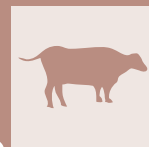


# Evaluation of mastitis risk through tank milk somatic cell count by the classification tree method in farms reared Brown Swiss breed cattle



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## SUMMARY

Mastitis is a serious disease that causes significant economic losses in dairy farming, negatively affecting milk yield and quality. This disease not only threatens the health of dairy cows but also reduces the economic efficiency of farms. The aim of this study was to investigate the impact of various factors on tank milk somatic cell count (TMSCC) and assess the associated mastitis risk. The research was designed to encompass a total of 170 farms, from which 680 tank milk (TM) samples were collected. The Classification Tree (CT) method was used to examine how factors such as season, milking type, and breeding conditions categorized by districts affected mastitis risk. Additionally, the effects of variables like barn type and udder cleaning practices on TMSCC were evaluated. One notable finding of the study was the statistically significant effect of seasonal variations on the TMSCC value. The season in which the milk samples were collected played an important role in determining the somatic cell count. The research results indicated that factors such as season, milking type, and breeding conditions had a significant impact on mastitis risk. However, certain variables, such as barn type and udder cleaning practices, were found to have no significant effects in this context. Additionally, analyses were conducted to compare the quantitative differences in factors that did not demonstrate statistically significant effects on TMSCC. The study reported an arithmetic mean of 228,997 cells/mL and a geometric mean of 107,094 cells/mL for TMSCC. These values were found to be compliant with the upper limits set by the European Union (EU) and Turkish Food Codex for somatic cell counts in milk. Therefore, from the standpoint of public health, the consumption of milk and dairy products originating from these farms was deemed safe and unlikely to pose any issues. The findings of this study suggest that efforts should be increased to reduce the somatic cell count values in milk produced in the region to below 100,000 cells/mL. This proactive measure would further enhance the safety and quality of dairy products, benefiting both consumers and producers in the long run. Future research and interventions should develop strategies to achieve these goals.

## KEY WORDS

Farm, tank milk, Brown Swiss breeds cow, somatic cell count, mastitis risk.

## INTRODUCTION

Milk and dairy products are the most preferred products as human food. The fact that these products are very diverse increases their importance in commercial terms and human nutrition. It is an obligation that the products are produced in a way that protects food safety and public health. Therefore, the products must be produced in a way that protects food safety and public health. The first rule for this is to produce quality milk [1]. The quality of milk, on the other hand, is measured by the lowness of somatic cell count (SCC) and the total bacterial count (TBC) in raw tank milk that has not been pasteurized. Somatic cell count (SCC) is the accepted international standard measure of milk quality. Bulk Tank Milk Somatic Cell

Count (TMSCC) is often used as a parameter in determining the quality of milk. TMSCC varies depending on the number and duration of mastitis infection in dairy cows. If the TMSCC level obtained from farms is above the standard threshold, the price paid for milk decreases, and milk producers are penalized in this way [2].

In developed countries, milk is mainly evaluated in terms of SCC. The legal TMSCC limit is 300,000 - 400,000 cells/mL in Canada, Great Britain, Germany, and Italy [3], and 750,000 cells/mL in the USA [4].

In 2017, in the Grade "A" Pasteurized Milk Ordinance, the SCC value was specified as  $SCC \leq 500,000$  cells/ml by the United States Food and Drug Administration [5]. In the European Union (EU) milk hygiene regulation, the use of milk with SCC and TBC higher than 400,000 cells/mL and/or 100,000 bacteria/mL is prohibited as human food. In addition, from the point of view of public health, it is desired that the geometric mean of the SCC obtained as a result of tests performed over a three-

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month period by taking at least one sample per month from tank milk is below 400,000 cells/mL [6].

According to the provisions of the Turkish Food Codex (TGK), after 2005, the use of milk with a TMSCC level of more than 500,000 cells/mL and/or a total bacterial count (TBC) of more than 100,000 cells/mL as human food was prohibited [7]. For many years, TMSCC has been an indicator in terms of the udder health of the herd in milk-producing farms. In addition to being an indicator of udder health and milk quality, and resistance and sensitivity to mastitis, SCC is also the most important indicator of subclinical mastitis [8]. The most important factor affecting the SCC of milk is mastitis. Mastitis simply means “udder inflammation”. It is formed especially by the effects of bacteria and causes great economic losses by reducing milk yield in cows [9].

