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Original Article
Özgün Araştırma

Investigation of the relationship between bacterial groups isolated from buffalo milk with subclinical mastitis and somatic cell counts

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Abstract: The aim of this study was to investigate the relationship between the bacterial groups obtained from the milk of buffaloes with subclinical mastitis and the milk SCC rate. For this purpose, this study was carried out on 60 milk samples collected from Water buffalo with subclinical mastitis and healthy. Milk samples were divided into three groups as healthy (Group 1; n=20), and with subclinical mastitis caused by Gram negative (Group 2; n=20) and by Gram positive bacteria (Group 3; n=20). SCC value was statistically lower in Group 1 compared to Group 2 and Group 3 (P<0.01). However, it was determined that there was no significant difference between Group 2 and Group 3 in terms of SCC (P>0.05). In conclusion, SCC value significantly increased in milk with sub-clinical mastitis compared to healthy milk. However, there was no difference in SCC value in milk samples with mastitis caused by gram negative and gram positive bacteria.

Keywords: Bacteria, buffalo, mastitis, SCC.

Subklinik mastitisli manda sütünden izole edilen bakteri grupları ile somatik hücre sayıları arasındaki ilişkinin araştırılması

Özet: Bu çalışmada subklinik mastitisli mandaların sütünden elde edilen bakteri grupları ile süt SHS oranı arasındaki ilişkinin araştırılması amaçlanmıştır. Bu amaçla, bu çalışma subklinik mastitisli ve sağlıklı mandadan toplanan 60 adet süt örneği üzerinde gerçekleştirildi. Süt örnekleri sağlıklı (Grup 1; n=20) ve Gram negatif (Grup 2; n=20) ve Gram pozitif bakterilerin neden olduğu subklinik mastitisli (Grup 3; n=20) gruplar olmak üzere üçe ayrıldı. Grup 2 ve Grup 3'e göre Grup 1'in SHS değeri istatistiksel olarak daha düşüktü (P<0.01). Ancak Grup 2 ile Grup 3 arasında SHS açısından anlamlı fark olmadığı belirlendi (P>0.05). Sonuç olarak subklinik mastitisli sütlerde SHS değeri sağlıklı süte göre anlamlı olarak arttı. Ancak gram-negatif ve gram-pozitif bakterilerin neden olduğu mastitisli süt örneklerinde SHS değerinde fark yoktu.

Anahtar kelimeler: Bakteri, bufalo, mastitis, SHS.

Introduction

Mastitis is known as the inflammatory reaction of the mammary gland against infectious and non-infectious agents (Bradley 2002). It is also known that mastitis is one of the most common and most important diseases of dairy herds. It directly affects the world dairy industry as it reduces milk quality and quantity in dairy herds (Fagiolo and Lai 2007). The mastitis occurs in subclinical and clinical forms in farm animals (Fagiolo and Lai 2007), it is known that their mean incidence in dairy cows are 30% and 14.2%, respectively (Kurt and Eşki 2021). In addition, the prevalence of mastitis in buffaloes varies between 66%-70.32% (Sharma et al. 2011). Subclinical mastitis is more critical than clinical mastitis because it is difficult to detect, does not show clinical symptoms and is long-lasting (Islam et al. 2011). The most important factors of mastitis are bacteria, but it is known that viruses, fungi and algae cause the disease (Dalanezi et al. 2017). Furthermore, mastitis has a multifaceted etiology (Dalanezi et al. 2017), and it is reported that the major mastitis agents are *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Streptococcus uberis* (*S. uberis*), *Streptococcus agalactiae* (*S. agalactia*) and *Streptococcus dysgalactiae* (*S. dysgalactiae*) in cows (Kurt et al. 2019). Diagnosis of mastitis is performed by a number of clinical and laboratory examinations. The most basic method in the determination of mastitis is based on the direct or indirect determination of the milk somatic cell counts (SCC) level. The SCC typically tends to increase in milk with mastitis and its elevation is

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considered a reflection of udder infection. It is also a characteristic parameter used to evaluate milk quality. Therefore, SCC can be considered as a tool that can be used to determine the quality of healthy milk (Kurt at al. 2022). It has been reported that the SCC value of raw buffalo milk must be less than 400.000 cells/ml according to European Union Directives (92/46CEE and 94/71 CEE) (Sharma et al. 2011). In addition, udder infections and bacterial groups that cause udder infection can affect the change in SCC level to different degrees (Karzis et al. 2017). On the other hand, the SCC level is also important for milk quality in healthy buffalo milk (Tripaldi et al. 2010). As the SCC level in buffalo milk decreases, the quality of products such as cheese obtained from milk increases (Tripaldi et al. 2010; Sharma et al. 2011). However, it is known that intramammary infections increase the level of milk SCC (Feng et al. 2021) and can affect the level of SCC in bacteria that cause the disease (Malinowski et al. 2006; Karzis et al. 2017).

