

## Mastitis causative agents and SCC relationship with milk yield and composition in dairy cows

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### PALAVRAS CHAVE ADICIONAIS

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### INFORMACIÓN

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### INTRODUCTION

The demand for food quality and safety is growing, given the increased consumption of customized products and governmental requirements for control strategies to reduce sanitary risks. Although milk has a perishable nature and is one of the most-consumed foods, it is also a food with high associated risk. Bovine mastitis is considered the most economically important disease for dairy production, providing hazards to food safety. Bradley (2002) and Sordillo (2011) define mastitis as the mammary gland inflammation through ceiling channel colonization by microorganisms, mostly bacteria, fungi, yeasts and algae. Due to high prevalence in some production systems, mastitis

### SUMMARY

This study aimed to evaluate the effect of mastitis pathogens prevalence and somatic cells count (SCC) on dairy cow milk yield (MY) and composition. Milk samples of Holstein cows (n=1163) with  $30.97 \pm 10.22$  kg·d<sup>-1</sup> of milk yield were collected and evaluated. *Staphylococcus* spp. was the most prevalent pathogen. In general, increased SCC decreased MY and lactose concentration and increased milk crude protein content. Coliforms had different SCC and milk crude protein concentrations relation. SCC and milk lactose content relationships were different for *S. aureus* and *Streptococcus* spp. Lactose and crude protein are the most sensitive milk components to SCC variation and this relationship is dependent on pathogen presence.

### Agentes causadores de mastite e relações entre a CCS com a produção e com a composição do leite em vacas leiteiras

### RESUMO

Este estudo teve por objetivo avaliar o efeito da prevalência de patógenos causadores de mastite e da contagem de células somáticas (CCS) sobre a produção e composição do leite. Amostras de leite de 1163 vacas da raça Holandesa com  $30.97 \pm 10.22$  kg·d<sup>-1</sup> de produção de leite foram coletadas e avaliadas. *Staphylococcus* spp. foi o patógeno mais prevalente. No geral, o aumento da CCS reduziu a produção de leite e a concentração de lactose e aumentou a concentração de proteína. Coliformes tiveram uma relação diferente entre CCS e teor de proteína no leite. A relação entre CCS e concentração de lactose foi diferente para *S. aureus* e *Streptococcus* spp. Lactose e proteína bruta são os componentes do leite mais sensíveis à variação da CCS e esta relação é dependente da presença do patógeno.

increases antimicrobial use and discards of milk and animals, and decreases milk yield (Bradley, 2002; Barkema *et al.*, 2009).

Factors that influence susceptibility to mastitis are related to the environment, milking management and animal characteristics, such as partum order, lactation stage and milk yield (Peeler *et al.*, 2000; Sordillo, 2011; Hertl *et al.*, 2014). Intramammary infections may manifest in clinical form, with systemic symptoms, physical changes in mammary gland and milk; or in subclinical form, with somatic cell count (SCC) increase, changes in milk composition and, consequently, decrease of milk yield and industrial efficiency (Halasa *et al.*, 2007; Contreras and Rodriguez, 2011).

Some studies have tried to relate the causative agents of mastitis, SCC and milk yield and composition; however the results are divergent. Olives *et al.* (2013) observed lactose concentration decrease and SCC increase with intramammary infection. Hertl *et al.* (2014) found higher milk yield loss with *E. coli* and *Klebsiella* spp. and lower for coagulase-negative staphylococci infection. According Hass *et al.* (2002), *S. aureus* infections provide high SCC for a long time. Intramammary infection showed SCC and protein concentration increase and lactose concentration and milk yield decrease (Leitner *et al.*, 2004). Therefore, this study aimed to evaluate the effect of mastitis pathogens prevalence and SCC on milk yield and composition in dairy cows.