It is expected that the SCC in a healthy cow is below 200,000 cells/mL. The fact that the SCC level in milk is above 200,000 cells/mL is considered abnormal and is evaluated as an indicator of a possible fever in the udder [10]. When there is a bacterial infection or any trauma in the udder, the SCC in the milk begins to increase [11]. Depending on SCC values in cow milk, Wattiaux [12] categorized the mastitis levels as no mastitis (SCC < 200,000 cells/mL), low level (200,000-500,000 cells/mL), widespread (500,000-1,000,000 cells/mL), and endemic (> 1,000,000 cells/mL).

High SCC affects the quality of milk and shortens the shelf life of pasteurized milk. Also, high SCC negatively affects the components of milk composition and complicates the processing of milk into products [9]. In addition to affecting milk production and quality, high SCC in milk may also pose a risk, especially in terms of the mineral content and coagulation properties of milk. Therefore, numerous scientific studies are needed on how low SCC affects milk quality. In this study, the compliance of SCC, which is an indicator of subclinical mastitis in animals, with the norms of the European Union (EU) and the communiqués of the Turkish Food Codex (TFC) was investigated in dairy cattle breeding farms in Iğdır province. In addition, the effects of environmental factors on SCC were discussed by comparing them on the basis of subgroups, and recommendations were made on reducing the SCC values of milk produced in the region and on measures that can be taken.

## MATERIAL AND METHOD

### Material

The Iğdır plain and its surroundings, where the animal subjects of the research were living, have a unique climatic characteristic that is hot in summer and dry in winter and a local microclimate on the scale of Turkey and Eastern Anatolia. In June-August, the temperature in the province varies between 39-42 °C. The lowest and highest temperatures observed between 1940 and 2016 were determined as -30.3 and +42.0 °C, respectively [14].

The research material constituted of tank milk samples taken every month for 12 months in 2015 from a total of 170 farms, including 51 farms in the central district, 37 in the Karakoyunlu district, 68 in the Aralık district, and 14 in the Tuzluca district.

In the study, by calculating seasonal means of TMSCC of milk samples taken monthly, tank milk SCC was examined based on seasonal factors. The farms that were the subject of the research

were farms that raised Brown Swiss cattle. In order to take milk samples from these farms, we went together with the vehicles of the companies collecting milk. During the putting of milk into the main tanks in the farms, milk samples were filled into sample dishes.

Since no records are kept in the farms, in order to determine the effect of some environmental factors on TMSCC detected in milk samples, the necessary breeding information such as milking method, barn condition, and udder cleaning were obtained from breeders.

### Determination of somatic cell count (SCC)

Milk samples were taken in 50 cc bottles from the milk obtained from daily milking in each farm, and the analyzes were performed with a Somatic Cell Counting device (DeLaval Cell Counter) located in the Zootechny Laboratory of the Faculty of Agriculture of Iğdır University. After a few drops of milk sample were taken into the DCC, the loaded cassette was placed in the DCC, and SCC measurements were performed. SCC values in milk were determined by taking into account the user manual of the device and detailed information about the method given by Gonzalo et al. [15] and Hamann et al. [16].

### Data organization and statistical analysis

In the study, seasons, the effective factors on the value of SCC, were operationalized as I. Season (December, January, and February), II. Season (March, April, and May), III. Season (June, July, and August), and IV. Season (September, October, and November, months). The barn type was examined under two categories as modern and conventional, the milking type was examined under two categories as machine and manual, and udder cleaning was also examined under two categories as yes and no. The districts of the farms where the animals were raised were examined under four categories: Central, Karakoyunlu, Aralık, and Tuzluca.