In this context, the presented study hypothesized that the bacterial group that causes disease in buffaloes with subclinical mastitis can lead to different changes in the SCC rate. For this reason, it was aimed to investigate the relationship between the bacterial group (Gram negative and Gram positive) obtained from the milk of buffaloes with subclinical mastitis and the milk SCC rate. It was also aimed to compare the SCC values obtained from milk samples with subclinical mastitis and the SCC values of healthy milk samples.

Material and Methods

Animals and groups

This study was carried out on 60 milk samples collected from Anatolian buffalo with subclinical mastitis and healthy. The buffaloes had similar age, lactation period, milk yield, and body condition score. The animals had free access to water. Also, no buffalo received any treatment before the study for mastitis. Milk samples were divided into three groups as healthy (Group 1; n=20), and with subclinical mastitis caused by Gram negative (Group 2; n=20) and by Gram positive bacteria (Group 3; n=20).

Udder examination and collection of milk samples

First, udder examination was performed with palpation and inspection. Animals with clinical signs of mastitis such as abnormal milk, blindness, quarter asymmetry, redness, swelling, hotness and painful sensation were not included in the study. Milk samples with subclinical mastitis and healthy were detected by california mastitis test (CMT). CMT test was performed for each quarter and then abtained results were classified as negative (0), trace, (1+), (2+) and (3+) (Baştan et al. 2008). The result was considered negative when a value of 0 was observed, and this milk sample was considered healthy. All milk samples (~20 ml) were collected aseptically from individual quarters of buffaloes with healthy and subclinical mastitis into sterile falcon tubes (Isolab®, Germany) for SCC analysis and bacterial evaluation (Hogan et al. 1999). Then, the milk samples were transported to the laboratory (~+4°C) and examined for SCC and bacterial analysis.

Microbiological examination

The milk samples with healthy and subclinical mastitis were prepared for inoculation with appropriate methods. The milk samples were inoculated separately into Edwards medium, MacConkey agar (Oxoid, CM0007) and blood agar (Oxoid, CM0055) by streaking method. After these procedures, the samples were incubated at 37°C during 24 h. Finally, Gram staining was performed to samples to identify the Gram reaction, and Gram negative and Gram positive bacteria were determined. If any bacteria were isolated in healthy milk samples, those samples were not included in the study. When the mixed pathogen was isolated, those milk samples were not included in the study.

Determination of milk somatic cell count

The Milk SCC values were determined using an automated somatic cell counting device (DeLaval Cell Counter DCC®, Sweden) and commercial counting cassettes (DeLaval Cell Counter Cassettes). All procedures of the analysis were carried out according to the manufacturer's instructions.

Statistical analysis

All statistical analyzes were performed with SPSS (IBM SPSS Statistics 24) package program.

The non-parametric Kruskal-Wallis test was used for statistical analysis. Multiple comparisons were made using the post-hoc Tamhane's T2 test, and results were given as mean ± standard deviation (mean ± SD). $P<0.05$ was considered significant.

Results

It was found that no bacterial isolation was obtained in Group 1. While Gram negative bacteria were isolated in Group 2, Gram positive bacteria were iso-

lated in Group 3. On the other hand, All groups were compared for SCC. SCC value was statistically lower in Group 1 compared to Group 2 and Group 3 ($p<0.01$). However, it was determined that there was no significant difference between Group 2 and Group 3 in terms of SCC ($p>0.05$). The SCC values of all groups are presented in detail in Table 1.

Table 1. SCC results obtained in Group 1, Group 2 and Group 3.