## MATERIAL AND METHODS

### ANIMALS AND SAMPLES

Holstein cows ( $n=1163$ ) with  $30.97 \pm 10.22$  kg d<sup>-1</sup> of milk yield and  $247.82 \pm 143.51$  days in milk from one commercial herd in the region of Araras, São Paulo, Brazil were used in this experiment.

For microbiological analysis, a composite sample of each mammary teat of every cow were sampled in a sterile and without preservatives bottles (Embalpharma, Sertão da Estiva, Brazil) after elimination of three first milk jets and teat disinfection with 70% alcohol (Oliver *et al.*, 2004). Milk samples were immediately frozen and sent to the microbiology laboratory at the Center of Agrarian Sciences of Federal University of São Carlos, in Araras, São Paulo, Brazil.

To analyze milk composition analysis, complete milking samples were collected and placed in 40 mL plastic bottles containing bronopol. Samples were sent to the laboratory of Milk Clinic, University of São Paulo, in Piracicaba, São Paulo, Brazil. Individual milk yield

was recorded from three milkings, one day after milk samples, through the animal identification and milk weighing in Alpro<sup>®</sup> system-milk (DeLaval, Jaguariúna, Brazil).

### MICROBIOLOGICAL ANALYSIS

Microbiological analysis was performed according to adapted National Mastitis Council methodology (Oliver *et al.*, 2004). Samples were defrosted at room temperature, 10 µL of milk was plated with a calibrated nickel handle in Mueller-Hinton (Kasvi, Curitiba, Brazil) agar media with 5% sheep blood and another 10 µL of milk on MacConkey media (Kasvi).

Plates were considered bacteriologically positive when they had three or more identical colonies after 48 h of incubation at 37°C (Oliver *et al.*, 2004). Colony growth characteristics were analyzed, such as size, type, color, hemolysis production, gram coloration test and arrangements, with electron microscope scanning. Colonies that grew only on MacConkey agar media and had negative result on gram test were plated in commercial media Rugai with lysine (NewProv, Pinhais, Brazil). Rugai with lysine media has biochemical assays for gram negatives bacteria differentiation, as urea oxidation, motility in sulfate media, indol motility, sulphide gas production, glucose, saccharose and lactose fermentation (Oliver *et al.*, 2004).

Colonies that were grown on blood agar media and had gram positive results were submitted to a catalase test through the addition of one drop of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), differentiating cocci catalase positive (*Staphylococcus* spp. and *Corynebacterium* spp.), and catalase negative (*Streptococcus* spp.). For *Staphylococcus aureus* and *Staphylococcus* spp. differentiation suspicious colonies were plated in agar mannitol media (6.5% NaCl) (BD Biosciences, New Jersey, USA) and incubated at 37° C for 12 h, for *Staphylococcus aureus*

**Table I.** Pathogens prevalence and milk yield and composition of dairy cows in a commercial herd (Prevalência de patógenos e produção e composição do leite de vacas leiteiras em um rebanho comercial).