In addition, based on the classification developed by Wattiaux [12], the mastitis risk levels by SCC were examined under four categories: I. no mastitis (SCC < 200,000 cells/mL), II. Low level (200,000-500,000 cells/mL), III. Widespread (200,000-500,000 cells/mL), and IV. Endemic (> 1,000,000 cells/mL). In the examination of the mastitis risk of these groups in terms of SCC on the basis of factors, the classification Tree (CT) analysis was used. CT, which was proposed and used by Akbulut et al. [17] in the evaluation of livestock data, is one of the non-parametric statistical analyses in which dependent and independent variable(s) can be considered categorical variables and is also very important for livestock studies (but not widely used). In the CT analysis, mastitis risk was used as the dependent variable, and the season, district, farm type, milking type, and udder cleaning were used as independent variables. The number of parent-child nodes was determined as 100:50. The maximum tree depth was taken as 2. SPSS statistical package software (Version 20.0) was used to evaluate the environmental factors affecting the SCC and analyze the data.

## RESULTS AND DISCUSSION

Somatic cell counting of milk obtained at farms is the gold standard in terms of identifying animals with mastitis and obtaining

information about the health of the herd. Quickly and accurately measuring the somatic cell count of the milk tank or animal milk gives the opportunity to start early treatment by allowing timely detection of infected animals, and it ensures animal welfare [18].

The factors affecting the TMSCC values in the milk obtained in this study, which was carried out in Brown Swiss cattle breeding farms, are given in Table 1. It was determined that the TMSCC was affected by the seasonal factor ( $\chi^2=1,164.83$ ,  $p<0.001$ ), while the barn type, milking type, the district where the animals were raised, and udder cleaning factors did not cause a statistical significant variation in terms of TMSCC.

On the other hand, when evaluated quantitatively, it was observed that farms that were conventional and where milking was made by hand and udder cleaning was not performed had more TMSCC (23,000 cells/mL, 34,000 cells/mL, and 14,000 cells/mL, respectively) compared to farms that were modern and where milking was made by machine and udder cleaning performed.

In addition, when seasons were considered, it was seen that the highest TMSCC was obtained in spring and the lowest TMSCC was obtained in autumn. In terms of the districts where cows were raised, the highest TMSCC was obtained from the farms acting in Karakoyunlu, and the lowest TMSCC was obtained from the farms in the Tuzluca district (Table 1).

The mean TMSCC value ( $228,990\pm 13,020$  cells/mL) obtained in this study was lower than value ( $264,200$  cells/mL;  $n=5646$ ) obtained by Kaya et al. [11], while it was higher than values (between  $96,130\pm 21,700$  and  $104,190\pm 16,000$  cells/mL) reported by Temelli and erbetçioğlu [19] for a 4-year period. In this study, the mean LogTMSCC was found to be 5.030. In their study conducted on the Brown Swiss breed, Çoban et al. [20] reported the LogTMSCC value as 5.710.

When TMSCC values obtained in some studies conducted abroad were compared with the TMSCC value ( $228,990\pm 13,020$  cells/mL) determined in the current study, it was seen that the

value of this study was lower than the value of  $383,314\pm 43,227$  cells/mL determined by DeLong et al. [21] in the Mississippi state in the southeast of the USA and higher than the value of  $212,940\pm 11,465$  cells/mL determined in the state of North Carolina in the same study. O'Hara et al. [22] reported mean Log-transferred SCC (LNSCC) values as  $4.72\pm 0.02$ ,  $4.42\pm 0.02$ , and  $4.52\pm 0.02$  in Swedish Holstein cows and  $4.54\pm 0.02$ ,  $4.27\pm 0.02$ , and  $4.35\pm 0.02$  in Swedish red cows for the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> monthly milk measurements, respectively.

