	ChronicPC[a]	ChronicNC[b]
Negative	357	165	141	171
Minor pathogens	0	45	111	0
Non-*aureus Staphylococci*	0	39	87	0
Corynebacterium spp.	0	6	24	0
Major pathogens	0	18	129	0
Streptococcus uberis	0	0	42	0
Streptococcus dysgalactiae	0	9	27	0
Staphylococcus aureus	0	6	24	0
Streptococcus agalactiae	0	3	36	0
Streptococcus like-bacteria[c]	0	6	15	0
Klebsiella spp.	0	0	6	0
Udder health status, total	357 (30%)	234 (20%)	402 (35%)	171 (15%)

[a]Chronic positive-culture.
[b]Chronic negative-culture.
[c]Streptococcus like-bacteria: *Enterococcus* spp. ($n = 10$), *Lactococcus* spp. ($n = 6$) and *Aerococcus* spp. ($n = 5$).
*Milk samples with more than one pathogen being detected were not included in the model ($n = 10$); in 4 milk samples, 2 different major pathogens were isolated, and in 6 milk samples, isolation of minor and major pathogens were observed. Milk samples with more than two pathogens being detected were considered contaminated ($n = 11$) and were eliminated from all analyses.

Table 3. Effect of non-chronic, chronicPC, chronicNC on milk and components yield in comparison with healthy cows

Item	Healthy			Non-chronic			ChronicPC[1]			ChronicNC[b]			*P*
No.	357			234			402			171			–
LnSCC [× 10^3 cells/mL]	4.13	D	(0.09)	5.13	C	(0.09)	6.63	A	(0.09)	6.08	B	(0.08)	<0.0001
	[62.21]			[168.80]			[756.20]			[437.07]			
Milk yield (kg/day)	23.76	A	(0.84)	21.62	B	(0.90)	18.03	C	(0.87)	19.65	B,C	(0.78)	<0.0001
Δ[c] Milk losses (kg/day)	reference			2.1 (9%)			5.7 (24.1%)			4.1 (17.3%)			
Milk components (g/100 g)													
Fat	4.13	B	(0.10)	4.38	A	(0.11)	4.30	A	(0.11)	4.48	A	(0.10)	0.0329
Total protein	3.45	B	(0.04)	3.57	A	(0.04)	3.64	A	(0.04)	3.62	A	(0.04)	0.001
Lactose	4.51	A	(0.03)	4.46	A	(0.03)	4.32	B	(0.03)	4.35	B	(0.03)	<0.0001
Total solids	13.05	B	(0.13)	13.36	A	(0.14)	13.22	A,B	(0.13)	13.40	A	(0.12)	0.0817
Solids non-fat	8.91	A	(0.04)	8.98	A	(0.05)	8.92	A	(0.04)	8.93	A	(0.04)	0.612
Milk components (g/cow/day)													
Fat	93.78	A	(3.46)	92.88	A	(3.69)	74.41	B	(3.58)	87.05	A	(3.18)	<0.0001
Total protein	78.53	A	(2.54)	74.95	A	(2.72)	63.42	B	(2.63)	69.09	B	(2.35)	<0.0001
Lactose	108.50	A	(3.93)	97.53	B	(4.20)	78.57	C	(4.07)	86.40	C	(3.63)	<0.0001
Total solids	303.34	A	(9.96)	285.82	A	(10.63)	233.68	B	(10.31)	261.08	B	(9.19)	<0.0001
Solids non-fat	209.56	A	(7.17)	192.99	A	(7.66)	159.35	B	(7.42)	174.18	B	(6.61)	<0.0001

LnSCC was back transformed and values of SCC were presented within brackets [× 10^3 cells/ml].
Standard error was presented within parentheses (SE). Different letters mean $P < 0.05$.
[a]Chronic positive-culture.
[b]Chronic negative-culture.
[c]Milk loss estimated by the difference of milk yield from healthy *vs.* non-chronic, chronicNC and chronicPC cows.

https://doi.org/10.1017/S0022029920000321 Published online by Cambridge University Press

Table 4. Effect of chronic subclinical mastitis caused by major and minor pathogens on milk and components yield in comparison with healthy cows