Item	Experimental group						p <sup>3</sup>
	Control	Coliforms	<i>Corynebacterium</i> spp.	<i>S. aureus</i>	<i>Staphylococcus</i> spp.	<i>Streptococcus</i> spp.	
N	801 (68.87%) <sup>a</sup>	11 (0.95%) <sup>d</sup>	49 (4.21%) <sup>c</sup>	54 (4.64%) <sup>c</sup>	186 (15.99%) <sup>b</sup>	62 (5.33%) <sup>c</sup>	<0.001
DIM <sup>1</sup>	248.3±5.12	297.8±43.74	218.0±20.73	284.1±19.74	250.8±10.64	265.0±18.13	0.196
	kg d <sup>-1</sup>						
Milk yield	31.288±0.359 <sup>a,b</sup>	25.773±3.059 <sup>b,c</sup>	32.598±1.450 <sup>a</sup>	28.596±1.381 <sup>c</sup>	31.234±0.744 <sup>a,b,c</sup>	28.726±1.289 <sup>c</sup>	0.058
	cel. mL <sup>-3</sup>						
SCC <sup>2</sup>	321±37.7 <sup>c</sup>	1046±326.4 <sup>a</sup>	284±149.0 <sup>c</sup>	179±149.0 <sup>c</sup>	343±77.4 <sup>b</sup>	1390±133.3 <sup>a</sup>	<0.001
	g kg <sup>-1</sup>						
Fat	34.82±0.28	35.59±2.41	35.47±1.10	35.22±1.10	34.89±0.57	35.30±0.98	0.984
Protein	29.98±0.10 <sup>b</sup>	31.11±0.89 <sup>a,b,c</sup>	29.93±0.41 <sup>a,b,c</sup>	30.82±0.41 <sup>a</sup>	29.52±0.21 <sup>c</sup>	30.56±0.37 <sup>a,b</sup>	0.024
Lactose	45.86±0.13 <sup>a</sup>	43.35±1.11 <sup>b</sup>	46.46±0.51 <sup>a</sup>	45.18±0.51 <sup>a,b</sup>	45.25±0.26 <sup>b</sup>	44.00±0.45 <sup>b</sup>	<0.001

\*Means superscript with similar letters in the same line, do not differ by PDIFF means test ( $p>0.05$ ).<sup>1</sup>DIM: days in milk; <sup>2</sup>SCC: Somatic cells cont. <sup>3</sup>P: straight comparison probability.

**Table II. Milk yield and composition relation with SCC, according to the mastitis causative pathogen (Relação entre produção e composição do leite com a CCS, de acordo com o patógeno causador de mastite).**

Item	General	Experimental group						p <sup>2</sup>
		Control	Coliforms	<i>S. aureus</i>	<i>Corynebacterium spp.</i>	<i>Staphylococcus spp.</i>	<i>Streptococcus spp.</i>	
Milk yield	p <sup>1</sup> <0.001							<0.001
Intercept	40.40±0.76	41.14±0.95	33.12±9.40	48.60±5.02	37.44±3.51	40.72±2.09	39.54±3.65	0.516
Slope	-5.58±0.44	-6.07±0.57	-2.56±4.04	-12.8±3.22	-3.14±2.27	-5.62±1.21	-4.63±1.44	0.192
Fat	p <sup>1</sup> =0.873							0.276
Intercept	34.7±0.6	34.8±0.8	43.1±4.5	39.8±3.9	35.2±2.9	34.0±1.9	33.7±3.3	0.627
Slope	0.1±0.4	0.0±0.5	-3.5±1.9	-3.0±2.5	0.2±1.9	0.6±1.1	0.7±1.3	0.611
Protein	p <sup>1</sup> <0.001							0.096
Intercept	28.5±0.2	28.2±0.3 <sup>b</sup>	35.3±2.6 <sup>a</sup>	29.1±1.6 <sup>b</sup>	28.6±1.2 <sup>b</sup>	28.1±0.6 <sup>b</sup>	29.1±0.9 <sup>b</sup>	0.050
Slope	0.9±0.1	1.1±0.2 <sup>a</sup>	-1.9±1.1 <sup>b</sup>	1.1±1.0 <sup>a</sup>	0.9±0.8 <sup>a</sup>	0.9±0.3 <sup>a</sup>	0.6±0.4 <sup>a</sup>	0.045
Lactose	p <sup>1</sup> <0.001							<0.001
Intercept	50.2±0.3	50.3±0.3 <sup>b</sup>	46.4±3.4 <sup>b</sup>	55.9±1.8 <sup>a</sup>	51.9±1.4 <sup>a,b</sup>	51.1±0.8 <sup>b</sup>	48.1±1.3 <sup>b</sup>	0.001
Slope	-2.8±0.1	-2.8±0.2 <sup>b</sup>	-1.4±1.5 <sup>a,b</sup>	-7.1±1.2 <sup>c</sup>	-3.7±0.9 <sup>a,b</sup>	-3.6±0.4 <sup>b</sup>	-1.7±0.5 <sup>a</sup>	<0.001

\*Means superscript with similar letters in the same line, do not differ by Fisher test ( $p > 0.05$ ); <sup>1</sup>p: Probability for general liner effect of the CCSL on milk yield and composition; <sup>2</sup>p: straight comparison probability.

and *Staphylococcus spp.* differentiation, according Silva *et al.* (2010).