In the current study, the geometric mean of TMSCC was calculated as 107,094 cells/mL. This value was higher than the geometric mean value (86,000 cells/mL) reported by Busato et al. [23] in Switzerland and lower than the geometric mean values (ranging between  $174,000\pm 25,000$  and  $205,000\pm 27,000$  cells/mL) reported by Toledo et al. [24] in Swedish Red cattle. When the factors that are thought to affect the TMSCC in farms were compared (Table 1), it was seen that the TMSCC value was low in the autumn season. This may have been caused by the fact that the weather is cold in this season. Aytekin and Boztepe [25] reported that the highest SCC values were detected in summer months. In this study, when the farm type was examined in terms of SCC values, it was found that the SCC was lower in modern farms compared to conventional farms. Similarly, Sarialioğlu and Laçın [26] also determined the mean SCC value at a lower level in modern dairy cattle farms compared to conventional dairy cattle farms. The fact that cleaning and hygiene conditions are more complied with in modern farms has had an impact on this result.

When the milking method applied in farms was examined, it was observed that machine milking made a positive contribution to being SCC low due to the fact that milking by machine is more controlled in terms of hygiene than manual milking. Similarly, Bhakat et al. [27] reported that in Jersey cows, SCC was significantly higher in manual milking compared to machine milking.

When the districts where the animals were raised were exam-

**Table 1** - Statistics for variation in TMSCC values by factors related to farms.

Variables		Survived calves	Deceased calves	P value <sup>1</sup>	P value <sup>2</sup>		
Factors	Groups	n	Descriptive Statistics			$\chi^2$ test <sup>1</sup> p-value	
			Geometric Mean x	Arithmetic Mean x $\pm$ SE	Median		LogSCC Log
Total		680	107,094	228,997 $\pm$ 13,020	114,000	5.030 $\pm$ 0.021	
Season	Winter	170	124,050	207,280 $\pm$ 15,370	158,500	5.094 $\pm$ 0.039	$\chi^2=1,164.80$ $p<0.001^{***}$
	Spring	170	144,750	276,630 $\pm$ 28,750	149,500	5.161 $\pm$ 0.041	
	Summer	170	94,034	239,500 $\pm$ 32,930	88,000	4.973 $\pm$ 0.045	
	Autumn	170	77,896	192,580 $\pm$ 23,500	77,000	4.892 $\pm$ 0.047	
Barn Type	Modern	212	106,560	213,380 $\pm$ 19,810	104,000	5.028 $\pm$ 0.038	$\chi^2=345.04$ $p=0.236^{ns}$
	Conventional	468	107,340	236,070 $\pm$ 16,660	118,500	5.031 $\pm$ 0.027	
Milking Type	Machine	436	103,250	216,720 $\pm$ 15,490	113,500	5.012 $\pm$ 0.027	$\chi^2=313.30$ $p=0.697^{ns}$
	Hand	244	114,330	250,920 $\pm$ 23,430	117,000	5.058 $\pm$ 0.037	
Districts	Central	204	93,170	219,090 $\pm$ 22,920	93,500	4.969 $\pm$ 0.042	$\chi^2=971.34$ $p=0.581^{ns}$
	Karakoyunlu	148	125,650	272,920 $\pm$ 33,430	144,000	5.099 $\pm$ 0.049	
	Aralık	272	111,540	223,250 $\pm$ 20,100	122,500	5.047 $\pm$ 0.033	
	Tuzluca	56	95,710	176,910 $\pm$ 25,070	92,000	4.981 $\pm$ 0.071	
Udder Cleaning	Yes	344	108,530	222,090 $\pm$ 16,880	113,500	5.036 $\pm$ 0.029	$\chi^2=353.35$ $p=0.152^{ns}$
	No	336	105,650	236,070 $\pm$ 19,910	121,000	5.024 $\pm$ 0.024	

<sup>1</sup>: In this study,  $\chi^2$  test and p-value it belongs to arithmetic mean values

