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Impact of Subclinical Mastitis-causing Bacterial Species on the Composition and Chemical Properties of Milk

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ABSTRACT

Background: Here we investigate whether the composition and some chemical properties of milk can correlate with bacterial species causing subclinical mastitis in cows.

Methods: One hundred and eighty cows were used in the study. The California Mastitis Test (CMT) was applied to the selected cows. The cows were divided into four groups: cows with negative CMT (n=45), the *Escherichia coli* (n=45), *Streptococcus agalactiae* (n=45) and *Staphylococcus aureus* (n=45) groups.

Result: Milk composition and some chemical properties were measured. The highest fat ratio (5.82±0.34%) was found in the *Escherichia coli* group ($p<0.05$). The proportions of solid-non-fat (9.67±0.09%), protein (3.51±0.03%), lactose (5.29±05%) and mineral matter (0.75±0.01%) were lowest in the *Escherichia coli* group ($p<0.05$). The electrical conductivity of milk was lowest in the CMT-negative group (4.23±0.02 mS/cm), while the *Streptococcus agalactiae* group had the highest value (4.61±0.02 mS/cm) ($p<0.05$). The specific gravity of the *Escherichia coli* group was lower than the CMT-negative (1,034.91±0.56 kg/m^3) and *Staphylococcus aureus* (1,034.24±0.65 kg/m^3) groups ($p<0.05$). Based on our findings, we propose that milk composition and some chemical parameters are altered in subclinical mastitis. However, these could not be standardized according to the responsible bacterial species. We recommend that these parameters are monitored regularly on dairy farms.

Key words: Bovine mastitis, Chemical properties, Milk composition, Raw milk.

INTRODUCTION

Milk is a unique biological fluid with high nutritional value. Milk composition, production and quality are important factors affecting the profitability of dairy farms. Also, the quality of dairy products largely depends on the composition of raw milk. Therefore, changes in the composition of raw milk are of great importance for milk producers (Li *et al*., 2014). The composition and some chemical properties of milk are among the criteria used to monitor milk quality. Milk production and composition can be affected by many genetic and non-genetic factors (Ivanov *et al*., 2017). The composition of milk (consisting of fat, solid-non-fat, protein, lactose and minerals), the electrical conductivity and the specific gravity of the milk also vary depending on multiple factors, such as breed, nutrition, season, lactation period and parity (Suzuki and van Vleck, 1994; Sharif *et al*., 2007; Pandey *et al*., 2021). However, the most important factor affecting milk production, composition, electrical conductivity and specific gravity is the development of mastitis. Subclinical mastitis manifests itself with changes in milk protein, milk fat, solid-non-fat, lactose, mineral matter, electrical conductivity and specific gravity (Lindmark-Mansson *et al*., 2006; Kasikci *et al*., 2012; Deshapriya *et al*., 2019). Depending on the type of bacteria that cause mastitis, changes may occur in the milk component, electrical conductivity and the specific gravity of the milk. Moreover, it has been observed that even the same bacteria can have different effects on milk components (Coulon *et al*., 2002; Vasil *et al*., 2016; Boas *et al*., 2017).

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In the present study, we aimed to determine how important subclinical mastitis-causing bacterial species, such as *Staphylococcus aureus* (*S. aureus*), *Streptococcus agalactiae* (*S. agalactiae*) and *Escherichia coli* (*E. coli*), affect the composition (fat, solid-non-fat, protein, lactose and mineral matter) and some chemical properties (electrical conductivity and specific gravity) in milk.

MATERIALS AND METHODS

Study groups and bacteriological culture

In this study, clinically healthy, mid-lactation, multiparous (between parities two and three) Holstein and Simmental cows [between 3 and 5 years old, weighing 450-500 kg,

with body condition scores of between 3.5 and 3.8, milked twice per day and with a daily milk yield of 10-25 L (mean= 14.54 L)] were selected among the cows in seven commercial dairy farms (four free-stall or loose and three tie-stall) in the Elazig province of Turkey. Statistical power analysis was performed to determine the total number of animals in the groups. In this study, the required minimum sample number was determined as 180 using effect size = 0.25, alpha = 0.05 and power = 0.80 (Cohen, 1988). For the study, the ethics committee approval was obtained from the Fırat University Animal Experiments Local Ethics Committee (FU-2018/98). The study was conducted between 01.11.2019 and 30.01.2020.