	Major pathogens							Minor pathogens						
Item	Healthy			ChronicPC[a]			*P*	Healthy			ChronicPC			*P*
No.	357			129			–	357			111			–
LnSCC [× 10^3 cells/ml]	4.13	B	(0.10)	6.68	A	(0.12)	<0.0001	4.10	B	(0.10)	6.47	A	(0.14)	<0.0001
	62.09			792.74				60.35			644.32			
Milk yield (kg/day)	24.12	A	(0.99)	17.00	B	(1.19)	<0.0001	24.42	A	(1.07)	19.24	B	(1.50)	0.0013
Δ^b Milk losses (kg/day)	7.12			(29.5%)				5.18			(21.2%)			
Milk components (g/100 g)														
Fat	4.26	A	(0.12)	4.36	A	(0.14)	0.5467	4.07	A	(0.13)	4.32	A	(0.18)	0.0966
Total protein	3.38	B	(0.04)	3.66	A	(0.05)	<0.0001	3.41	B	(0.04)	3.58	A	(0.06)	0.0022
Lactose	4.57	A	(0.03)	4.40	B	(0.04)	0.0014	4.55	A	(0.03)	4.27	B	(0.05)	<0.0001
Total solids	13.17	A	(0.14)	13.37	A	(0.17)	0.0044	12.99	A	(0.16)	13.13	A	(0.22)	0.1003
Solids non-fat	8.89	A	(0.05)	9.01	A	(0.06)	0.1921	8.91	A	(0.05)	8.82	A	(0.07)	0.0638
Milk Components (g/cow/day)														
Fat	99.45	A	(3.71)	72.76	B	(4.40)	<0.0001	96.32	A	(4.14)	80.89	B	(5.79)	0.0439
Total protein	79.40	A	(2.84)	61.62	B	(3.39)	0.0001	80.56	A	(3.15)	67.18	B	(4.41)	0.0096
Lactose	110.61	A	(4.59)	75.46	B	(5.50)	<0.0001	111.92	A	(4.90)	83.17	B	(6.86)	0.0002
Total solids	312.60	A	(10.89)	225.96	B	(13.02)	<0.0001	312.26	A	(12.05)	249.53	B	(16.87)	0.0013
Solids non-fat	212.78	A	(8.27)	153.36	B	(9.91)	<0.0001	215.66	A	(8.97)	168.85	B	(12.56)	0.0008

LnSCC was back transformed and values of SCC were presented within brackets [× 10^3 cells/ml].
Standard error was presented within parentheses (SE). Different letters mean $P < 0.05$.
[a]Chronic positive-culture.
[b]Milk loss estimated by the difference of milk yield from healthy *vs.* chronicPC cows.