<sup>\*\*\*</sup>: statistically significant at a level of  $P<0.001$

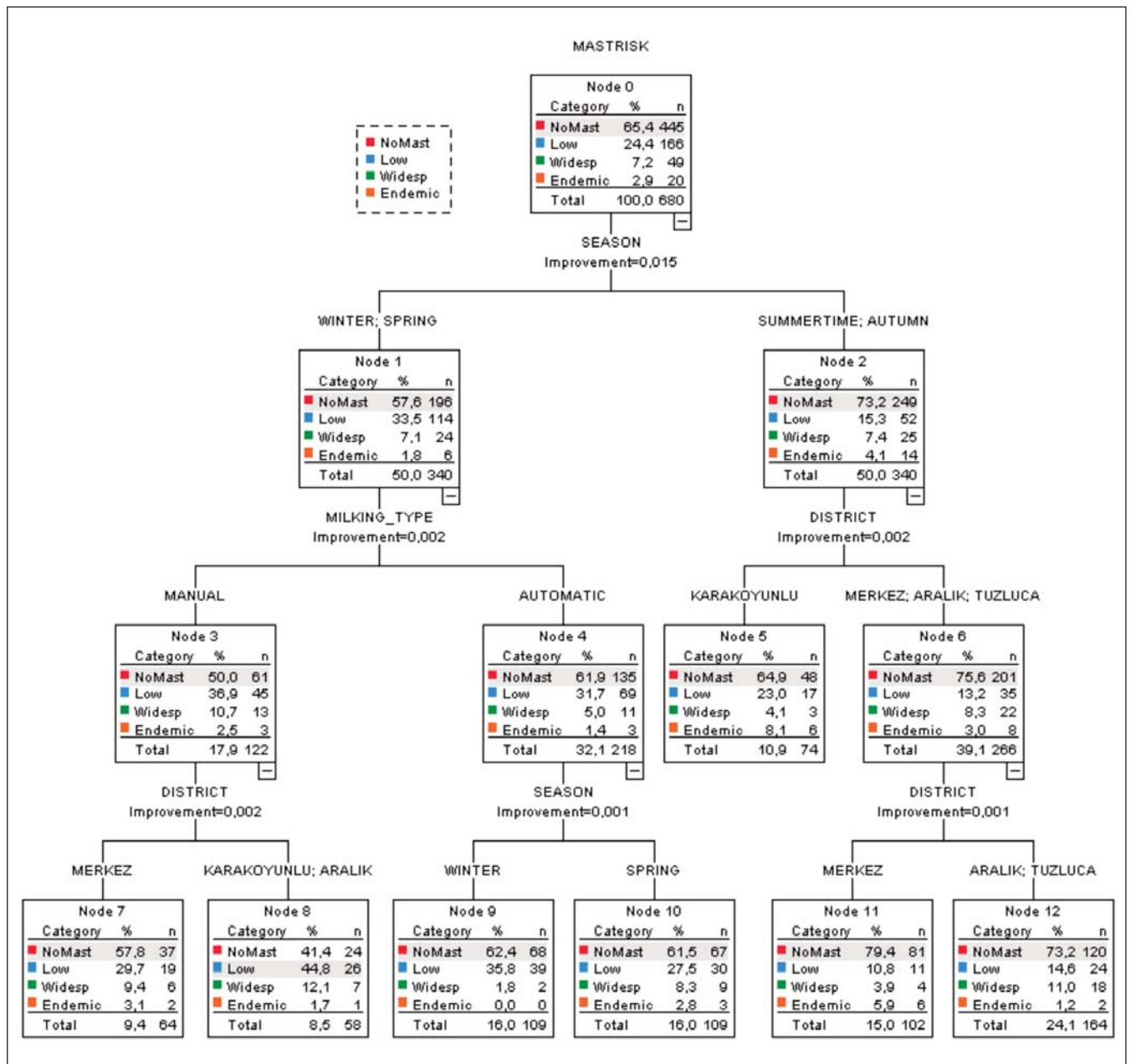
ns: not significant

ined, it was seen that the mean TMSCC value in the milk obtained from the farms in the Tuzluca district was lower than the TMSCC values in the milk obtained from the farms in other districts. This may have been due to the fact that the Tuzluca district has lower temperatures compared to other districts. It is clear that temperature stress affects the immune system of animals. The fact that dairy cows are under temperature stress leads to an increased susceptibility to udder infection. This can also cause SCC to increase by increasing the number of dead epithelial cells and immune cells in milk [28]. In terms of the udder cleaning factor, the SCC values of the milk obtained from farms where udder cleaning was performed were low because the cleaning rules were complied with. In their study conducted in farms raising Anatolian mandate, Sel et al. [29] also reported that the milk obtained from the farms where udder cleaning was performed had statistically significantly lower SCC compared to others that did not apply udder cleaning ( $p < 0.001$ ).

