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Some Factors Affecting the Somatic Cell Count in the Milk of Anatolian Water Buffalos *(Bubalus bubalis)* raised in Igdir Province

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ABSTRACT

The aim of this study was to investigate the role of some environmental factors affecting the somatic cells count (SCC) in the farms of Anatolian water buffalos in Igdir. For this purpose, a total of 637 milk samples gathered from 91 animals raised in 54 farms were analyzed and statistically evaluated. The age and education level of the farmers and the effect of the milker on the SCC were found to be significant (P <0.001). SCC value (65.930 ± 2.484 cells/ml) was found to be the lowest when the milker is the person from the household. The udder cleaning before milking was a significant factor (P <0.001), SCC was 66.330±2.570 cells/ml for the producers that applied hygiene and was 125.860±9.500 cells/ml for the ones that did not. Season was determined to be significant factor (P <0.001) and SCC values were 52.290 ± 5.653 cells/ml, 69.990 ± 3.557 cells/ml and 104.810 ± 6.258 cells/ml for winter, spring, and summer seasons, respectively. In addition, correlations (Pearson correlation) between SCC and change in some environmental factors were weak but significant (P <0.01). The arithmetic and geometric means and standard errors of SCC were 82.4±3.3x10^3 cells/ml and 60.1±3.4x10^3 cells/ml, respectively. These results indicate that 100% the SCC of buffalos' milk sample are in accordance with the limits indicated in the related regulations of the Turkish Food Codex and of the European Union Commission. It was determined that SCC in raw milk obtained from Anatolian Water Buffalos grown in Igdir province was suitable for both Turkey and EU norms.

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Authors' Contribution
SV executed the experimental work and collected the data. IY and MY designed the study, analyzed the data and wrote the article.

Key words
Anatolian water buffalo, Environmental factors, Somatic cells count, Raw milk.

INTRODUCTION

Water buffalos are animals capable of adapting to various environmental conditions, resistant to diseases and not demanding, and thus for centuries were utilized for meat, milk, skin, and farming. Water buffalos consume inexpensive feeds with low quality and high cellulose content in coarse feed and convert them into quality animal products (Sekerden, 2001; Soysal, 2009; Damé *et al*., 2010). Owing to this features, water buffalo husbandry is very economical.

Buffalo milk has superior nutritional value because it contains high levels of protein, fat and minerals (especially calcium and phosphorus) compared to cow milk. High milk quality and processing of milk products (especially mozzarella, cream, yoghurt, *etc*.) increase the demand for water buffalo (Soysal, 2009; Damé *et al*., 2010). However, in order for milk to be processed into commercial products, to have a long shelf life and to minimum product loss, the SCC and total bacteria should be below the internationally acceptable standards with elevated breast health (Mundan *et al*., 2015).

The determination that buffalo milk has SCC within normal limits also reveals that consumption does not pose a risk for human health (Manlongat *et al*., 1998).

Significant differences in the SCC among buffalo flocks have been reported to occur due to management differences, milk hygiene practices, and barn conditions (Roy *et al*., 2003; Soysal, 2009; Sahin *et al*., 2016; Hussain *et al*., 2018). In order to reduce the levels of SCC, it is necessary to take some precautions and measures (Sahin *et al*., 2016), such as improving the milking management while paying attention to breast health. These precautions can be categorized as improvement of hygiene and barn conditions, milking at even intervals, application of mastitis control program, training of milking personnel.

In Turkey, a little attention has been paid to buffalo milk composition and/or a SCC. It is necessary to set a standard for somatic cell count threshold in Turkey. As stated in the related regulations of the Turkish Food Codex (Com. No: 2017/20) (Anonymous, 2018a) and European Union Commission (Com. No. 1662/2006) (Anonymous,

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2018b). Geometric average in the raw cow milks of SCC must be less than 400,000 cells/ml.

Therefore, in this study, the effects of environmental factors such as season, milker, and milk hygiene practices on SCC level in Anatolian water buffalo milk in Igdir Province were investigated.

MATERIALS AND METHODS

The animals were selected from plains that is located in Eastern Anatolia Region of Turkey and substantially differed from the surroundings mainly having the local microclimate characterized by distinctive climate characteristics of hot summers and dry winters with a relatively temperate climate. Igdir Province is also situated in the foothills of Mount Ararat, Turkey's highest mountain (Karaoğlu, 2011). The temperature ranges from 39-42°C in June-August and the lowest and highest temperatures observed between 1940 and 2016 are -30.3 and +42.0°C, respectively. Igdir province correspond to a total of 3.588 km^2 and the altitude ranges between 800 and 900 m with an average annual temperature of 12.1°C and rainfall of 258.6 mm. We therefore evaluated the current situation of the milk production practices of the water buffalos farming in the context of the SCC (Anonymous, 2018c). The research material consisted of 637 milk samples collected for 7 months from 91 Anatolian water buffalos of which 26 were from Igdir Central District, 50 were from Karakoyunlu, and 15 from Aralik district. Since no records were kept in the production facilities, the necessary information was regarding the practices were obtained through a face-to-face mini-questionnaire with producers to determine the effect of certain environmental factors affecting the amount of somatic cells obtained from milk samples.

