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## **RESEARCH ARTICLE**

# Determination of the Effect of Somatic Cell Count on Udder Measurements and Subclinical Mastitis with Data Mining Method

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## ABSTRACT

In this study, it was aimed to determine the effect of somatic cell count (SCC) on udder measurements and subclinical mastitis in Holstein cows by data mining method. In the study, the udder measurements and the SCC values of milk samples taken monthly from 79 Holstein cows were used. The Bayesian Net, Decision Table and Nearest Neighbors algorithms were used in the classification of the udder measurements, and model validation is determined by the simple validation method. In the study, it has been found that the best classification model was formed according to the Nearest Neighbors algorithm with the accuracy rate of 97.95% [Root Mean Square Error (RMSE):0.07, Mean Absolute Error (MAE):0.01, Root Relative Squared Error- RRSE (%):22.20, Relative Absolute Error -RAE (%): 5.78, Kappa statistic: 0.95]. The effect of udder measurements on subclinical mastitis was found significant for the front teat length (FTL), the distance between rear teats (DBRT), the distance between side teats (DBST), the rear teat height (RTH) (P<0.01) and the rear teat diameter (RTD) (P<0.05).

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#### **INTRODUCTION**

One of the diseases widely seen in modern dairy farms and that caused huge economic losses is mastitis (Kaya et al., 2011). The more common form of mastitis is subclinical mastitis (Park et al., 1982). The subclinical mastitis can be diagnosed by observing the SCC and the bacteria levels in the milk (Yalcin et al., 2010). The SCC is  $\leq 100 \times 10^3$  cell/ml in healthy cow's milk (Hillerton, 1999), and the leukocytes constitute approximately 75-85%, and the epithelial cells constitute 15-25% of these cells (Barret, 2002). There is an increase in the number of macrophages, lymphocytes, neutrophils, as well as decrease in the number of epithelial cells in the milk of cows with subclinical mastitis (Coban et al., 2008), and neutrophils constitute more than 90% of the leukocytes (Ten Napel et al., 2009). The increase in SCC has been considered to be an early stimulus of inflammatory changes in the mammary gland, and the mammary gland has been evaluated as the subclinical mastitis suspicious when the SCC in the milk is  $\geq 200 \times 10^3$  cell/ml (Wattiaux, 2005). The lactation number (Eyduran et al., 2005), the

udder structure (Uzmay *et al.*, 2003), the breeds, the milking frequency, the season, the milking hygiene and the education level of breeders have been considered among the factors that affect the SCC other than the infectious agents. A deterioration in the udder health, a decrease in the quality of milk and 5-20% decrease in the amount of milk produced occur depending on the increase in SCC (Juozaitiene *et al.*, 2006).

At the same time, automatically determining some parameters such as the SCC and the number of bacteria that are important indicators for detecting subclinical mastitis, which constitutes more than 70% of the losses induced by mastitis in dairy cattle breeding, (Harmon, 2001) via measurement devices leads to an increase in the number and the types of data to be collected in this way.

Today, one of the methods used in the evaluation of such large data sets is Data Mining (DM). This method can be defined as obtaining the information, which is previously unknown and remained hidden but at the same time useful, from the data stacks in a non-monotonic process (Baykal, 2006). There are many studies showing that data mining gives successful results in the classification processes in the field of dairy cattle breeding. For instance, Ortiz-Pelaez and Pfeiffer (2008) used three different data mining techniques including logistic regression, classification trees and factor analysis in order to classify the cattle herds according to the disease risk. Kamphuis *et al.* (2009) used the classification tree which is a data mining method to determine subclinical mastitis, and could be used as an appropriate method in diagnosing this disease. Grzesiak *et al.* (2011) used the Naive Bayes classifier (NBC), and classification and regression trees (CART) methods of the data mining in order to detection of difficult conceptions in dairy cows, and they stated that these methods can be useful for the breeders.

The aim of this study was to classify the fact of subclinical mastitis in Holstein cows with the Bayesian Net, Decision Table and Nearest Neighbors algorithms of data mining depending on SCC and to determine the udder measurement characteristics that are effective in catching subclinical mastitis according to the best classifier model that is formed.

