Turkish Journal of Agriculture - Food Science and Technology, 6(5): 557-560, 2018

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X
www.agrifoodscience.com,
Turkish Science and Technology

Relationships Between Dye Reduction Test Scores and Somatic Cell Count in Bovine Raw Milk

Baris Kadir Yalcin, Savas Atasever*

Department of Animal Science, Faculty of Agriculture, Ondokuz Mayıs University, 55139 Atakum/Samsun, Turkey

ARTICLE INFO

Research Article

Received 14 December 2017
Accepted 13 April 2018

Keywords:
Cow milk
Methyelene blue
Resazurin
Somatic cell count
Raw milk quality

*Corresponding Author:
E-mail: satasev@omu.edu.tr

ABSTRACT

The aim of this study was to reveal the relationships between dye reduction test scores and somatic cell count (SCC) in bovine raw milk. The SCC, methyelene blue reduction scores (MTS) and resazurin reduction test scores (RTS) were determined at biweekly intervals in four test days (TD) between March and April 2017 in a total of 89 raw milk samples sold in Samsun province as unpacked. While SCC values were recorded by an automatic counter, all SCC values were transformed to log10 base before statistical analysis. In MTS method, the time for the change of the color of milk from blue to white was noted and milk quality was assessed using a 1 to 4 point scale (1=>5h-good/excellent; 2=2-5h-medium; 3=0.5-2h-bad and 4=<0.5h-very bad). The change of milk color caused by resazurin solution after one-hour incubation at 37°C was evaluated by a 1 to 3 point scale (1=blue-very well; 2=mauve/deep pink-medium and 3=light blue/uncolored-poor). While significant differences were determined among logSCC means by TD groups after one-way analysis of variance (One-way ANOVA), effects of TD on MTS or RTS were insignificant. Besides, when the samples were examined by lower or higher than 500×10^3 cells/ml that assumed as the threshold for SCC in this study, no significant effect of SCC on the MTS or RTS. In this study, correlation and determination coefficients of SCC with MTS and RTS were calculated to be r=0.279; R^2=0.149 and r=0.18; R^2=0.097 and, it was concluded that dye reduction tests are not suitable to reliably determine the quality of bovine raw milk.

DOI: https://doi.org/10.24925/turjaf.v6i5.557-560.1755

Introduction

In spite of the main target in livestock farms has been regarded boosting animal products until recently, a great effort has been shown to elevate not only quantity but also quality of the products in today's enterprises. Moreover, preventing animal products away from bacterial invasion may be seen a positive approach by public health. In this context, dairy sector is seen as the dominant structure among the animal sections. Milk with low bacteria or antibiotic residues and no abnormal character by smell or taste is assumed to be favourable. Today, some indirect but rapid techniques have been developed to detect milk quality instead of bacteria counting. Of these, electrical conductivity, pH, coagulation score and somatic cell count (SCC) have commonly been used. Study results (Koc, 2008; Atasever et al., 2012; Mikone Jonas et al., 2016) emphasized that SCC increased according to many factors such as breed, age, stage of lactation, location or management differences. While elevated levels of SCC reflect an abnormality in milk or mammary gland of cow, a punishment-reward system according to SCC has been applied in many countries. The highest SCC limit for bovine raw milk than can be consumed by human has been manifested to be 400×10^3 cells/ml by EU directives and this level has been declared to be 500×10^3 cells per ml by Turkish Food Codex in Turkey (Erdem et al., 2010).

To determine milk quality, dye reduction observations have also been performed in some circumstances. Of these, methylene blue and resazurin reduction have been known as rapid and easy examination methods, however, their reliabilities have still been argued. While the time for colourless of milk is recorded in the first method, the change of colours in milk within an hour is noted after adding dye materials. De Silva et al. (2016), who reported a high (0.91) correlation between methylene blue test score (MTS) and total bacteria count (TBC), emphasized that MTS data could be used as reliable parameter to detect raw milk quality. In contrast, Kramomtong et al. (2007) reported low associations of MTS with TBS. These studies clearly indicate the low reliability or validity of dye tests. While Bilkis et al. (2013) informed that MTS values might be benefitted to

decide milk quality, Muliro et al. (2013), revealed that RTS data were not suitable as a quality test method. As seen, investigating the associations of dye reduction tests with SCC will ensure considerable information to dairy owners and food analysts. Moreover, this type studies are expected to lead to other researchers via contributing to the literature.

