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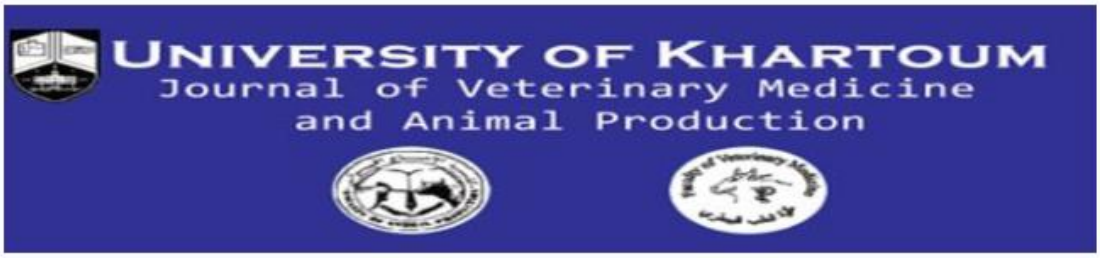

Physicochemical Properties and Microbial Load of Cow Milk Collected from Milk Supply Chain during Winter and Summer in Khartoum State, Sudan

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Abstract

Milk is considered an important chain for transmission of pathogenic microorganisms to human beings unless it is produced and handled under good hygienic conditions. Thus, hygienic production of milk has to get due attention in order to provide high quality milk to the consumers. This study was conducted to evaluate the quality of milk available to the consumers in Khartoum State during winter and summer seasons. Two hundred samples of raw cow's milk were collected and evaluated for the physicochemical properties of milk (solids not fat; SNF, fat, protein, lactose, acidity and density). In addition the bacteriological examinations (total bacterial, coliform and psychrotrophic bacterial) counts were carried out. The results showed significantly ($P\leq0.001$) higher fat (5.03%) and SNF (11.52%) content of cows' milk samples obtained during winter. However, highly significant ($P\leq0.001$) values were found in the milk samples collected during summer for content of lactose (4.72%) and acidity (0.19%). Highly significant ($P\leq0.001$) counts for total bacteria (TBC), coliform and psychrotrophic bacteria were obtained for cow's milk samples collected during summer (log10 7.58, log10 5.54 and log10 2.30, respectively). Hence the study suggested that more efforts are needed to improve milk hygiene and quality by regular monitoring and raising awareness among dairy owners in addition to initiation of milk collection centers coupled with cooling facilities.

المستخلص

يعتبر اللبن وسيلة مهمة لنقل الكائنات الحية الدقيقة المسببة للأمراض للبشر إلا إذا تم إنتاجه والتعامل معه في ظل ظروف صحية جيدة. وبالتالي فإن الإنتاج الصحي للالبان يجب أن يحظى بالاهتمام من أجل توفير البان ذات جودة عالية للمستهلك. أجريت هذه الدراسة بغرض تقييم جودة الالبان المتاحة للمستهلكين في ولاية الخرطوم خلال فصلي الشتاء والصيف. تم جمع مائتين عينة من لبن البقر الخام وتم تقييمها الخصائص الفيزيائية الكيميائية للبن (المواد الصلبة اللادهنية، الدهون، البروتين، اللاكتوز، الحموضة والكثافة). كما تم إجراء الفحص البكتريولوجي (العدد الكلي للبكتريا، بكتيريا القولون والبكتيريا المقاومه للبروده). أظهرت النتائج نسب أعلى معنويا ($P\leq0.001$) في محتوى الدهون (5.03٪) والمواد الصلبة اللادهنية (11.52٪) في عينات لبن الأبقار التي تم الحصول عليها خلال فصل الشتاء. وجدت فروقات معنوية عالية ($P\leq0.001$) في عينات اللبن التي تم جمعها خلال الصيف في محتوى اللاكتوز (4.72٪) والحموضة (0.19٪). وجدت فروقات معنوية عالية ($P\leq0.001$) في عينات اللبن التي تم جمعها خلال الصيف. تم الحصول على فروقات معنوية عالية ($P\leq0.001$) في العدد الكلي للبكتريا، بكتيريا القولون والبكتيريا المقاومه للبروده للعينات التي تم جمعها خلال الصيف (log10 7.58، log10 5.54 و log10 2.30، على التوالي). اقترحت الدراسة ضرورة بذل المزيد من الجهود لتحسين صحة وجودة الألبان عن طريق الرقابة المنتظمة ورفع مستوى الوعي فى مجتمع أصحاب مزارع الألبان بالإضافة إلى إنشاء مراكز تجميع للألبان مزودة بأجهزة التبريد.

