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Full Length Research Paper

Relationship between somatic cell count and catalase activity in raw milk of Anatolian buffaloes

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The objective of this study was to determine the relationships between somatic cell count (SCC) and catalase (CAT) activity of milk samples of Anatolian buffaloes. The data were collected in two smallholder farms of Samsun Province, Turkey. A total of 64 samples of bucket milk was analyzed for SCC and CAT during October to November 2008. SCC analyses were performed using direct microscopy, and CAT values were obtained from the observation of enzyme activity scores. The data were tested by one-way analysis of variance (ANOVA), and farms were compared by t-test. While, no significant differences in each parameter were determined by test days (TD), SCC values tended to elevate with higher CAT scores. In herd level, two farms had similar levels by SCC, but significant differences were obtained in CAT values. Estimated high (r=0.806) correlation in the present work indicated the possibility of using CAT values to determine quality of buffalo raw milk.

Key words: Somatic cell count, catalase activity, milk quality, correlation, Anatolian buffalo.

INTRODUCTION

Buffalo raising ensures a great financial gain to the national economy of many countries in the world. However, there has been a drastic reduction in buffalo population of Turkey and the only breed referred to as Anatolian buffalo, is known as an important genetic resource. In this point, enhancing quality and quantity of milk obtained from buffaloes may be seen as an obligation for saving this breed. Despite evaluating, milk quality can be accurately performed by microbial analyses, some easy, rapid and reliable methods have been developed (Pyörälä, 2003). Of these methods, somatic cell counting in raw milk is assumed as the most reliable marker to detect quality of milk and to reflect general health status of the herds. Somatic cells are body cells and are present at normal levels in normal milk. Numbers of these cells per ml is referred to as somatic cell count (SCC) and high levels of SCC in milk reflect abnormal conditions (Koc, 2008). Sharif and Muhammad (2008) emphasized that inflammation of udder markedly increases the SCC in milk, leading to inferior processing characteristics and reduced acceptance of dairy products because of changes in components and properties of raw milk.

The European Union Directives (46/92 and 71/94) set a limit of 400 000 cells ml^{-1} for SCC in buffalo milk when the milk is used for products made with raw milk (Moroni et al., 2006). Harmon (1994) clearly indicated that elevated SCC in both serum and raw milk are influenced by many factors such as parity, stage of lactation, season, daily variation, breed, etc. Because many SCC analysis methods need much time and are labor consuming, many dairy owners are interested in alternative screening tests. Riener et al. (2009) reported that there are around 70 indigenous enzymes in milk and several of these are significant in relation to quality of milk.

Similar to this, Fox and Kelly (2006) reported that lactoperoxidase, catalase, amylase, lipases, esterases, proteinases and xantine oxidoreductase are the indigenous enzymes that are in milk. Kang et al. (2002) estimated a high correlation (r: 0.89) between microbial load and catalase (CAT) activity in pasteurized milk. However, of the enzymes related to quality of milk, CAT has not been widely used, and its efficiency is still unclear. Moreover, in spite of many studies that have been carried out on the association of SCC with several significant enzymes of bovine raw milk (Kováč et al., 2007; Asadpour et al., 2008; Najafi et al., 2009), there is no report on the relationship between SCC and catalase activity in buffalo milk.

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Table 1. Distribution of SCC and CAT values (mean ± standard deviation) by test day.

TD	n	logSCC	CAT
1	16	5.78±0.07	2.81±0.24
2	16	5.86±0.08	3.13±0.25
3	16	5.83±0.05	2.88±0.25
4	16	5.88±0.06	3.06±0.30
General	64	5.84±0.03	2.97±0.13

SCC: somatic cell count, CAT: catalase, TD: test day.

Table 2. Changes of SCC values by CAT groups.

CAT Groups	n	logSCC
1	9	5.40±0.01[a]
2	9	5.60±0.03[a]
3	21	5.88±0.03[b]
4	25	6.04±0.04[b]
General	64	5.84±0.03

Different superscript letters in the same column indicate statistically significant differences (P<0.001).