The objective of the present study was to determine the association of MTS and RTS with SCC, which has been assumed as the most reliable indirect quality parameter, in bovine raw milk samples.

Materials and Methods

Milk samples were collected from buckets after morning milking in Samsun province, which is located in the Black Sea region of Turkey, in four different test days (TD). All samples were produced by Jersey and its crosbreds with Turkish native cows those reared in the similar breeding, barning and feeding conditions. In the study, SCC data were obtained by an automatic cell counter and due to high variation among the values, all numbers were transformed to logarithm 10 base (logSCC) to ensure homogeneity. Of dye reduction tests, RTS data were achieved by applying resazurin solution to the raw milk samples (Braide et al., 2015). The solution was prepared by 2.5 mg resazurin within 50 ml distilled water. For the test, 1 ml solution was added to 10 ml milk and after 1 h incubation at 37 °C, change of colour was recorded using a score scale (1=blue, extra; 2= dark pink or lilac, first class; 3=pink or uncoloured, second class). In MTS evaluation, decolorization time of milk samples those including methylene blue solution according to bacterial load was recorded. Dye solution that composed of methylene blue and distilled water was added to milk samples and disappearing time of blue colour was recorded (Mahari and Yemane, 2016). Thus, test results were obtained with scores where higher ones indicates poor milk quality (4=< ½ h, 3= <1/2-2h, 2= <2-5 h and 1=<5h). All milk quality parameters were evaluated to determine the effect of TD by one- way analysis of variance (One-way ANOVA). The mathematical model was as follows:

$$Y_{ij}=\mu+T_i+e_{ij}$$

Where: Y_{ij}: is dependable variable (logSCC, RTS and MTS), μ: population mean, T_i: effect of TD *(i=1,2,3,4)* and e_{ij}: random residual.

The means of the groups were compared with Duncan's multiple comparison test. Also, the change in RTS and MTS data were assessed in two SCC subgroups (1=lower SCC; $\leq 500x10^3$ cells/ml and 2= higher SCC; $>500x10^3$ cells/ml) by independent *t*-test. To reveal the relationships among three parameters, Kandall's tau-b correlation coefficients were calculated. The statistical analyses were computed with SPSS 17.0 for Windows package program at the significance level of $P<0.05$.

Results and Discussion

Descriptive of parameters used in this study are seen in Table 1. As mentioned in Materials and Methods, calculated RTS mean indicates to moderate quality level of milk samples. Besides, MTS mean points out to moderate/poor quality. In the analysis, logSCC and SCC means were obtained to be 5.58 and 648×10^3 cells/ml, respectively. These levels could be evaluated as high, according to EU directives. It was observed that milk samples investigated in the present work had moderate/low quality level when all quality parameters of the study were assessed together.

Changes of the traits according to TD are presented in Table 2. As understood, highest RTS and MTS means were obtained in the 1st and 4th TD, respectively. However, no difference was found among the groups, statistically. In contrast, logSCC means between 1st and 2nd, 1st and 4th or 2nd and 3rd TD were differed from each other ($P<0.05$). The lowest and highest logSCC means were calculated from 2nd and 1st TD groups, respectively. These findings clearly showed that TD was an effective environmental factor only for SCC among three traits. Really, Miller et al. (2004) emphasized day to day variation among the SCC values. Relatively higher numerical structure of logSCC by DRTS might be one of the main reasons of this result.