The California Mastitis Test (CMT) was applied to the cows, as described by Ruegg and Reinemann (2002). The commercial reagent (BOVI-VET, CMT-Test, Kruuse, Denmark) equal to the milk was added to each cup of the container. Then, according to the consistency of the gel formed in each cup, CMT scoring was done (always by the same person). According to the score, the results were divided into CMT-negative (-) and positive (+, ++ and +++ degrees). If at least one-quarter received one of the +, ++ and +++ values, the cow was considered to have subclinical mastitis. If all mammary quarters were negative, they were evaluated as CMT (-), healthy cows (n=45) (Tolosa *et al*., 2013). To establish the other groups, milk samples (approximately 2 ml) were collected aseptically after disinfection of the teat with 70% alcohol on a cotton ball. Milk samples were bacteriologically tested in the Microbiology Laboratory of the Faculty of Medicine, Firat University, according to the National Mastitis Council (NMC) guidelines (Hogan *et al*., 1999). Milk samples were centrifuged at 4000 rpm for 15 minutes. The precipitate formed at the bottom was sampled with a Pasteur pipette, inoculated on blood agar and MacConkey agar (Oxoid, Hampshire, United Kingdom) media and incubated at 37°C for 24-48 hours under aerobic conditions. Colony morphologies were identified using Gram staining technique. Bacterial species were identified using Catalase, Coagulase, Esculin and Christie-Atkins-Munch-Peterson (CAMP) tests. After isolation and identification of microorganisms, the *E. coli* group was designated as cows with only *E. coli* growing in milk samples of CMT-positive (n=45), *S. agalactiae* group as cows with only *S. agalactiae* growing in milk samples of CMT-positive (n=45) and *S. aureus* group as cows with only *S. aureus* growing in milk samples of CMT-positive (n=45). In the bacteriological examination of milk from healthy cows, no growth of any microorganisms was observed. The cows that grew two or more bacteria in their milk samples were excluded. Two-thousand teats belonging to 500 cows were examined until the number of cows in the groups was completed.

Milk analysis

Fat (%), solid-non-fat (%), protein (%), lactose (%), mineral matter (%), specific gravity (kg/m^3) and electrical conductivity (mS/cm) in the milk samples were measured using a Lactoscan Milk Analyser (Milkotronic/EUROPE) device. Measurements were made in fresh milk and as described by Alam *et al*. (2018). Milk analysis were performed in the Obstetrics and Gynecology Milk Laboratory of Fırat University.

Statistical analysis

Statistical analyses were performed using SPSS software (Statistical Package for the Social Sciences for Windows SPSS 22.0 Edition for Windows, Chicago, Illinois, USA).

The normality distribution of the fat, solid-non-fat, protein, lactose, mineral matter, specific gravity and electrical conductivity were tested using the visual (histogram and probability graphs) and Kolmogorov-Smirnov test. It was determined whether the data showed a normal distribution or not. The protein and mineral matter data were non-normally distributed in all groups. Therefore, the Kruskal-Wallis test was used for group comparison. The Bonferroni corrected Mann-Whitney-U test was used for the post-hoc pairwise group comparisons after the Kruskal-Wallis test. As a result of the Bonferroni correction, the value obtained as a result of dividing the statistical significance limit value of 0.05 by the number of comparisons to be made (four groups are compared in six different ways) was used as the significant value in the pairwise group comparisons. The Bonferroni corrected Mann-Whitney-U significance value was calculated as $p=0.05/6=0.0083$; however, when a significance value of 0.01 or less is used for this test, the power of the Mann-Whitney-U test decreases. Therefore, a value of $p<0.01$ was accepted in the pairwise group comparisons.

It was determined that the data of fat, solid-non-fat, lactose, specific gravity and electrical conductivity parameters showed a normal distribution and provided the parametric test assumptions. Thus, a one-way analysis of variance (ANOVA) was performed on the data of these parameters. Post-hoc analysis was performed to determine the difference between groups after ANOVA. The Levene test was applied to determine the post-hoc analysis to be made on fat, solid-non-fat, lactose, specific gravity and electrical conductivity data.