The milk was taken in the evening and morning from the production facilities and placed in the tubes of 50 CC. Each tube was coded with the animal ear tag identity number and transported to the Department of Animal Science, Faculty of Agriculture, Igdir University, on an ice bucket. The SCC measurements were made by DCC (DeLaval Somatic Cell Meter, somatic cell counting device, Igdir University, Faculty of Agriculture). After placing a few drops of milk sample in the DeLaval counting kit, the samples were loaded cassette and was placed in the DeLaval cell counter. The SCC device is "DNA specific flourescent probe Propidium lodite" (Gonzalo *et al*., 2006). Further details are can be accessed for the use of the device Hamann *et al*. (2010).

Both SCC analysis results and the data gathered via mini questionnaire were prepared for further analyses using Excel spreadsheet. In addition to the descriptive statistics, comparisons were made with respect to different groups in each variable (*e.g.* Student t-test). Non-parametric test (Kruskal-Wallis) were used for non-normal distributed characteristics of groups (Orhan *et al*., 2004; Yildiz *et al*., 2011). The significance levels of the differences between the groups were compared with Tamhane -s T2 of Post Hoc Multiple Comparison tests, assuming that the variances are not equal. In this study, the data were analyzed with SPSS statistical package program (Ver. 17.0).

RESULTS

As a part of high quality agricultural practice, there is a need for a qualified workforce for the maintenance of farm, proper feeding and sheltering of the water buffalos, the experience of the farmer, milker, following of health and hygiene rules, the determination of the quality of the milk products obtained, and the processing and marketing of the products. Full exploitation of the current workforce in an enterprise can be realized by keeping milk and fertility records of animals. Having a healthy production in the enterprise, reaching a proper animal husbandry information level and being able to apply that information can only be achieved with and elevated education level of the labor force. For this reason, age, education and some farming practices of the business owners were surveyed and the findings were summarized in Table I.

The ages of the producers are divided into three groups (20-40 of age, 41-60 of age, and 61+). The results revealed that the effect of age groups on SCC was very important ($P<0.001$). The detailed comparisons revealed that the produced milk had the highest SCC in the enterprises operated by the owners at the 20-40 age group with mean SCC value of 86.860±8.515 cells/ml whereas the lowest number of somatic cells in the milk was observed in the enterprises with the 61+ age group of SCC value of 75.370±4.151 cells/ml.

Considering the education levels, the lowest SCC (44.460±4.700 cells/ml) was obtained from the enterprises with associate degree, the highest SCC (130.510±11.2 20 cells/ml) was obtained from the enterprises of the literate (but does not have a diploma). The highest education levels of farm producers were recorded as literate, primary school, junior high school, high school, and associate degree, and it was determined that all of the growers (100%) were literate. It must also found that the effect of educational status on SCC is significant ($P <0.001$). Among all the education groups, the lowest number of somatic cells were observed in the milk produced in the farms operated by persons with a associate degree (44.460± 4.700 cells/ml) whereas the highest SCC was observed in the milk produced by the producers who were literate without formal educationor diploma (130.510 ±

11.220 cells/ml). Because the education level is the key to access the accurate and up-to-date information on hygiene practices on the farm, lower level of SCC of milk produced can be linked to high-level education among farmers.

The seasonal effect on SCC values was found to be significant (P<0.001) and the mean values and standard deviations for each season were 52.290±5.653 cells/ml, 69.990±3.557 cells/ml, and 104.810±6.258 cells/ml for winter, spring, and summer, respectively. According to these results, the highest SCC values were observed in the milk gathered in summer, while the lowest values were in winter milk.

In order to determine the effect of the milking personnel on SCC, the milking personnel was classified into three groups. These were external workers, household members female/male and external workers+household members female/male. The statistical analysis was subsequently performed on grouping in respect to the milk SCC levels. According to these test results, effect of the milking personnel on SCC was highly significant (P<0.001).

Table I shows that the status and the gender of the milking personnel on resulting milk SCC was very significant (P <0.001). The lowest milk SCC was obtained from the milking process conducted by housewives (65.930±2.484 cells/ml) and the highest number of somatic cells was obtained from milk resulted from the process maintained by outside workers (194.540±15.376 cells/ml).