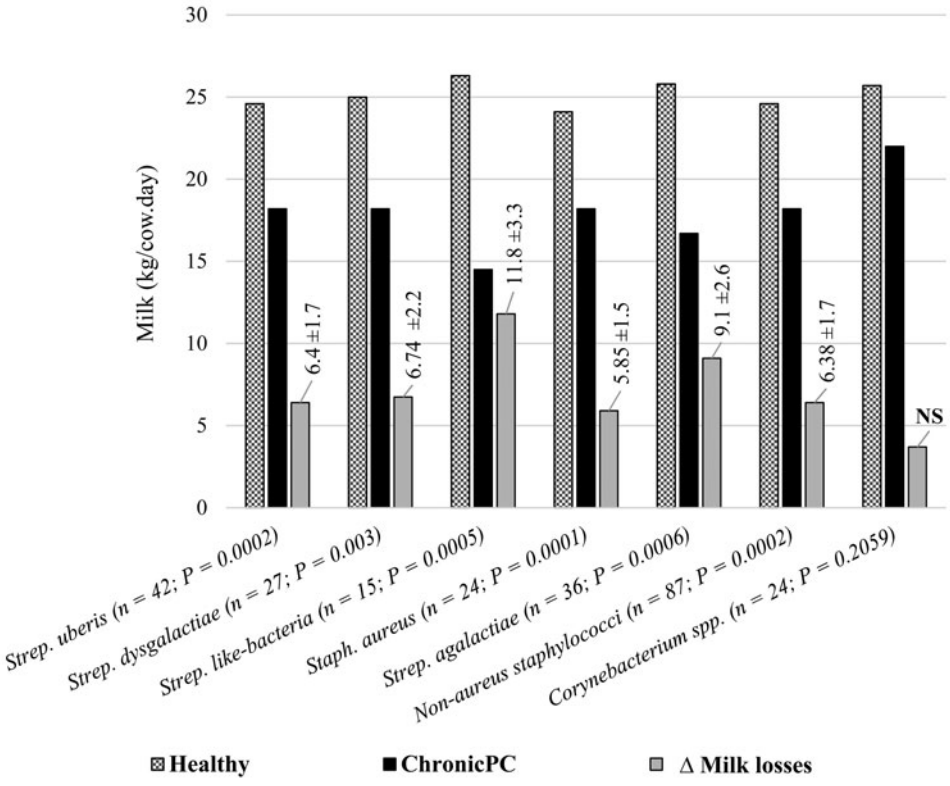

Fig. 1. Effect of chronic subclinical mastitis caused by type of pathogens on milk yield in comparison with healthy cows and, *Δ milk loss estimated by the difference of milk yield from healthy *vs*. chronicPC cows. ±SE and NS, not significant.

(+5.2 kg/cow/day) and major pathogens (+7.1 kg/cow/day). ChronicPC cows had lower milk components yields when infected by minor and major pathogens in comparison with healthy cows (Table 4).

Cows with chronicPC caused by *S. uberis*, *S. dysgalactiae*, *Streptococcus* like-bacteria, *S. aureus*, *S. agalactiae* and Non-*aureus Staphylococci* had lower milk yields when compared with healthy cows ($P < 0.05$; Fig. 1). Milk losses varied from 5.8 ± 1.5 to 11.8 ± 3.3 kg/cow/day depending on the pathogen. Milk losses per type of pathogens were 24.5% when chronicPC was caused by *S. aureus*; 26% for *S. uberis* and non-*aureus Staphylococci*, 27% for *S. dysgalactiae,* 35.3% for *S. agalactiae* and 44.9% for *Streptococcus* like-bacteria. On the contrary, chronicPC cows infected by *Corynebacterium* spp. had similar milk yields when compared with healthy cows.

Cows with chronicPC (except those infected by *Corynebacterium* spp.) had lower milk component yields than healthy cows (Fig. 2). Milk protein yield losses of chronicPC cows varied from 17.6% to 33.2%, and equivalent figures for losses of other components were, fat 16.5 to 39.6%, lactose 18.5 to 47.5%, solids non-fat 17.5 to 41.8% and total solids 17.9 to 41.5%, depending on the pathogen (Fig. 2).

Discussion

We questioned if milk yield and milk composition is altered more by CSM than by non-chronic (SM) in dairy cows, and if any differences are pathogen dependent. It is very well known that ML occurs due to damage caused by pathogens to the secretory tissues of the mammary gland and the breakdown of cell junctions, which may result in the permanent loss of milk synthesis capacity (Forsback *et al*., 2009). ML found in the present study varied from 24.5 to 44.9% in comparison with healthy cows and had a strong dependency on the pathogen type.

Non-chronic cases frequency (20.1%) are consistent with what we find in other dairy herds in Brazil,. but surprisingly higher for chronicPC cases (34.5%). Most of the composite milk samples were culture-negative (>65%) and we believe that culture was positive in 35% of the cases because cows had chronic infection. The interesting results were not only the reduced milk yield of chronicPC cows, it was that the reduction of milk yield was larger in chronicPC cows than in the other SM or SCM cows. Therefore, our obtained results emphasize the importance of the bacteriology results for preventing and controlling pathogens that cause chronic mastitis. ChronicNC had less accentuated effects on milk yield and composition which might be due to the intermittent shedding of bacteria which varies with the type of pathogen. Alternatively, the immune system of the cows may already have solved the problem prior to the milk sampling.