According to this test, it was determined whether the variances were homogeneously distributed in the data. While variances for fat and specific gravity showed a homogeneous distribution ($p<0.05$), it was determined that variances for solid-non-fat, lactose and electrical conductivity did not show a homogeneous distribution ($p<0.05$). Therefore, Tukey HSD (honestly significant difference) was used as a post-hoc test for fat and specific gravity, while Tamhane's T2 test was used for solid-non-fat, lactose and electrical conductivity. P-values<0.05 were considered significant in Tukey's HSD and Tamhane's T2 analysis.

RESULTS AND DISCUSSION

Mastitis causes serious economic losses for milk producers through a decrease in milk quality and production.

It continues to be an important problem frequently encountered in the dairy industry (Petrovski *et al*., 2006). Due to mastitis, changes occur in the composition and chemical properties of milk. These changes significantly affect the quality of milk and products derived from milk (Kul *et al*., 2019). When evaluating the relationship between mastitis and milk composition, differences such as breed, season, lactation number and period should be taken into account (Malek dos Reis *et al*., 2013; Alhussien and Dang, 2018).

Table 1 summarizes the statistical analyses of milk composition. The highest fat ratio (5.82±0.34%) was found in the *E. coli* group ($p<0.05$). On the other hand, the rate of solid-non-fat (9.67±0.09%), protein (3.51±0.03%), lactose (5.29±05%) and mineral matter (0.75±0.01%) were significantly lower in the *E. coli* group compared to the other groups ($p<0.05$). In a study conducted to determine the changes in milk composition in mastitis caused by *Staphylococcus xylosus* and *Staphylococcus warneri*, it was stated that protein and solid-non-fat decreased, while it was claimed that there was no change in the ratio of lactose and fat (Vasil *et al*., 2016). In a study conducted on milk with mastitis caused by Coagulase Negative Staphylococci (CNS), the lactose ratio was low and the protein ratio was high. In the same study, high protein and low lactose levels were detected in *E. coli* and *S. aureus* mastitis (Coulon *et al*., 2002). In our study, it was determined that while the rate of fat in milk with mastitis caused by *E. coli* increased, the rate of solid-non-fat, protein and lactose decreased. In the *S. aureus* group, there was no change in milk components compared to healthy milk, while only an increase in fat was observed in *S. agalactiae*. Malek dos Reis *et al*. (2013) found that the ratio of solid-non-fat and protein in milk with mastitis caused by *S. aureus* decreased and the lactose ratio was unaffected. Moreover, it was reported that there was a decrease in the ratio of fat and solid-non-fat, but no change in the ratio of lactose and protein in milk from CNS and *Streptococcus* spp. In the same study, it was stated that in *Corynebacterium* spp. mastitis, there was an increase in protein rate, a decrease in lactose and fat, but no change in the ratio of solid-non-fat. In mastitis caused by *Streptococcus uberis* (*S. uberis*), it is reported that while the ratio of fat and protein increases, the lactose ratio is not different from healthy cows (Pecka-Kielb *et al*., 2016). In a study conducted in CNS-infected milk, where *Staphylococcus chromogenes* was the most prevalent CNS species isolated from mammary quarters with subclinical mastitis, it was reported that these factors caused an increase in somatic cell count, but did not affect milk components (Tomazi *et al*., 2015).

Due to the differentiation of the ion value of the milk, there is a change in the electrical conductivity of the milk (Norberg *et al*., 2004; Alhussien and Dang, 2018).

The electrical conductivity of milk was lowest in the CMT (-) group (4.23±0.02 mS/cm), while the *S. agalactiae* group had the highest value (4.61±0.02 mS/cm) ($p<0.05$). There was no statistical difference between the *E. coli* (4.49±0.03

Table 1: Milk composition between groups (Mean±Standard error of mean).