## MATERIALS AND METHODS

Animal and feeding management: In the study, 30 cows in the 1<sup>st</sup> lactation and 49 cows in the 2<sup>nd</sup> lactation, as a total of 79 Holstein cows in a farm in Nigde province (Turkey) were used. It was ensured that the trial material cows were not taken between the 3<sup>rd</sup> and the 30<sup>th</sup> of the lactation, and there was no structural (anatomical) defects and clinical mastitis in their udders. Cows were grouped according to lactation period and fed by total mix ration (TMR) having different feed ingredients (Table 1).

 $\ensuremath{\text{Table1:}}$  Total Mix Ration (TMR) and calculated nutrient content for lactating cows

Feed ingredients	TMR				
	The beginning of lactation (I month)	Peak yield Period (2-5 months)	Low yield Period (6-10 months)		
Roughage /concentrated feed rate (%)	43:57	40:60	46:54		
Calculated nutrient content (Dr	ry Matter %)				
Crude protein (%)	17	17.3	16		
Metabolic energy (kcal/kg)	2968	2932	2661		
ADF (%)	19.8	19.1	24.2		
NDF (%)	32.3	33.1	40.5		

**Udder measurements:** The udder measurements were taken before milking. Front teat length (FTL), rear teat length (RTL), distance between front teats (DBFT), distance between rear teats (DBRT), distance between side teats (DBST), front teat diameter (FTD), rear teat diameter (RTD), front teat height (FTH) and rear teat height (RTH) were measured. The measurements were taken according to the method reported by Kuczaj (2003) and Kul *et al.* (2006) using measurement stick and measurement strip.

**Somatic cell count (SCC):** Individual milk samples were collected monthly with in-line milk meters and transferred into 50 ml plastic tubes, and SCC were measured from fresh milk samples by DeLaval Cell Counter (DeLaval Int. AB, Tumba, Sweden). The relationship of the SCC values of the milk samples taken from the cows with

subclinical mastitis was separated into classes as; not seen  $(<200 \times 10^3 \text{ cell/ml})$ , low  $(200-500 \times 10^3 \text{ cell/ml})$ , widespread  $(500-1000 \times 10^3 \text{ cell/ml})$  and epidemic  $(>1000 \times 10^3 \text{ cell/ml})$  in the light of the information existed in the literature (Wattiaux, 2005; Kul *et al.*, 2006).

**Data mining method:** Data mining (DM) is an important process step for obtaining meaningful information from the data, in other words, is a process of information discovery. In this aspect, the DM can be regarded as a step in the realization of the discovery part of the core information discovery as well as regarded as an independent process in databases (Tuzunturk, 2010). There are many methods (techniques) in DM and the categories that are accepted as widely used were conducted by J. Han. According to him, these categories were made in six different ways, including Characterization and Discrimination, Association Analysis, Classification and Prediction, Cluster Analysis, Outlier Analysis and Evolution Analysis (Ertugrul et al., 2013). The DM used in this study is a classification application, and first the data was optimized for analysis. Then, Bayesian Net, the Decision Table and the Nearest Neighbors algorithms, which are included in Weka program, were used and the best classifier model within the models made by these algorithms was chosen. The simple validation method was used for testing the model validation (Weka, 2013). This model was used in order to classify the subclinical mastitis incidence and to estimate it. Secondly, udder measurement characteristics effective in the risk of developing subclinical mastitis were determined using chi-square test statistics. In addition, the effect of SCC on developing this disease was analyzed.

In order to compare the Bayesian Net, the Decision Table and the Nearest Neighbors algorithms, the Root Mean Squared Error (RMSE), the Mean absolute error (MAE), the Root relative squared error (RRSE), the Relative absolute error (RAE) and the Kappa statistic (K) were used. According to these criteria, the model giving high K and low RMSE, RRSE and RAE value has shown a better fit. The equations and the equation terms are given below.

$$\begin{split} \mathbf{K} &= \frac{\mathbf{P}_{G} - \mathbf{P}_{B}}{1 - \mathbf{P}_{B}}, \quad \mathbf{P}_{G} = \frac{\sum_{i=1}^{n} \mathbf{G}_{i}}{n}, \qquad \mathbf{P}_{B} = \frac{\sum_{i=1}^{n} \mathbf{R}_{i} \mathbf{C}_{i}}{n^{2}}, \\ \mathbf{MAE} &= \frac{1}{n} \sum_{i=1}^{n} \left| \mathbf{t}_{i} - \mathbf{o}_{i} \right|, \quad \mathbf{RMSE} = \sqrt{\frac{1}{n} \sum_{i=1}^{n} \left| \mathbf{t}_{i} - \mathbf{o}_{i} \right|^{2}}, \\ \mathbf{RAE} &= \frac{\sum_{i=1}^{n} \left| \mathbf{t}_{i} - \mathbf{o}_{i} \right|}{\sum_{i=1}^{n} \left| \mathbf{t}_{i} - \overline{\mathbf{t}} \right|}, \quad \mathbf{RRSE} = \sqrt{\frac{\sum_{i=1}^{n} \left| \mathbf{t}_{i} - \mathbf{o}_{i} \right|^{2}}{\sum_{i=1}^{n} \left| \mathbf{t}_{i} - \overline{\mathbf{t}} \right|^{2}}} \end{split}$$