In addition, it was also determined that the districts where the operations were located had an effect on SCC, and the effect of milk from different regions of Province on SCC was statistically significant (P <0.001). As a descriptive parameter, milk samples obtained from different districts of Igdir Province where water buffalo dairy operations were present. The mean milk SCC values of the Central, Karakoyunlu, and Aralik districts were found to be 97.900±5.590 cells/ml, 64.710±3.060 cells/ml and 108.300±13.010 cells/ml, respectively.

Table I.- Statistics and factors effected on SCC according to factor groups in the farms.

Factors	Groups	Statistics						
		n	$\bar{x}$±SE	Min.	Median	Max.	χ^2/t-test	P*/Z**
Age groups	20-40 years	176	86.860±8.515 [a]	8.000	52.000	773.000	χ^2=9.375	P=0.009
	41-60 years	203	87.400±4.874 [a]	11.000	70.000	372.000		
	61+ years	258	75.370±4.151 [b]	5.000	59.500	625.000		
Education levels	Literate	70	130.510±11.220 [a]	20.000	89.000	372.000	χ^2=39.911	P=0.000
	Primary school	301	71.540±3.480 [b]	5.000	56.000	625.000		
	Middle school	168	93.270±8.790 [ac]	8.000	60.000	773.000		
	High school	70	69.910±6.650 [c]	17.000	59.500	338.000		
	Associate degree	28	44.460±4.700 [d]	11.000	41.500	143.000		
Seasons	Winter	91	52.290±5.653 [a]	5.000	33.000	273.000	χ^2=75.819	P=0.000
	Spring	273	69.990±3.557 [b]	8.000	53.000	348.000		
	Summer	273	104.810±6.258 [c]	14.000	75.000	773.000		
Milker	Household woman	455	65.930±2.484 [ab]	5.000	53.000	625.000	χ^2=71.156	P=0.000
	Household woman +male	42	80.000±5.041 [bd]	20.000	70.500	143.000		
	Male	21	88.000±20.858 [bd]	17.000	28.000	338.000		
	Household woman + worker	84	124.550±16.400 [d]	9.000	75.500	773.000		
	Worker	35	194.540±15.376 [c]	20.000	201.000	372.000		
Districts	Central	182	97.900±5.590 [a]	8.000	76.500	372.000	χ^2= 9.003	P=0.000
	Karakoyunlu	350	64.710±3.060 [b]	5.000	51.000	625.000		
	Aralık	105	108.300±13.010 [a]	9.000	63.000	719.000		
Udder cleaning	Yes	476	66.330±2.570 [a]	5.000	51.000	625.000	Z=3.687	P=0.000
	No	161	125.860±9.500 [b]	9.000	86.000	719.000		

*Kruskal Wallis test; **, Kolmogorov-Smirnov test. Means with the same letter are not statistical significant different (P<0.05).

Table II.- Arithmetic and geometric averages of SCC (cells/mlx1000) values by districts.

Districts	n	Arithmetic avg. ($x10^3$)	Geometric avg. ($x10^3$)	Turk Food Codex relevance	EU norms relevance
Merkez	182	97.8±6.6	73.9	+	+
Karakoyunlu	350	66.3±3.1	52.4	+	+
Aralık	105	109.3±13.5	66.7	+	+
Total	637	82.4±3.3	60.1	+	+

It has been determined that the difference of SCC resulting from breast cleaning before milking operation as oppose to not cleaning has a very significant (P<0.001) effect on the milk SCC level in enterprises that produce buffalo milk. The mean number of somatic cells was found to be 66.330±2.570 cells/ml in the milk obtained from the enterprises where breast cleansing is routinely applied whereas the mean number was 125.860±9.500 cells/ml in the milk obtained from the producers that do not conduct a routine breast cleansing before milking. It can be deduced that the hygiene practices are more prevalent among the enterprises that clean the breasts.

According to Table II, the arithmetic mean of SCC of collected raw milk was 82.4 ± $3.3x10^3$ cells/ml and the geometric mean was 60.1±$3.4x10^3$ cells/ml. These values were found to be much lower than 400.000 cells/ml indicated by the EU Commission (Anonymous, 2018b) and Turkish Food Codex (Anonymous, 2018a).

DISCUSSION

Within the scope of the study, the ages of the producers were examined and SCC values were determined according to age groups. According to this, the highest SCC is obtained from the enterprises with 20-40 age group, the lowest is obtained from the enterprises with SCC 61+ age group. This difference was highly statistically significant (P <0.001). It is possible to refer to the experience of the producers as a factor to achieve a low number of somatic cells (*e.g.* 61+ age group). However, in a study conducted by Barkema *et al*. (1999) that used 300 Dutch dairy herds to determine the management style and its association with bulk milk somatic cell count (SCC) and the incidence rate of clinical mastitis. The study concluded that younger, higher educated, and more enthusiastic farmers to invest in animal husbandry had better performance to control mastitis and lower SCC.