Similar to our study, ML based on results of a single SM (non-chronic) episode caused by *S. aureus, S. agalactiae* and environmental streptococci had a reduction of 0.6, 1.3 and 0.5 kg/day (Wilson *et al*., 1997), respectively, which were lower if we compare them with the observed ML caused by the same pathogens isolated from chronicPC cows. A previous study showed ML of 26.3% by *S. aureus*, 13.8% by CNS, 11.7% by *Streptococcus* spp. and 8.4% by *Corynebacterium* spp. (França *et al*., 2017). This ML was higher than we observed from non-chronic mastitis (14% *vs*. 9%). On the other hand, the ML observed here due to CSM was greater than the estimation of ML based on studies

https://doi.org/10.1017/S0022029920000321 Published online by Cambridge University Press

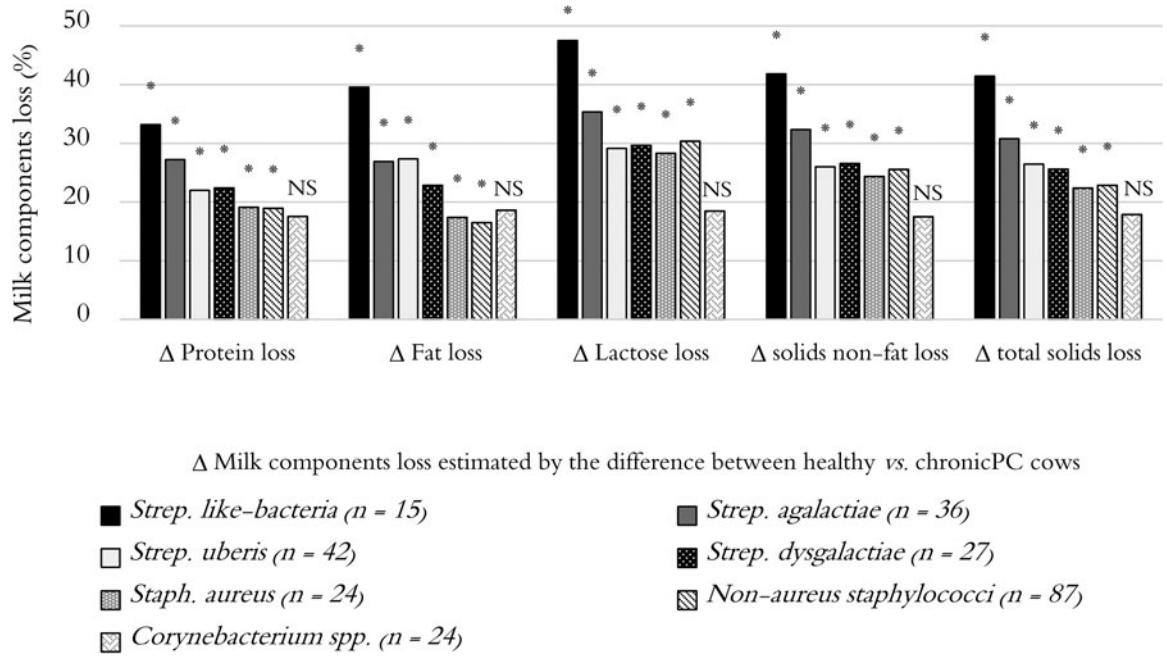

Fig. 2. Effect of chronic subclinical mastitis caused by type of pathogens on milk components yield in comparison with healthy cows and, *Δ milk components loss estimated by the difference between healthy *vs.* chronicPC cows. ±SE. *$P < 0.05$. NS, not significant.

using only SCC evaluation (Hagnestam-Nielsen *et al.*, 2009; Hand *et al.*, 2012; Gonçalves *et al.*, 2018*a*). Hand *et al.* (2012) described ML varying from 1.8 to 7.6% for first parity and 2.5 to 10.9% for second parity cows when SCC ranged from 200 to 1000×10^3 cells/ml and Hagnestam-Nielsen *et al.* (2009) found ML varying from 1.4 to 11.5% depending on the cow parity and the SCC ranging from 100 to 1000×10^3 cells/ml. To summarize, in the present study, we observed that ML in cows with CSM was more intense than cows with SM, which might depend on the persistence of the causative agent in the mammary gland.

We also observed lower milk component yield, but higher milk component concentration when healthy *vs.* chronicPC cows were compared. The higher concentrations of fat and total protein in chronicPC cows may be partially caused by the compensation effect of lower milk yield. It is known that intramammary infection changes the blood-milk barrier permeability, provoking a concurrent efflux of lactose and K+ into the bloodstream and an increase from the bloodstream of Na^+, Cl^- and whey proteins in milk (Gonçalves *et al.*, 2018*b*). Consequently, it explains why casein concentration is reduced when SCC is elevated, since there is an increase in proteolytic enzymes (Urech *et al.*, 1999; Leitner *et al.*, 2006). Our results suggest that the reduction of milk component yields varied according to the type of mastitis causing pathogens (Table 4 and Fig. 2).