Where,  $P_G$  and  $P_B$  refer to the observed and expected probabilities, respectively, and others indicate the followings:  $G_i$ : the observed frequency of the i<sup>th</sup> row and column,  $R_i$ : the total frequency of i<sup>th</sup> row,  $C_i$ : the total frequency of i<sup>th</sup> column, n: the total number of observations,  $\overline{t}$ : the mean of target values,  $t_i$ : estimated value of the i<sup>th</sup> class, and  $o_i$ : the actual value of the i<sup>th</sup> class

Table 2: The results of the milk samples classification of Bayesian net, Decision Table and Nearest Neighbors algorithm

Algorithm total	Classification result of each algorithm			Error variance values				
(391)	Correctly Classified	Incorrectly classified	Time taken	Карра				DDCE%
	Samples (%,Value)	samples (%,Value)	(seconds)	statistic	INAE	KIIJE	NAE/0	KKJE/0
Bayesian net	73.65(289)	26.35 (102)	0.03	0.02	0.21	0.33	98.37	99.38
Decision table	74.42(291)	25.58 (100)	0.09	0.06	0.20	0.32	97.01	98.49
Nearest Neighbors	97.95(383)	2.05 (8)	0.01	0.95	0.01	0.07	5.78	22.20

 Table 3: Irregularity matrix according to the result of the Nearest

 Neighbors algorithm

Classes	Low	Not	Epidemic	Widespread	Total	The success
		seen				rate of number
						of correctly
						classified
						samples (%)
Not seen	0	288	0	0	288	100
Low	52	6	0	0	58	89.65
Widespread	0	2	0	25	27	92.59
Epidemic	0	0	18	0	18	100
A	0/	- (200			-0/	

Accuracy rate % = (288+52+25+18)/391=97.95%

(Weka, 2013). The degree of compliance of the Kappa statistic is classified as [<0] bad, [0:01-0:20] poor, [0:21-0:40] not bad, [0.41-0.60] medium, [0.61-0.80] good and [0.81-0.92] very good, [0.93-1] excellent (Boyacioglu and Guneri, 2006).

The 3.6.9 version of Weka software package was used in evaluating performances of data mining algorithms during the process of the classification of subclinical mastitis (Weka, 2013). The SPSS 15.0 package program was used for chi-square analysis (SPSS, 2006).

### RESULTS

The milk instances that subclinical mastitis observed in Holstein cows were determined as a result of the classification of udder measurement values according to three different Data Mining algorithms. The results of the classification and the error variance values of Bayesian Net, Decision Table and Nearest Neighbors algorithms were as in Table 2. Accordingly, the RMSE, MAE, RRSE (%) and RAE (%) values of the created models were found in the range of 0.07-0.33, 0.01-0.21, 22.20-98.38 and 5.78-98.37, respectively (Table 2). When the models compared in terms of error criteria, the kappa statistic of the Nearest Neighbors Algorithms which has the lowest value was found as 0.95. Also, in revealing the relationship that exists between variables, the accuracy rate was determined to be higher than the others (Table 2).

According to this model, 383 milk samples from a total of 391 were classified accurately with the accuracy rate of 97.95%. The accuracy of subclinical mastitis class values was found as in Table 3 according to the Nearest Neighbours algorithm that successfully separates classes from each other accurately and with minimal errors. According to these results, only 8 of the milk samples were incorrectly classified according to this algorithm. Considering the measured udder values, it has been determined that 26.4% of cows in the herd can have subclinical mastitis, while 73.6% of them do not have (Table 3).