Table 1 Descriptives of the parameters (x ± Sx)

Parameter	n	Min	Max	x±Sx
RTS	87	1	3	2.09±0.60
MTS	87	1	4	2.54±0.77
LogSHS	87	4.45	6.67	5.58±0.46

Table 2 Change of the parameters by test days

Test day	n	Parameter (x±Sx)		
		RTS	MTS	LogSHS
1	22	2.31±0.64	2.54±0.67	5.82 ± 0.53^{c}
2	22	2.04±0.48	2.40±0.73	5.36 ± 0.33^{a}
3	22	2.04±0.65	2.50±0.74	5.69 ± 0.27^{bc}
4	21	1.95±0.58	2.71±0.95	5.46 ± 0.53^{ab}
General	87	2.09±0.60	2.54±0.77	5.58±0.46

Different superscript letters in the same column indicate statistically significant differences ($P<0.05$)

Table 3 Means (x±Sx) of RTS and MTS by SCC subgroups

SCC groups	n	RTS	MTS
<500 cells/ml	51	2.03±0.59	2.37±0.74
≥500 cells/ml	36	2.16±0.60	2.77±0.76

Table 4 Correlation coefficients among the traits

Parameter	MTS	logSCC
RTS	0.322*	0.180
MTS		0.279

(*): P<0.05

Table 5 Regression values between RTS and SCC

Coefficient	SE	t	P
-0.162	0.750	-0.216	0.830
0.403	0.134	3.015	0.003

Table 6 Regression values between MTS and logSHS

Coefficient	SE	t	P
-1.058	0.935	-1.131	0.261
0.644	0.167	3.860	0.000

Variation in DRTS by two SCC threshold groups is seen in Table 3. The threshold for SCC (500×10^3 cells/ml) was declared by Turkish Food Codex as the legal limit of SCC value which may be accepted to consume milk by humans (Davut and Atasever, 2017). The means of both traits were lower in the first SCC group. But, similar to Table 2, no statistically significant difference was determined between two SCC groups. The low sample sizes of the groups might be assumed to be a reason for this result obtained here. Demirci et al. (2010) reported that RTS method is not suitable to decide an abnormality in milk with high microbial load due to relatively rapid declorasition. That's why, raw milk samples with low quality might be caused to some false results in the obtained data.

Variation in DRTS by two SCC threshold groups is seen in Table 3. The threshold for SCC (500×10^3 cells/ml) was declared by Turkish Food Codex as the legal limit of SCC value which may be accepted to consume milk by humans (Davut and Atasever, 2017). The means of both traits were lower in the first SCC group. But, similar to Table 2, no statistically significant difference was determined between two SCC groups. The low sample sizes of the groups might be assumed to be a reason for this result obtained here. Demirci et al. (2010) reported that RTS method is not suitable to decide an abnormality in milk with high microbial load due to relatively rapid declorasition. That's why, raw milk samples with low quality might be caused to some false results in the obtained data.

At the end of the present study, associations among three traits were determined using Kendall's tau-b correlation estimation method. The correlation coefficients of the traits were estimated for both two SCC threshold groups (partial correlations) and also for the total samples (Table 4). As seen, a weak (r=0.322) correlation coefficient was determined between MTS and RTS. Also, both DRTS values had statistically insignificant correlations with logSCC. Actually, these associations might be expected to be stronger, however, because of the raw milk samples of this study had been collected from open bazaars where hygiene and storage conditions were poor, these findings obtained here might be regarded as a normal result. In other words, wide variation of SCC between 28×10^3 cells/ml and 4686×10^3 cells/ml might be assumed as the main reason for relatively low correlations. Nevertheless, when SCC was assumed as the control group, MTS may be preferred by RTS.

In the view of the obtained finding here, regression equailities were estimated to determine the relationship level of SCC with RTS and MTS. The descriptives of regression values between RTS and logSCC are seen in Table 5. According to the calculations, the suitable regression model was achieved to be; Y=0.403X-0.162 and determination coefficient (R^2) was determined to be 0.097. In other words, revealed association may be assumed as very low.