Introduction

Currently the consumers want clean, wholesome and nutritious food that is produced and processed in a sanitary manner and free from pathogens (Khan *et al.*, 2008). The presence of food borne pathogens in milk is due to direct contact with contaminated sources in dairy farm environment and excretion from the udder of infected animal (Oliver *et al.*, 2005 and El Zubeir *et al.*, 2006). Quality milk production is necessary in order to get milk, which is free from pathogenic bacteria and harmful toxic substances, sediment and extraneous substances in addition to the good flavor, normal composition, adequate keeping quality and low bacterial counts (Khan *et al.*, 2008). Bacterial contamination appears from different sources, air, milking equipment, feed, soil, feces and grass (Coorevits *et al.*, 2008). Milking management aims to minimize microbiological, chemical and physical contamination, and this practice covers all aspects of the process of obtaining milk from cows quickly and effectively, while assuring the health of the cow and the quality of the milk (Morgan, 2004).

Mirzadeh *et al.* (2010) evaluated raw milk composition by some dairy farms in Lordegan region of Iran compared with global and Iran milk average and found fat, SNF and acidity content of cow milk were 3.90±0.97%, 8.67±0.69% and 0.19±0.02%, respectively. Czerniewicz *et al.* (2006) found the SNF and density of Friesian cows and Jersey cows were 9.25 % and 9.86 %, and 1.030 g/cm3 and 1.029 g/cm3, respectively. The fat and SNF content of raw cow milk were 8.40±0.54 and 4.05±0.37%, respectively (Bille *et al.*, 2009). Landi *et al.* (2011) found the SNF, fat and protein were 9.48±0.05%, 4.64±0.10% and 3.75±0.06% respectively, for cow milk when studying the effects of biotype, grazing management and different methods of feeding on milk composition. The lactose was 2.3±0.5% and 2.1±0.7% for milk obtained from cows with subclinical and clinical mastitis, respectively (Hamid *et al.*, 2012). They also found that the lactose was 2.42±0.6%; 1.67±0.7% and 2.3±0.6% for Friesian, crossbred and local breed cows, respectively. Bashir and El Zubeir (2013) evaluated milk production and reproduction of Baggara cattle in South Kordofan State, Sudan and found that the SNF was 9.19±0.78% and density was 1.031± 0.003 g/cm3. The stage of lactation was significantly ($P \leq 0.05$) affected protein and solids not fat (SNF) content of milk from local cows, while all milk constituents (except protein) had affected milk of crossbred cows in South Darfur State, Sudan. Sudan (Shuiep *et al.*, 2012).

El Zubeir and El Owni (2009) found high average of total bacterial counts during summer season than winter. Magnusson *et al.* (2006) reported that not all bacterial spores are removed even with the best cleaning method, therefore it is important to maintain good hygiene at all stages of milk production. Elmagli and El Zubeir (2006) concluded that storage conditions have significant effects on bacterial count.

Milking udder with subclinical mastitis and wet environment lead to contamination of bulk tanks milk and hence raw milk reaches the consumers with colifrom counts (FAO, 2008). Moreover Leitner et al. (2008) indicated that refrigerated storage of good quality milk from a single cow resulted in moderate deterioration of its quality, low level of bacterial growth standard plate counts and psychrotrophic counts and small losses of curd yield. Mohamed *et al.* (2016) encouraged the use of lactoperoxidase enzyme system in preservation of raw milk as it has been found useful in extending the shelf life of milk. They concluded that adequate management schemes at the level of production, processing and marketing should be applied alongside the lactoperoxidase enzyme system for a better dairy development in rural areas of Sudan.

Materials and methods

Sources and number of milk samples

This investigation was based on collecting 200 milk samples from cow's milk that obtained from different farms, collection centers and sale points in Khartoum State. The samples were collected during summer and winter seasons in order to determine compositional and hygienic quality of milk samples. The samples were collected into clean sterile bottles and transported in an ice box (4-5oC) to the laboratory of the Department of

Dairy Production, Faculty of Animal Production, University of Khartoum for physicochemical analysis.