The distributions of the milk samples with subclinical mastitis according to the udder measurements were presented Table 4. The effect of udder measurement groups on subclinical mastitis was significant in the groups FTL, DBST, DBRT, RTH and in RTD, while it

was non-significant in the groups RTL, FTH, DBFT and FTD. When the FTH and RTH increases, the risk of having subclinical mastitis decreases, but it has been seen that this effect is non-significant (P>0.05) in the FTH group, while this is significant in the RTH group (P<0.05). When the diameter of teat analyzed, it was noted that the FTD of 69.5 % of the cows with subclinical mastitis is between 2.5-3 cm, in 7.4% is more than 3.1 cm, although the difference was non-significant. For RTD, the teat diameter of 66.3 % of the cows ranges between 2.5-3 cm, and of 7.4% had >3.1 cm, while the difference was significant. It was further observed that in 55.8% of the cows according the DBFT was between 16-24 cm, and in 1.1% was >32 cm and difference was non-significant. However, the difference was significant for DBRT and DBST (P<0.01) (Table 4).

In this study, RTH and FTH had negative correlation with not seen (P<0.01) group, RTH and FTH had positive correlation with low (P<0.05, P<0.01) group. Also, that the RTD was found to be significant positive correlation with epidemic (P<0.05) group (Table 5).

### DISCUSSION

The discrepancies in udder structure increase the risk of many diseases such as mastitis and shorten the life of the cow. It induces economic costs, mainly consisting of discarded milk, increased health care costs and reduced milk quality (de Haas, 2003). High favorable genetic correlations are estimated between teat length, udder depth, fore udder attachment and udder balance and cases of clinic mastitis in several populations of dairy cows (Lund *et al.*, 1994; Rupp and Boichard, 1999). Cows with shorter teats, higher udders and tighter fore-udder attachment were genetically less likely to develop mastitis (de Haas, 2003). Also the udder size cow has increased the milk yield (Sekerden *et al.*, 2009).

In the study, the subclinical mastitis status according to the SCC values is divided into 4 different classes including not seen, low, widespread and epidemic. The Bayesian Net, Decision Table and Nearest Neighbours algorithms were used in the classification. It was found that 26.4% of the cows in the herd may have subclinical mastitis, while 73.6% of them do not when cows are classified according to the udder measurement values. This also shows that about 1/4 of the cows in the herd could have an intra-mammary infection. The rate of 26.4% of the cows having subclinical mastitis found by data mining method is lower than the rate of 36% of cows having subclinical mastitis found in the study reported by Kaygisiz and Karnak (2012), while it has been found higher than the rate of 0-5.3% reported by Kaya et al. (2011). These differences are thought to be sourced by factors such as the season, nutrition, lactation stage, other infections that the animal has and the type of bacteria that causes subclinical mastitis.

Table 4: The distribution of the milk samples with subclinical mastitis according to the udder measurements

Groups	Low	Widespread	Epidemic	Groups	Low	Widespread	Epidemic
	f (%)	f (%)	f (%)		f (%)	f (%)	f (%)
FTL	**			RTL	Ns		
4-5	34 (65.4)	6 (24.0)	0 (0)	3-4	19 (36.5)	6 (24)	7 (38.9)
6-7	9 (17.3)	17 (68.0)	3 (16.7)	5-6	31 (59.6)	16 (64)	11 (61.1)
8-9	7 (13.5)	2 (8)	14 (77.8)	7-8	l (l.9)	3 (12)	0 (0)
>10	2 (3.1)	0 (0)	I (5.6)	>9	l (l.9)	0 (0)	0 (0)
DBFT	Ns			RTH	**		
7-15	18 (34.6)	10 (40)	5 (27.8)	34-40	2 (3.8)	0 (0)	3 (16.7)
16-24	28 (53.8)	15 (60)	10 (55.6)	41-47	17 (32.7)	4 (16)	4 (22.2)
25-31	5 (9.6)	0 (0)	3 (16.7)	48-54	16 (30.8)	6 (24)	7 (38.9)
>32	l (l.9)	0 (0)	0 (0)	>54	17 (32.7)	15 (60)	4 (22.2)
FTH	Ns			FTD	Ns		
41-47	18 (34.6)	4 (16)	7 (38.9)	1-2.5	13 (25)	5 (20)	4 (22.2)
48-54	23 (44.2)	10 (40)	6 (33.3)	2.51-3	38 (73.1)	17 (68)	l I (61.Í)
>54	11 (21.2)	11 (44)	5 (27.8)	>3.1	l (1.9)	3 (12)	3 (16.7)
DBRT	**		( )	DBST	**	( )	· · · ·
4-6	2 (3.8)	0 (0)	3 (16.7)	7-12	9 (17.3)	6 (24)	0 (0)
7-9	15 (28.8)	8 (32)	3 (16.7)	13-18	37 (71.2)	16 (64)	2 (11.1)
10-12	28 (53.8)	11 (44)	4 (22.2)	19-24	6 (11.5)	3 (12)	2 (11.1)
> 3	7 (Ì 3.5)	6 (24)	8 (44.4)	>25	0 (0) <sup>´</sup>	0 (0)	14 (77.8)
RTD	*		( )				( )
1-2.5	14 (26.9)	5 (20)	6 (33.3)				
2.51-3	38 (73.I)	16 (64)	9`(50)				