Table 3. Comparison between SCC and CAT values by farms

Farm	n	logSCC	CAT
1	32	5.88±0.05	3.28±0.15[a]
2	32	5.79±0.04	2.66±0.19[b]
General	64	5.84±0.03	2.97±0.13

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

The aim of the present study was to investigate the variation of SCC and CAT levels and to estimate the relationship between SCC and CAT enzyme activity in bucket milk samples of Anatolian buffaloes.

MATERIALS AND METHODS

Sampling

Data were obtained by collecting bucket (composite) milk samples from two smallholder farms in Samsun Province of Turkey, between October and November 2008. The farms had similar conditions by feeding and husbandry applications and both of them had three lactating buffaloes. Milk samples were collected during the morning milking with two weeks intervals at four times. Animals were hand-milked once a day and samples taken from the buckets. No preservative included samples were kept in an ice-cooled box and immediately transported to the laboratory on the same day.

Somatic cell counting

In SCC analysis, direct microscopic counting method (Koc, 2008) was performed. For each test day, 8 samples were collected from two farms for analyses and thus, a total of 64 samples were assessed in the study. For each slide, 0.01 ml raw milk sample was dyed with methyelene blue solution. Dye solution used in the study was composed from 0.6 g of certified methylene blue chloride to 52 ml of 95% ethyl alcohol, 44 ml of tetrachlorethane and 4 ml glacial acetic acid.

For preparation of methylene dye, to stain somatic cells and leukocytes, ethyl alcohol (54 ml) and tetrachloroethane (40 ml) were mixed in a bottle and heated in a water bath at 60 to 70°C for 15 min. Methylene blue dye was added to the solution carefully and kept in a refrigerator at 4°C for 30 min and then glacial acetic acid was added. The dye solution so prepared was filtered using a filter paper with a pore size of 10 to 12 micron and stored in a colored bottle. Only those cells, which possessed a blue stained nucleus, were counted. Total number of fields counted per slide was 50 and the working factor (WF) was 10604.

Catalase activity testing

In CAT analysis, hydrogen peroxide degradation by catalase to water and oxygen according to following reaction was utilized:

$$2\ H_2O_2 \rightarrow 2H_2O + O_2$$

Firstly, about 10 ml of raw milk was put into a standard tube that is included Durham's tube as reserved. After addition of 1 ml H_2O_2, severity of free O_2 occurrence with bubble formation was recorded to be: 1) CAT negative (-), 2) CAT weak (+), 3) CAT moderate (++), and 4) CAT strong (+++) (Atasever and Erdem, 2008).

Statistical analysis

SCC values were transformed to $\log_{10}$ for normality and homogeneity of variances. The data were tested by one-way analysis of variance (ANOVA) and means were compared by Duncan's multiple range test based on the 0.05 level of probability. The model was as follows:

$$y_{ij} = \mu + a_i + e_{ij}$$

where; y_{ij} is observation value for SCC or CAT, μ is population mean, a_i is effect of the test day (i= 1, 2, 3 and 4), and e is the random residual effect. At the farm level, comparison was performed by t-test. To compute correlations between SCC and CAT values, Pearson's correlation coefficient analysis was applied. All statistical analyses were performed using SPSS 10.0 for Windows (SPSS, 1999).

RESULTS

In the present study, logSCC and CAT means (±SD) and distribution of both parameters by test day (TD) were given in Table 1. As seen, there was no significant difference by TD groups. Obtained untransformed SCC mean was calculated to be SCC 829 359±54 730 cells ml^{-1} and CAT mean was determined to be 2.97±0.13. In analysis, SCC values by CAT subgroups, significant differences (P<0.001) was found among the groups (Table 2).

In farm level, no significant differences were determined by logSCC (Table 3). Besides, statistically