Physicochemical analysis

The chemical analysis of milk samples was determined by using milk analyzer according to the manufacture instructions twice by LactoScan milk Analyzer (Milkotronic LTD, Europe) to determine fat, protein, lactose, SNF and density of the milk samples. Twenty five ml of the samples were taken in the sample holds after mixed gently (4-5times). The sample holder was put in the analyzer in the recess position and the analyzer sucked the milk and makes the measurement. When the measurement was finished, the sample returned in the sample-holder and the digital indicator showed the specified result.

The acidity of the samples determined according to Foley *et al.* (1974).

Microbiological examination of milk samples

The samples were collected in clean sterile bottles then evaluated for total bacterial count, coliform bacterial count and psychrotrophic bacterial count according to Harrigan and McCance (1976). Plate count agar No. 298 (Biomark laboratories) was used to enumeration of TBC and psychrotrophic count and MacConkey agar no. 779 (Biomark laboratories) was used to determine coliform count. Plates for enumeration of TBC and coliform count incubated at 32° C for 48 hours. Plates for enumeration of psychrotrophic count were incubated at 7° C for ten days. The counting of the colonies was done manually by using a colony counter and reported as colony forming units per milliliters (cfu/ml). The total number of the colonies in the dilution was multiplied by the reciprocal of the dilution (Marshall, 1992).

Statically analysis

The collected data were analyzed by factorial design using Statistical Packages for Social Sciences (SPSS) computer program.

Results

Fat content during different seasons showed highly significant differences (Table 1). The obtained values were higher than those obtained by Mohamed and El Zubeir (2007); Ahmed and El Zubeir (2007) and Shuiep *et al.* (2016). However it was lower than those obtained from Baggara cattle (5.08±1.05%) in South Kordofan State, Sudan (Bashir and El Zubeir, 2013) Similarly Rhone *et al.* (2008) reported that milk fat was higher ($P<0.05$) during winter and lower during the summer and rainy seasons. Also, Heck *et al.* (2009) found lower milk fat content during summer season compared with winter season. However Butler (2011) indicated that the differences in fat composition of milk were greater for summer than winter season in three milk samples. The fat content of cow milk from different sources showed highly significant differences (Table 2 and 3). Pavel1, E. R. and Gavan (2011) reported that nutrition can be regarded as one of the most important sources of variation in the yield and composition of milk. They stated that climatic conditions and seasonal variation as well as regional differences can play an important role. Similarly Shuiep *et al.* (2016) reported that variations between milk fat content could be due to different management, feeding regimes, production systems and breed of cattle. On the other hand, Bille *et al.* (2009) indicated that fat content of milk decreases as the weather becomes warmer and increases again with the approach of winter.

The protein content of cow milk samples collected from different sources during different seasons revealed no significant differences (Table 1, 2 and 3). The obtained values supported who found the mean of protein of milk samples collected from Baggara cattle in South Kordofan State, Sudan was 3.62± 0.31% (Bashir and El Zubeir, 2013). However the present results were higher than those reported by Pavell and Gavan (2011) who found that protein content in milk of dairy cow was 3.4% and 3.3%, for spring and summer respectively. The result of protein content (3.5±0.9%) was near to that reported by Soliman (2005) who found that protein content was 3.5±0.03% in lactating dairy cows. Bille *et al.* (2009) showed that mean for protein content of cow milk was 3.2±0.6%. Stergiadis *et al.* (2010) reported that protein content of milk was

not significantly influenced by either management or season. Shuiep *et al.* (2016) found that within local cows, stage of lactation and parity order were significantly (P≤0.05) affecting protein, while among crossbred cows, the protein content was not affected by stage of lactation and parity order.