A negative relationship between SCC and content of milk components, similar to what we observed in the present study, was reported by Park *et al.* (2007) when they compared cows culture-positive and SCC $\geq 500 \times 10^3$ cells. França *et al.* (2017) observed similar fat content between healthy and SM cows, but they reported an increase of 3.15% in total protein content and a decrease of 5.6% in lactose content. Milk component alteration results described by França *et al.* (2017) were much lower than those observed from chronicPC cows in the present study.

Pathogens that elicit a greater somatic cell response are considered 'major' ones (e.g. *S. agalactiae*, *S. aureus*, *S. uberis*, and *S. dysgalactiae*), in contrast to 'minors' (*Corynebacterium* spp. and non-*aureus Staphylococci*) that are more likely to result in an SCC level below 200 000 cells/ml (Bradley and Green, 2005). We have adopted this classical pathogen grouping based on species identification by MALDI-TOF, which might be considered one limitation of our study since some minor pathogens (e.g. *S. chromogenes*) have presented similar adhesiveness capacity on the mammary secretory tissue as *S. aureus* (Taponen and Pyorala, 2009).

Finally, the dairy farms used in the present study were considered to be well managed Brazilian farms. However, based on our results of chronicPC cases we may infer that Brazilian herds had more chronic cases than expected and these facts suggest the adoption of a control plan including preventive measures, e.g. monthly monitoring of SCC and culturing cows with persistent elevated SCC might help in making treatment decisions against the chronic mastitis-causing pathogen. Identifying the responsible pathogens is important, because this might suggest what treatment protocol(s) to use. For example, if the pathogen is *S. agalactiae* it is known that the use of antibiotics can give a 95% cure rate. However, the presence of an environmental pathogen may indicate the need for a change in bedding management or pre-milking holding area.

In conclusion, lactating cows with CSM had higher SCC, lower milk yield and altered milk composition when compared to cows diagnosed with SM or healthy cows, and this difference was even higher when the CSM case presented as culture-positive. The changes observed in milk yield and compositions were dependent on the type of mastitis (CSM vs. non-chronic) and the type of pathogen causing subclinical mastitis (major and minor pathogens).

Acknowledgments. The authors acknowledge FAPESP for the scholarship (Proc. 2013/23613-8, Proc. 2015/00142-5 and Proc. BEPE 2015/04570-1), and project funding (Proc. 2014/17411-6). We thank 'Qualileite', Milk Quality Laboratory (School of Veterinary Medicine and Animal Science – USP, Brazil) team and all milk producers, for their assistance with milk sampling period.

https://doi.org/10.1017/S0022029920000321 Published online by Cambridge University Press

References

Archer SC, Mc Coy F, Wapenaar W and Green MJ (2013) Association between somatic cell count early in the first lactation and the lifetime milk yield of cows in Irish dairy herds. *Journal of Dairy Science* **96**, 2951–2959.

Barcelos MM, Martins L, Grenfell RC, Juliano L, Anderson KL, dos Santos MV and Gonçalves JL (2019) Comparison of standard and on-plate extraction protocols for identification of mastitis-causing bacteria by MALDI-TOF MS. *Brazilian Journal of Microbiology* **50**, 849–857.

Barkema HW, Green MJ, Bradley AJ and Zadoks RN (2009) Invited review: the role of contagious disease in udder health. *Journal of Dairy Science* **92**, 4717–4729.

Bradley A and Green M (2005) Use and interpretation of somatic cell count data in dairy cows. *In Practice* **27**, 310–315.

Coulon JB, Gasqui P, Barnouin J, Ollier A, Pradel P and Pomiès D (2002) Effect of mastitis and related-germ on milk yield and composition during naturally-occurring udder infections in dairy cows. *Animal Research* **51**, 383–393.

Dürr JW, Cue RI, Monardes HG, Moro-Méndez J and Wade KM (2008) Milk losses associated with somatic cell counts per breed, parity and stage of lactation in Canadian dairy cattle. *Livestock Science* **117**, 225–232.