### SCC and Risk Factors

The determination of the mastitis risk level in terms of SCC contents of milk obtained from the farms was examined by the classification tree (CT) method, which is one of the decision tree methods. The risk levels, on the other hand, were determined based on the method proposed by Wattiaux [12] and described in the method section above. The created CT is shown in Figure 2. During the CT analysis process, only season, milking type, and district variables could be included in the model, while the other variables (farm type, udder cleaning) were determined as non-significant (Figure 1). The correct classification percentage of the CT method was calculated as 65.7%. Considering all the data, the proportions of farms that had no, low, widespread, and endemic mastitis in Igdir province were determined as 65.4% (Node 0), 24.4%, 7.2%, and 2.9% respectively. The environmental factor that most affected the risk of mastitis was determined to be the season. 57.6% of the data (Node 1) were in the no mastitis group in winter

Figure 1 - CT diagram of the mastitis risk.



and spring, while 73.2% of the data were in the no mastitis group in summer and autumn. However, the endemic rate increased by 4.1% in the summer and autumn months compared to the winter and spring months (Node 2) (Figure 1).

It was determined that after the seasonal factor, the milking type and district factors were effective on the risk of mastitis. The rate of no mastitis was found to be 61.9% (Node 4) in machine-milking, while the rate of no mastitis was found to be less (50%) in hand milking (Node 3). When examining the risk of mastitis by districts, the rate of no mastitis in milk obtained from farms located in Karakoyunlu district was found to be 64.9% (Node 5), and no mastitis in milk obtained from farms in the central and other districts was found to be 75.6% (Node 6) (Figure 1).

In this study, the risk rates of mastitis were found to be 65.4%, 24.4%, 7.2%, and 2.9% for the categories of no mastitis, low, widespread, and endemic, respectively (Figure 1). In another study conducted with the same method, mastitis risk rates were reported as 64%, 18%, 7%, and 10% for the same categories, respectively [30]. On the other hand, by studying as described by Wattiaux [12].

## CONCLUSION

As a result, in this study, it was determined that the SMSCC value was affected by the season, barn type, milking method, udder cleaning, and breeder conditions (on the basis of districts). In addition, it was also revealed that the TMSCC values obtained in the context of the study were lower than the values specified in the EU and TGK norms. This shows that the consumption of milk and dairy products obtained from cows as food will not pose a problem from the point of view of public health and it can be used safely. Moreover, it was also observed that the awareness of breeders about healthy milk production was formed. In this context, it may be recommended to gradually reduce the SMSCC to below the level of 100,000 cells/mL in dairy cattle breeding farms in Iğdır province in terms of compliance with the quality milk criterion. Reducing somatic cell counts (SCC) in dairy cows is essential for maintaining milk quality and ensuring the health of the animals. Farmers should implement strict hygiene practices during milking and udder preparation. They should regularly test for SCC and promptly treat any cases of mastitis. Additionally, they should ensure that cows receive a balanced diet that supports their immune systems. Farmers should train staff in proper milking techniques and provide an environment that minimizes udder stress. They should provide clean and comfortable living conditions with good ventilation and keep detailed records to monitor SCC levels and overall cow health. Farmers should consider genetic selection for improved udder health traits in breeding decisions. By following these practices, farmers can significantly improve milk quality, enhance profitability, and promote the well-being of their dairy herd.

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in 2021 due to Covid-19.

## Ethical statement

No animals were harmed during the collection of milk samples. We collected the samples after the milking of Animals. We were not involved in the milking process of these animals.

## Conflict of Interest

As authors, we declare that there is no conflict of interest between us.

## Author contributions

IY and MMT designed the study. IY collected data. AEK made the statistical analysis. The article was written by IY, MMT, and AEK. The language of the article was checked by MMT. All authors contributed to the critical revision of the article.

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