#### MILK COMPOSITION

Milk fat, lactose and crude protein concentrations were analyzed by infrared absorption (Foos, Hillerød, Denmark). Somatic cell count was analyzed by flow cytometry in optoelectronic equipment (Foos, Hillerød, Denmark), according Tomazi *et al.* (2015).

#### STATISTICAL ANALYSIS

Data of milk yield and composition were subjected to analysis of variance using MIXED procedure of SAS (Statistical Analysis System, version 9.2.), considering in the model the fixed effect of identified agent and random error. Identified coliforms (*Escherichia coli*, *Klebsiella spp.* and *Enterobacter spp.*) were grouped by similar epidemiological characteristics and low prevalence on analyzed milk samples. Comparisons between the agents were performed by the PDIFF means test. Agents prevalence was compared by qui square non parametric test.

The SCC was linearized according to Dabdoutb and Shook (1984) methodology:

$$LSCC = [\text{Log}_2 (\text{SCC} / 100.000)] + 3$$

The straight comparison method, of PROC MIXED of SAS (Statistical Analysis System, version 9.2.) were used to compare the regression functions of milk yield and composition in function of SCC linear obtained for each of the identified agents. A significance level of 0.05 was considered for all analyses performed.

#### RESULTS

##### PREVALENCE

The most prevalent agent was *Staphylococcus spp.*, identified in 15.99% of evaluated samples (**figure 1**;

$p < 0.05$ ). *Staphylococcus aureus*, *Corynebacterium spp.* and *Streptococcus spp.* showed similar prevalence ( $p > 0.05$ ), higher than coliforms ( $p < 0.05$ ).

*Streptococcus spp.*, coliforms and *Staphylococcus spp.* increased SCC (**table I**;  $p < 0.05$ ). *Streptococcus spp.* and coliforms had higher SCC than *Staphylococcus spp.* ( $p < 0.05$ ). *S. aureus* and *Corynebacterium spp.* had no effect on SCC ( $p > 0.05$ ).

##### PATHOGENS AND MILK YIELD AND COMPOSITION

*Streptococcus spp.* and *S. aureus* decreased milk yield ( $p < 0.05$ ; **table I**). Pathogens had no effect on milk fat concentration ( $p > 0.05$ ). *S. aureus* increased milk protein content, while *Staphylococcus spp.* decreased ( $p < 0.05$ ). *Staphylococcus spp.*, *Streptococcus spp.* and coliforms decreased milk lactose content ( $p < 0.05$ ). *Corynebacterium spp.* had no effect on milk composition ( $p > 0.05$ ).

##### LINEAR REGRESSIONS

Overall, there was an increase in crude protein content and decrease in milk yield and lactose concentration, with LSCC increase (**table II**;  $p < 0.05$ ). Every unit increase of LSCC resulted in +0.9 g kg<sup>-1</sup>, -5.58 kg day<sup>-1</sup> and -2.84 g kg<sup>-1</sup> of crude protein content, milk yield and lactose concentration, respectively. Milk fat content was not altered by LSCC ( $p > 0.05$ ).

##### REGRESSION FUNCTIONS COMPARISON

Pathogen isolation had no effect on LSCC relation with milk yield and fat content (**table II**;  $p > 0.05$ ). Pathogens isolation had effect on crude protein content and LSCC relationship ( $p < 0.05$ ). Coliforms had higher intercept and lower slope than other groups ( $p < 0.05$ ).