Forsback L, Lindmark-Mansson H, Andren A, Akerstedt M and Svennersten-Sjaunja K (2009) Udder quarter milk composition at different levels of somatic cell count in cow composite milk. *Animal: An International Journal of Animal Bioscience* **3**, 710–717.

França MM, Del Valle TA, Campana M, Veronese LP, Nascimento G and Morais JPG (2017) Mastitis causative agents and SCC relationship with milk yield and composition in dairy cows. *Archivos de Zootecnia* **66**, 45–49.

Gonçalves JL, Cue RI, Botaro BG, Horst JA, Valloto AA and Santos MV (2018a) Milk losses associated with somatic cell counts by parity and stage of lactation. *Journal of Dairy Science* **101**, 4357–4366.

Gonçalves JL, Kamphuis C, Martins CMMR, Barreiro JR, Tomazi T, Gameiro AH, Hogeveen H and dos Santos MV (2018b) Bovine subclinical mastitis reduces milk yield and economic return. *Livestock Science* **210**, 25–32.

Gudding R, McDonald JS and Cheville NF (1984) Pathogenesis of *Staphylococcus aureus* mastitis: bacteriologic, histologic, and ultrastructural pathologic findings. *American Journal of Veterinary Research* **45**, 2525–2531.

Hagnestam-Nielsen C, Emanuelson U, Berglund B and Strandberg E (2009) Relationship between somatic cell count and milk yield in different stages of lactation. *Journal of Dairy Science* **92**, 3124–3133.

Halasa T, Huijps K, Osteras O and Hogeveen H (2007) Economic effects of bovine mastitis and mastitis management: a review. *Veterinary Quarterly* **29**, 18–31.

Halasa T, Nielen M, De Roos AP, Van Hoorne R, de Jong G, Lam TJ, van Werven T and Hogeveen H (2009) Production loss due to new subclinical mastitis in Dutch dairy cows estimated with a test-day model. *Journal of Dairy Science* **92**, 599–606.

Hand KJ, Godkin A and Kelton DF (2012) Milk production and somatic cell counts: a cow-level analysis. *Journal of Dairy Science* **95**, 1358–1362.

IDF (2013) International Dairy Federation. 2013. Guidelines for the use and interpretation of bovine milk somatic cell counts (SCC) in the dairy industry. Bulletin of the International Dairy Federation. 466/2013. IDF, Brussels, Belgium.

Leitner G, Krifucks O, Merin U, Lavi Y and Silanikove N (2006) Interactions between bacteria type, proteolysis of casein and physico-chemical properties of bovine milk. *International Dairy Journal* **16**, 648–654.

National Mastitis Council (NMC) (2017) *Laboratory Handbook on Bovine Mastitis*. Wisconsin, USA: NMC, 148p.

Park YK, Koo HC, Kim SH, Hwang SY, Jung WK, Kim JM, Shin S, Kim RT and Park YH (2007) The analysis of milk components and pathogenic bacteria isolated from bovine raw milk in Korea. *Journal of Dairy Science* **90**, 5405–5414.

Schukken YH, Wilson DJ, Welcome F, Garrison-Tikofsky L and Gonzalez RN (2003) Monitoring udder health and milk quality using somatic cell counts. *Veterinary Research* **34**, 579–596.

Seegers H, Fourichon C and Beaudeau F (2003) Production effects related to mastitis and mastitis economics in dairy cattle herds. *Veterinary Research* **34**, 475–491.

Taponen S and Pyorala S (2009) Coagulase-negative *Staphylococci* as cause of bovine mastitis – not so different from *Staphylococcus aureus*? *Veterinary Microbiology* **134**, 29–36.

Urech E, Puhan Z and Schallibaum M (1999) Changes in milk protein fraction as affected by subclinical mastitis. *Journal of Dairy Science* **82**, 2402–2411.

Viguier C, Arora S, Gilmartin N, Welbeck K and O'Kennedy R (2009) Mastitis detection: current trends and future perspectives. *Trends in Biotechnology* **27**, 486–493.

Wilson DJ, Gonzalez RN and Das HH (1997) Bovine mastitis pathogens in New York and Pennsylvania: prevalence and effects on somatic cell count and milk production. *Journal of Dairy Science* **80**, 2592–2598.

https://doi.org/10.1017/S0022029920000321 Published online by Cambridge University Press