	Group 1 (n=20) (mean ± SD)	**Group 2 (n=20)** (mean ± SD)	**Group 3 (n=20)** (mean ± SD)	**P value**
SCC x 10^3 (cells/mL)	83.50 ± 7.80[a]	465.15 ± 118.79[b]	502.45 ± 126.46[b]	p<0.01

Different lower-case letters (a, b): in the same line indicate the statistical difference between measurements. SCC: Somatic Cell Count, SD: Standard Deviation.

Discussion and Conclusion

In the presented study, it was investigated that change of the milk SCC level of buffaloes with healthy and subclinical mastitis which caused by different types of bacteria. For this purpose, three study groups were formed from buffalo milk with healthy, and with subclinical mastitis caused by Gram negative and by Gram positive bacteria.

It has been reported that milk SCC value is closely related to udder health in farm animals (Alhussien and Dang 2018; Costa et al. 2020; Costa et al. 2021; Kurt et al. 2022). Moreover, SCC analysis is a routinely used tool to evaluate milk quality (Paape et al. 2007; Ruegg and Pantoja 2013). An acceptable SCC value for healthy buffalo milk cannot be expressed exactly. It is thought that the hygienic values of buffalo milk should be detailed (Pasquini et al. 2018). A previous study reported that the SCC value must be less than 400x10 cells/ml in healthy buffaloes (Sharma et al. 2011), but another study stated that there was no definite limit for buffalo raw milk (Pasquini et al. 2018). On the other hand, Tripaldi et al. (2010) reported that the threshold value of SCC in buffalo milk is 200x10 cells/ml and that a SCC level above this value is an indicator of the presence of inflammation. In addition, they also reported that buffalo milk with an SCC value of less than 100x10 cells/ml can generally be considered healthy. This information supports the results of our study because the SCC value of Group 1 was observed to be less than 100x10 cells/ml. We also think that the lower the SCC value, even in healthy milk, the higher the quality of the milk. It is known that buffalo milk is mainly used in cheese production, especially in Italy (Pasquini et al. 2018). At the same time, it was stated that milk SCC level affects cheese production (Barbano et al. 1991), and Sharma et al. (2011) informed that the decreased SCC value increased the obtained cheese product. Therefore, it is desirable to have the SCC level as low as possible in healthy buffaloes. On the other hand, Feng et al. (2021) reported that the main reason for the increase in milk SCC level was intramammary infection. The most insidious of udder infections is subclinical mastitis because it can persist for a long time without clinical signs and can reduce milk production and milk quality (Islam et al. 2011; Dhakal and Nagahata 2018). In addition, the bacterial species can affect the SCC level differently (Malinowski et al. 2006; Karzis et al. 2017). Fagiolo and Lai (2007) stated that Gram-negative and Gram-positive bacteria can use different mechanisms of adhesion to epithelial cells in the udder gland. Lavon et al. (2011) informed that *E.coli*, a major Gram-negative bacteria, increased milk SCC at a different rate than *S. aureus*, a major Gram-positive bactera. So, we thought that Gram-negative and Gram-positive bacterial groups would change the milk SCC level at different levels. However, in the presented study, it was observed that there was no significant difference between Group 2 and Group 3 in terms of milk SCC value. We thought that this could be related to the fact that bacterial groups in both groups did not cause significant damage to the mammary gland yet. Moreover, it is thought that the milk SCC difference between the groups can increase in favor of Group 2 as the disease progresses. There are different opinions for the SCC value of healthy buffalo milk. However, Dhakal and Nagahata (2018) reported that that the milk SCC standard value could be 200x10^3 cells/ml for the diagnosis of subclinical mastitis in buffaloes in the future. Although similar (Tripaldi et al., 2010) or different results have been expressed (Sharma et al. 2011), these results supports the results of this study because the milk SCC values of Group 2 and Group 3 were determined as 465.15±118.79 x 10^3 cells/ml and 502.45±126.46 x 10^3 cells/ml, respectively. Nevertheless, we think that milk SCC value should be investigated more comprehensively according to the pathogen in buffaloes with subclinical mastitis.

In conclusion, SCC value significantly increased in milk with subclinical mastitis compared to healthy milk. However, there was no difference in SCC value in milk samples with mastitis caused by gram-negative and gram-positive bacteria. On the other hand, we think that SCC value should be investigated comprehensively according to the specific bacterial species causing mastitis in further studies.

Ethical approval: This article has not been published previously and is not under consideration for publication elsewhere. The publication of this article is approved by all authors and explicitly by the responsible authorities where the work was carried out.

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