Lactose content and LSCC relationship also had no similarity between pathogens ( $p < 0.05$ ). *S. aureus* increased the intercept and decreased slope of LSCC and lactose content linear regression ( $p < 0.05$ ). *Strepto-*

*coccus* spp. increased slope between SCC and lactose content ( $p < 0.05$ ).

## DISCUSSION

Milk yield and composition were evaluated in function of pathogen identification and somatic cell count. With the increase in LSCC, there was milk yield and lactose content decrease and crude protein content increase. Pathogens had no effect on SCC and milk yield relationship. Coliforms changed LSCC and milk crude protein content relationship. *S. aureus* increased and *Streptococcus* spp decreased SCC effects on milk lactose content.

*Staphylococcus* spp. was the most prevalent evaluated pathogen (15.99%), followed by *Streptococcus* spp., *S. aureus* and *Corynebacterium* spp. Pathogen prevalence obtained in this study was similar to that found by Wilson *et al.* (1997), who found 11.23, 10.1, 9.1, 7.1 and 1% of *Staphylococcus* spp., *Streptococcus agalactiae*, *S. aureus*, *Streptococcus* spp. and coliforms, respectively. According to Santos and Fonseca (2007), under conditions such as those found when there is milking system vacuum fluctuation, *Staphylococcus* spp. colonizes epithelium ceilings and accesses the gland channel, becoming one of the most isolated pathogens.

*Staphylococcus* spp. and *Corynebacterium* spp. can be considered commensal pathogens of mammary gland (Gillespie *et al.*, 2009) being characterized as secondary pathogens due to low pathogenicity (Harmon, 1994). In the present study, *Corynebacterium* spp. had no effect on milk yield and composition. *Staphylococcus* spp., however, decreased milk protein and lactose concentrations. Although low in pathogenicity, *Staphylococcus* spp. can cause changes in the mammary gland secretory tissue and thus, alter milk composition (Taponen *et al.*, 2007), as observed in this study.

*S. aureus* had no effect on SCC and decreased milk yield. Santos and Fonseca (2007), said that *S. aureus* induce variables changes in SCC. Jones *et al.* (1984) observed that this pathogen did not always increase SCC, with 60% of cows with this agent showing less than

200.000/mL somatic cells. *S. aureus* produces cytolytic toxins, exfoliative toxin (TSST-1) and hyaluronidases responsible for epithelial damage. Thus, injuries can result in breast parenchyma necrosis, decreasing productive capacity (Barkema *et al.*, 2009). In addition, *S. aureus* infection is characterized as persistent, being isolated days after the acute phase of infection. We believe that this characteristic of persistent infection caused by *S. aureus* and epithelial degradation may be related to milk yield decrease.

*Streptococcus* spp. increased SCC and milk protein content and decreased milk yield. According Guerin-Fauble *et al.* (2002), this agent infection is generally subclinical, providing wide SCC increase. Haas *et al.* (2002) observed SCC elevation, 21 days before the clinical phase, persisting for smaller periods, after the clinical case, in relation to other evaluated agents. *Streptococcus* spp. promotes milk yield decrease due to mammary gland cistern and ducts infection, causing secretor epithelium fibrosis (Santos and Fonseca, 2007).