\*: P<0.05; \*\*: P<0.01; Ns: P>0.05; FTL: Front teat length, RTL: Rear teat length, DBFT: Distance between front teats, DBRT: Distance between rear teats, DBST: Distance between side teats, FTD: Rear teat diameter, RTD: Rear teat diameter, FTH: Front teat height, RTH: Rear teat height.

3 (16.7)

Table 5: The correlation between udder characteristics of the cows and individual log<sub>10</sub>SCC

4 (16)

0 (0)

>31

	010			
Traits	Not seen	Low	Widespread	Epidemic
FTL	-0.003	-0.21	-0.09	0.28
RTL	0.07	-0.09	-0.20	0.26
FTH	-0.15**	0.34*	0.16	-0.06
RTH	-0.15**	0.43**	0.25	-0.03
DBFT	0.03	-0.23	-0.22	-0.24
DBST	0.17**	-0.16	-0.25	0.28
DBRT	-0.009	0.06	0.08	-0.33
FTD	0.04	0.21	-0.06	0.38
RTD	0.03	0.23	0.07	0.68*
*: P<0.05;	**: P<0.01			

The values of FTL, DBST, DBRT, RTH (P<0.01) and RTD (P<0.05) of the cows in the study are suggestive in having subclinical mastitis, and consequently also an increase has been found in their SCC values, and this shows similarities with the statement of Rogers and Hargrove (1993) that the cows with higher SCC have a high udder base. Furthermore, it has been found consistent with the statement of Bardakcioglu et al. (2011) that rear udder diameter, the rear udder height from the ground have an important effect on mastitis, but has been found inconsistent with the statement that front teat height from the ground has an important effect. In the cow groups of RTL, FTH, DBFT and FTD, it has been observed that the subclinical mastitis case increases depending on SCC values; however, it has been determined that it is not statistically important.

At the same time, that this correlation has been found higher in groups with subclinical mastitis can be considered as an indicator for the infection occurs in udder structures. In the group that subclinical mastitis is not seen the relationship between DBFT and DBRT with SCC was 0.03 and -0.009, respectively (Table 5), and these values were lower than the correlation levels of 0.08 and 0.22 reported by Kuczaj (2003). The correlation level of DBST and SCC has been found statistically important (P<0.01) with the value of 0.17, and was higher than the value of -0.07 reported by Kuczaj (2003). A negative relationship was found with the value of -0.15 between FTH and SCC, and RTH and SCC. This value was lower for FTH, and higher for RTH than the values of 0.29 and -0.30 reported by Kuczaj (2003). This has been thought to result from the differences in cow breeding and feeding conditions.

Conclusion: The measurements of FTL, RTH, RTD, DBST and DBRT in cases of subclinical mastitis in Holstein cows corresponded with SCC values and were significant. This data mining study conducted in the field of dairy cow breeding is thought to be an alternative method for the researchers working in this field in determining subclinical mastitis by correlating udder measurements with the SCC values, especially in decision-making and large-scale data analysis. We suggest that the Nearest Neighbors algorithm could be used to the classification and with the highest accuracy rate. This information can be help to identify mastitis risk factors for herd health control program. It will also serve as a guide for developing sustainable milk quality goals and provide the means for monitoring progress towards those goals. In addition, our study results show that SCC values in the cows are in compliance with Republic of Turkey Ministry of Food, Agriculture and Livestock and EU affairs norms.

Author's contribution: AC and MC undertook experimental work, designing the experiment and acquisition of data (milk samples, SCC and teat measurements), HK and FU analyzed the statistical data and interpreted the results of analysis. Additionally, all authors interpreted during referee's progress critical revision of the manuscript.

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