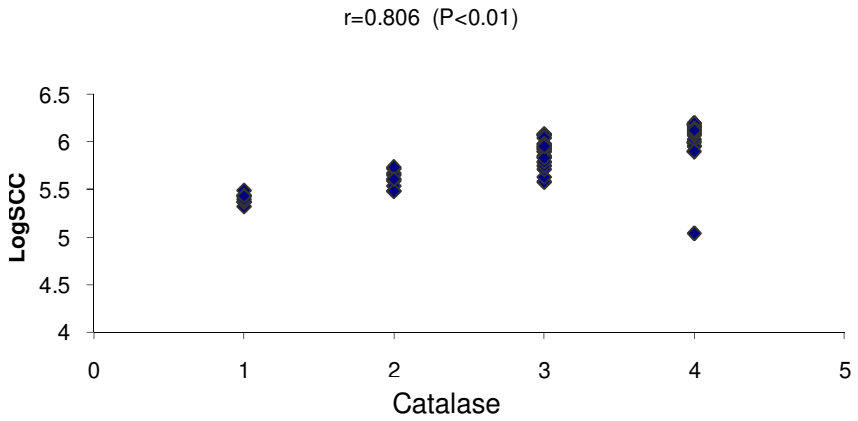

Figure 1. Correlation of somatic cell count with catalase.

significant differences (P<0.05) were observed by CAT. Additionally, positive and high correlation (r: 0.806; P<0.01) was determined in this study (Figure 1). As seen, distribution of this correlation between two parameters were linear.

DISCUSSION

In an earlier study, TD variation, an important reflector of any abnormal thresholds, has been estimated to be 8% for Zebu cattle (Millago et al., 2009). In the present study, it was observed that there was no significant difference by TD, which shows that management applications related to milk production were balanced. While CAT mean estimated in this work was assumed to be in the moderate level, obtained untransformed SCC mean was higher than the threshold level recommended by the EU directive (92/46) for dairy cattle (Juozaitiene et al., 2006). The mean logSCC of this study was fairly higher than that calculated in Murrah buffaloes by Dhakal (2006), and also, this level was higher than the result of a study conducted on Zebu cattle (Millago et al., 2009) and the result of a study on camels (Guliye et al., 2002). When we consider that the udder is more pendulous and teats are longer in buffaloes in comparison with cattle (Moroni et al., 2006), obtained values were not surprising.

Moreover, when the present study carried out in October and November months is considered, SCC values of other seasons would be expected to be relatively higher. This case was in agreement with those reported by the findings of Kelly et al. (2000). Besides, each unit increase in logSCC, results in a dramatic milk yield loss (Koldeveij et al., 1999), when calculated this value apparently points out to economic losses in the buffalo farms. Such that, Lindmark-Månsson et al. (2005) emphasized that elevated SCC is accompanied by decreasing milk yield and changes in milk composition that may affect milk processability, increased rennet clotting time, loss of moisture in cheese, delayed growth of starter cultures, reduced curd stability and yield.

As seen from Table 2, 1st and 2nd groups, and also, 3rd and 4th groups were statistically similar to each other, respectively. This case clearly indicated that negative and weak or moderate and strong CAT activities were determined to be at a similar level. In other words, reaction severity related to enzymatic activity can easily be recorded in buffalo raw milk. Normally, barn size, water scarcity, residual suckling, single udder-towel using and dairy laborers are seen as the most substantial risk factors for smallholder farms (Kivaria et al., 2004). Although both farms were in similar conditions by environmental factors, Farm 1 has relatively higher SCC and CAT values in the study (Table 3). This case clearly shows that several factors related to hygienic status associated with milk collecting and storing might be effective between farms.

For instance, different equipment cleaning frequency or technique might be effective on this case. As parallel to this concept, Dhakal (2006) emphasized that unhygienic conditions of dairy farms caused elevation of SCC in milk up to eight fold. Although no correlation was determined between SCC and CAT activity of milk in an earlier study (Phillips and Griffiths, 1987), our finding was found in parallelism with study results of some researchers (Kováč et al., 2007; Asadpour et al., 2008; Najafi et al., 2008), who investigated the relationships between SCC and other enzymes of milk. The estimated high correlation between the two parameters, reflects that CAT levels are useful indicators to measure milk quality (Figure 1). However, it should be regarded that investigations dealing with more data and time are needed to confirm this result.

Conclusions

The present study shows an association between SCC and CAT activity in Anatolian buffalo raw milks. Estimated high correlation between SCC, a reliable parameter to

detect raw milk quality, and CAT values, indicates the possibility of using catalase enzyme activity in determining raw milk quality of buffaloes. However, rechecking managemental practices related to hygiene, should be considered as the profitable approach by the herd owners. In addition, further investigations are needed on this topic using more data with quarter or animal basis to confirm the efficiency of CAT activity values in buffalo raw milk.

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