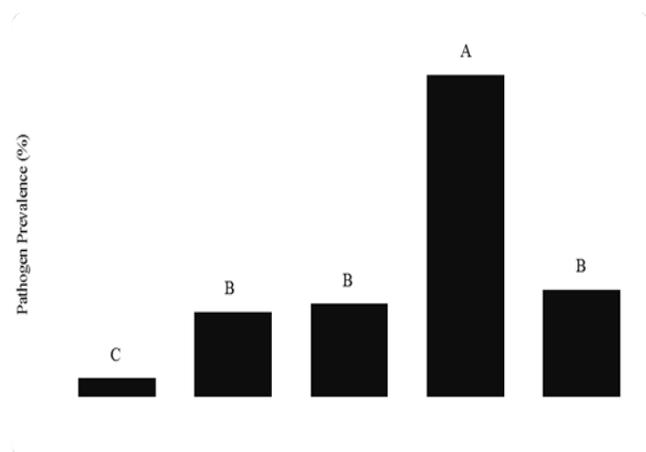
Coliforms increased SCC, decreasing milk yield and lactose concentration. The production of endotoxin, during multiplication phase, is responsible for clinical signs such as fever, weight loss, dehydration and, mainly, milk yield decrease, after the great leukocytes migration to the mammary gland in the acute phase (Santos and Fonseca, 2007). Due to these characteristics, we believe that animals with *E. coli*, *Klebsiella* spp. and *Enterobacter* spp. (coliform agents) presented a milk yield and lactose concentration decrease due to the acute phase infection identification at the sampling time.

According to Silanikove *et al.* (2000) and Leitner *et al.* (2004), infection presence and increased SCC basal levels promote the plasmin enzyme complex activation, which performs casein conversion to whey components. Peptides from enzymatic hydrolysis of true protein decrease lactose and other osmotic components output to lumen, resulting in decreased milk yield. Regardless of the milk pathogen isolated, increased LSCC results in milk yield decrease.

There was no relationship observed between SCC and milk fat content. Leitner *et al.* (2003) found no effect on milk fat concentrations with intramammary infection. Milk fat secretion occurs by direct incorporation and de novo synthesis of fatty acids, which are very sensitive to metabolic condition of the animal, becoming fat, the most variable component of milk (Sutton, 1989). We observed variation coefficients for fat, protein and lactose of 21.75; 9.69 and 7.74%, respectively.

Milk protein content increased with LSCC increase for all pathogens evaluated, except for coliforms. Milk protein content presented with increasing LSCC is related to changes in membrane permeability and increased serum proteins proportion on milk crude protein (Silanikove *et al.*, 2000; Leitner *et al.*, 2004). Due to SCC increase, there is proteolysis of casein, and it can reduce industrial performance of milk (Olives *et al.*, 2013). According to Silanikove *et al.* (2000), plasmin complex activation degree defines the changes that will occur with milk yield and composition. Concentrations of

**Figure 1.** Pathogens prevalence on evaluated dairy cows milk samples (Prevalência de patógenos nas amostras de leite avaliadas).



protein may be increased when the activation of plasmin was moderate and decrease when plasmin activity increases. Plasmin activation is dependent on infection character and animal immune response (Leitner *et al.*, 2004), allowing us to associate coliform infection isolated with immunosuppressed cows. We believe that milk protein concentration did not increase with coliforms as a result of increased plasmin complex activation, reflecting also in significant milk yield decrease.

The relationship between LSCC and milk lactose content was dependent on the isolated pathogen. Overall, the increased LSCC led to milk lactose content reduction. This reduction was greater in animals with *S. aureus* and lower in animals with *Streptococcus* spp., which presented -1.7 and 7.1 g·kg<sup>-1</sup> of milk lactose for each unit LSCC increment. We believe that this fact is related to the virulence of *S. aureus*, which while not promoting SCC variations in all infections (Jones *et al.*, 1984), produces toxins responsible for epithelial damage (Barkema *et al.*, 2009) and persistent infections (Haas *et al.*, 2002). As highlighted earlier, *Streptococcus* spp., on the other hand, have lower pathogenicity and invariably with significant elevation of the SCC.

Milk lactose and protein concentrations are more sensitive to SCC variations and this relationship is dependent on the pathogen presence. Study of this relationship can facilitate the advancement of knowledge of the pathophysiology of mammary infections and could permit future prediction of the agents present from the simplest analysis of SCC and the levels of protein and lactose, directing the strategies of control and prophylaxis.

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