Item	CMT (-) (n=45)		*Escherichia coli* (n=45)		*Staphylococcus aureus* (n=45)		*Streptococcus agalactiae* (n=45)		P
	$\bar{x}\pm S_{\bar{x}}$	Median	$\bar{x}\pm S_{\bar{x}}$	Median	$\bar{x}\pm S_{\bar{x}}$	Median	$\bar{x}\pm S_{\bar{x}}$	Median	
Fat (%)	3.38±0.36^{A}	2.20	5.82±0.34^{C}	6.30	4.18±0.34^{AB}	3.80	4.75±0.32^{BC}	4.60	***
Solid-non-fat (%)	10.16±0.08^{B}	10.20	9.67±0.09^{A}	9.60	10.16±0.13^{B}	10.00	10.05±0.11^{AB}	10.20	**
Protein (%)	3.68±0.03^{b}	3.70	3.51±0.03^{a}	3.50	3.69±0.04^{b}	3.60	3.64±0.04^{ab}	3.70	**
Laktose (%)	5.55±0.04^{B}	5.60	5.29±0.05^{A}	5.30	5.56±0.07^{B}	5.50	5.48±0.06^{AB}	5.60	**
Mineral matter (%)	0.79±0.01^{b}	0.80	0.75±0.01^{a}	0.80	0.79±0.01^{ab}	0.80	0.78±0.01^{ab}	0.80	*

*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$.

a,b: The difference between the groups with different letters on the same row is statistically significant, ($p<0.01$).

A,C: The difference between the groups with different letters on the same row is statistically significant, ($p<0.05$).

Table 2: Comparison of specific gravity and electrical conductivity in milk between groups (Mean±Standard error of mean).

Item	CMT (-) (n=45)		*Escherichia coli* (n=45)		*Staphylococcus aureus* (n=45)		*Streptococcus agalactiae* (n=45)		P
	$\bar{x}\pm S_{\bar{x}}$	Median	$\bar{x}\pm S_{\bar{x}}$	Median	$\bar{x}\pm S_{\bar{x}}$	Median	$\bar{x}\pm S_{\bar{x}}$	Median	
Specific gravity (kg/m^3)	1,034.91±0.56^b	1,036.00	1,031.00±0.50^a	1,031.00	1,034.24±0.65^b	1,3034.00	1,033.31±0.55^{ab}	1,034.00	***
Electrical conductivity (mS/cm)	4.23±0.02^a	4.30	4.49±0.03^b	4.50	4.46±0.03^b	4.50	4.61±0.02^c	4.60	***

***: p<0.001.

a,b,c: The difference between the groups with different letters on the same row is statistically significant, (p<0.05).

mS/cm) and *S. aureus* (4.46±0.03 mS/cm) groups in terms of the electrical conductivity of the milk (p<0.05) (Table 2). In the study conducted by Kuplulu *et al*. (1995), the electrical conductivity values of milk infected with *E. coli*, *S. uberis* and *Streptococcus dysgalactiae* were 4.86±0.08 mS/cm, 5.24±0.06 mS/cm and 5.54±0.11 mS/cm, respectively. These authors also found the highest electrical conductivity (7.53±0.22 mS/cm) in milk infected with *S. aureus*. In the presented study, the highest electrical conductivity was observed in the *S. agalactiae* group with the highest SCC. There was a positive correlation between the electrical conductivity of milk and SCC and a negative correlation between specific gravity and SCC. The findings of our study support previous studies (Kasikci *et al*., 2012; Boas *et al*., 2017) describing the relationship between the electrical conductivity of milk and mastitis. However, in another study, it was reported that there was no difference in density between subclinical mastitis and healthy milk (Panda *et al*., 2019). In our study, while there was no difference in specific gravity between milk with mastitis caused by *S. agalactiae* and *S. aureus* and healthy milk, a decrease was found in the specific gravity in the *E. coli* group. Vasil *et al*. (2016) investigated the effect of bacteria differences on the specific gravity of milk and no difference was found between milk with mastitis caused by CNS and healthy milk. In another study, no difference in specific gravity was found in mastitis caused by *S. uberis* compared to healthy milk (Pecka-Kielb *et al*., 2016).

Conclusion

We detected changes in milk composition, specific gravity and electrical conductivity in milk with subclinical mastitis in our study. However, these changes were not standardizable according to the responsible bacterial species (*E. coli*, *S. aureus* and *S. agalactiae*). We recommend that these parameters must be monitored regularly in dairy farms.

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