The results in this research revealed that the effect of education level of business owners on the milk SCC is highly significant (P <0.001). The lowest number of somatic cells in the milk was observed in the enterprises operated by associate degree graduates (44.460±4.700 cells/ml) whereas the highest SCC was found in the milk produced by the producers who were literate but not formally educated (130.510±11.220 cells/ml). That can be attributed to the ability to reach accurate and up-to-date health and hygiene information. Similarly, Delong *et al*. (2017) compared the number of low somatic cells in studies conducted in seven regions of the US in regard of the education level and with the same aim. Barkema *et al*. (1999) conducted a study among 300 Dutch dairy herds arriving the same conclusion that higher education of producers results better hygiene practices.

In this study also found that the effect of season on the SCC was significant (p<0.001), and mean SCC in milk ranked from the lowest to highest as the season progressed from winter to spring and to summer. According to these results, the highest SCC values were in summer, while the lowest values were in winter. Some researcher reported significant seasonal impact on milk SCC. Singh and Ludri (2001) found that SCC values were for the low in winter (76.000 cells/ml), and higher in summer (May, June and July) and autumn (August, September and October) as 108.000 cells/ml and 135.000 cells/ml, respectively. Another study targeting the effect of season on SCC of water buffalo milk (De *et al*., 2011) investigated the summer, autumn, and winter months found that mean SCC in milk was 119.000 cells/ml, 117.000 cells/ml and 83.000 cells/ml, respectively. These differences were all statistically significant (P<0.01). Similarly, Ozdede (2009) reported that the seasons were significantly effecting SCC in milk gathered from the producers that are the members of the Ankara Livestock Dairy Cattle Breeding Association (P<0.01). The results of the abovementioned studies were found to be similar to those obtained in the present study.

The status and the gender of the milking personnel on milk SCC was very significant (P <0.001). The lowest milk SCC was obtained from the milking process conducted by female household members whereas the highest number of somatic cells was obtained from milk resulted from the milking by outside workers. These values are similar to those obtained by Ozdede (2009) in which the number of somatic cells is lower for women in dairy cattle companies (P <0.05).

It has been determined that the difference of SCC

resulting from breast cleaning before milking operation as oppose to not cleaning has a very significant effect (P <0.001) on the milk SCC level in enterprises that produce buffalo milk. The number of somatic cells is lower in breast cleansing producers than in non-udder cleansing producers. Ozdede (2009) reported that the number of somatic cells in breast cleaning operations before and after milking decreased in dairy cattle decreased, indicating that the minimum number of somatic cells can be achieved via cleansing breasts before and after milking.

Another descriptive values analyzed here was the localities where milk samples were obtained. The three districts were Central, Karakoyunlu, and Aralik and SCC levels showed statistically significant (P <0.001) differences among districts. Similarly, Delong *et al*. (2017) showed regional differences among cow milk producing enterprises in seven regions in the United States and Patir *et al*. (2010) detected SCC level differences in raw cow milk samples from Elazig, Samsun, Malatya, Sanliurfa and Erzurum provinces of Turkey (P <0.05).

Healthy and high quality milk should have a SCC number that is below 200.000 cells/ml. Otherwise it is considered abnormal and a sign of subclinical mastitis (Harmon, 2001). Variables affecting the presence of somatic cells of milk in positive or negative direction in the enterprises that produce Anatolian water buffalos are season, education level of the producer, milking procedures, milking personnel, and breast cleansing practices. When the correlations of these factors with SCC were statistically evaluated, it was determined that there were significant and high correlations between these factors and SCC (P <0.01).

In this study, the number of somatic cells compared to previous studies was found very low. It can be caused by experienced growers' awareness of breast cleaning and high level of education. Because, the training of milk producers on hygiene affects SCC (Kaygisiz and Karnak, 2012).

CONCLUSION

As a result, the number of somatic cells in the Anatolian Water Buffalos raised in the Igdir Province of Turkey me*et al*l the world standards. Therefore, it is suitable to be processed in milk and milk products. Also, It has been determined that Buffalo milk production is safe for human health.

It was determined that SCC in raw milk obtained from Anatolian Water Buffalos grown in Igdir province was suitable for both Turkey and EU norms. That is, it show that results obtained in this study, 100% the SCC of buffalos' milk sample are in accordance with the limits indicated in the related regulations of the Turkish Food Codex and of the European Union Commission.

For this production to be sustainable, there is a need for qualified labor for the maintenance, feeding and sheltering of the water buffalos, elevating the agricultural production, maintaining the health of the shelter, the experience of the farmers, the maintenance of health and hygiene rules, increasing the quality of milk products, and successful processing and marketing.

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Ethical statement

None of the animals were harmed during the collection of milk sample. We were collected the samples during the milking of Buffalos. We were not involved in the importation process for these animals.

Statement of conflict of interest

We have declared no conflict of interest.

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