Also, other descriptives of regression values between MTS and logSCC are given in Table 6. Thus, the regression model and R^2 for MTS and logSCC was calculated to be; Y=0.644X-1.058 and 0.149, respectively. This finding clearly indicated that the change about 15% in MTS values was caused by logSCC values. Actually, this level might be assumed as higher than the value calculated between RTS and logSCC.

In normal, estimated relationships of DRTS with SCC could be expected as higher than obtained levels here. However, unsuitable storage conditions of bucket milk samples those used as the study material in the present study might be regarded to be the main reason for this case. Moreover, examined milk samples had a wide variation by SCC in the study. Such that, Demirci et al. (2010) emphasized that raw milk samples with high leucocyte number are not suitable for RTS method. Nevertheless, of two methods, MTS may be preferred to perform milk quality assessment with respect to obtained findings.

Conclusions

To determine bovine raw milk quality level, two dye induction techniques were examined in the present research. It was concluded that that MTS has a predominance to RTS, but, both DRTS values are not suitable to decide quality degree of bovine raw milk samples as unique test method. However, further studies including more data, especially on MTS should be carried out to confirm the obtained findings here.

References

Atasever S, Erdem H, Kul E. 2012. Using viscosity values for determining somatic cell count in cow milk. Asian J. Anim. Vet. Adv., 7(4): 741-745.

Bilkis T, Islam M, Sumy MC, Mandal NA, Uddin GN. 2013. Rapid estimation of quality of raw milk for its suitability for further processing in the dairy industries of Bangladesh. Int. J. Dairy Sci., 8(1): 1-11.

Braide W, Awiya H, Akien-Ali IJ, Lugbe PB , Oranusi US, Ayebabohoa M. 2015. Bacteriological examination of fresh cow milk and fura de nunu using rapid dye reduction test. Pyrex J. Microbiol. Biotechnol. Res., 1 (3): 28-37.

Davut M, Atasever S. (2017). Seasonal changes of composition and somatic cell count of bucket milk from Jersey crossbred cows in Northern Turkey, 11th Int.Symp. - Modern Trends in Lives. Prod. (Oct. 11-13), Belgrade-Serbia.

Demirci M, Oksuz O, Simsek O, Kurultay S, Kıvanc M, Gunduz HH, Ucan N. 2010. Controlling Milk and Its Products, Published by University of Anadolu, No: 2064, 254, Eskisehir, Turkey (In Turkish).

De Silva SASD, Kanugala KANP, Weerakkody NS. 2016. Microbiological quality of raw milk and effect on quality by implementing good management practices. Procedia Food Science, 6, 92-96.

Erdem H, Atasever S, Kul E. 2010. Determination of Milk Production Characteristics and Milk Losses Related to Somatic Cell Count in Jersey Cows Raised in the Black Sea Region of Turkey. Asian J. Anim. Vet. Adv., 5: 217-222.

Koc A. 2008. Factors influencing daily yield, somatic cell count and non-fat dry matter content of milk. Indian Vet. J., 85: 630-632.

Kramomtong I Tripipat T Koowatananukul C, Rangwises S, Ajariyakhajorn K. 2007. Relation between microorganisms, lactic acid production, and dye reduction tests in raw milk. Proceedings Chula. Univ. Vet. Sci. Ann. Con., 26-27 April, Thailand.

Mahari AT, Yemane H. 2016. Cow Milk Handling Practices and Factors Contributing to Quality Deterioration in Ethiopia. Food Sci. Qual. Man., 48: 14-17.

Mikone Jonas E, Atasever S, Graff M, Erdem H. 2016. Non-genetic factors affecting milk yield, composition and somatic cell count in Hungarian Holstein cows. J. Fac. Vet. Med. Kafkas Univ., 22 (3): 361-366.

Miller RH, Norman HD, Wiggans GR, Wright JR. 2004. Relationship of test day somatic cell score with test day and lactation milk yields. J. Dairy Sci., 87: 2299-2306.

Muliro PS, Shalo PL, Kutima PM. 2013. Quality assessment of raw camel milk using dye reduction tests. African J. Food Sci. Techn., 4(5): 116-121.