Table 1: Comparison of physicochemical characteristics of cow milk samples during winter and summer season

Chemical content	Measurement	Season		Total
		Winter	Summer	
Fat (%)	**Means +SD**	5.03±0.04	4.30±0.6	4.66±0.3
	Minimum	4.8	4.1	4.0
	Maximum	5.0	4.4	5.1
Protein (%)	**Means +SD**	3.50 ±0.95	3.50 ±0.95	3.50±0.95
	Minimum	1.6	2.7	2.3
	Maximum	5.3	3.5	3.8
Lactose (%)	**Means +SD**	4.62 ±0.2	4.72±0.2	4.67±0.2
	Minimum	4.5	4.5	4.1
	Maximum	4.6	4.7	4.8
Solid not fat (%)	**Means +SD**	11.52 ±2.3	11.21±0.6	11.36±1.4
	Minimum	10.9	9.3	9.2
	Maximum	11.6	11.2	11.5
Acidity (%)	**Means +SD**	0.14±0.0	0.19±0.0	0.17±0.0
	Minimum	0.15	0.16	0.14
	Maximum	0.16	0.19	01.9
Density(g/cm^3)	**Means +SD**	1.033 ±0.00	1.032±0.00	1.03 ±0.00
	Minimum	1.031	1.022	1.022
	Maximum	1.033	1.032	1.032

The lactose of the milk samples collected from different sources (Table 2) and during different seasons (Table 1) showed high significant differences (Table 3). Ahmed and El Zubeir (2007) found that the lactose was 3.95% during summer season and 4.01% during winter season. However the mean of milk lactose from Baggara cattle in South Kordofan State, Sudan was 4.89± 0.33% (Bashir and El Zubeir, 2013). The lactose content was ranged from 5.21 to 5.15% and from 5.33 to 5.02%, in local and crossbred cows, respectively (Shuiep *et al.*, 2016). This might be due to the fact that lactose of milk is affected by different locations and feedstuff that animals utilized (Kittivachra *et al.*, 2007). Also differences in milk composition could be attributed to biotype and system of production (Landi *et al.*, 2011). Shuiep *et al.* (2016) found that stage of lactation and parity order had no significant (P>0.05) influence on lactose content of milk from local cows. On the other hand, lactose content of milk samples from crossbred was significantly (P≤0.05) influenced by stage of lactation but not by the parity order.

Table 2: Comparison of physicochemical characteristics of cow milk samples from different sources

Chemical content	Measurements	Sources			Total
		Sale Points	Collection Points	Farms	
Fat (%)	Means +SD	4.8±0.4	4.5±0.5	4.4±0.7	4.56+0.5
	Minimum	4.5	4.4	4.2	4.2
	Maximum	5.0	4.6	4.6	5.0
Protein (%)	Means +SD	4.15±0.9	3.5± 1.8	5.3±1.6	4.31±1.4
	Minimum	3.4	5.4	3.6	3.2
	Maximum	4.9	5.9	5.3	5.9
Lactose (%)	Means +SD	4.6±0.0	4.7±0.2	4.6±.03	4.63±0.6
	Minimum	4.6	4.6	4.5	4.0
	Maximum	4.7	5.4	4.6	4.7
Solid not fat (%)	Means +SD	11.2±0.2	9.1±0.2	14.4±0.0	10.5±0.7
	Minimum	9.3	9.1	14.2	9.0
	Maximum	11.2	9.2	14.6	14.6
Density (g/cm^3)	Means +SD	1.03±0.00	1.033+0.00	1.03± 0.00	1.03±0.0
	Minimum	1.01	1.031	1.032	1.03
	Maximum	1.02	1.02	1.03	1.03
Acidity (%)	Means +SD	0.16±0.00	0.17±0.00	0.16±0.00	0.16±0.0

Solids not fat of milk samples collected during different seasons revealed significant differences, it was higher during winter season than summer (Tables 1), which might be due to the fact that SNF content of the milk generally follow the variation of the fat content, the higher the fat content the higher was the SNF but lower the density (Bille *et al.*, 2009). The non significant differences (P>0.05) of fat content of the milk samples (Table 3) were in accordance with results of El Zubeir and Ahmed (2007). The mean of SNF was 9.19± 0.78% for Baggara cattle milk (Bashir and El Zubeir, 2013). However this result was higher than that reported by Pavel1 *et al.* (2011) who found that SNF was 8.70% during summer period, in lactating dairy cows. The solids not fat of milk samples collected from different sources revealed non significant differences (Table 2). This might be due to effect of breed, feeding and management as reported by Shuiep *et al.* (2016). Moreover Nickerson (1999) stated that synthetic secretary tissue of the mammary gland, the initiation and establishment of lactation the milk ejection reflex the breeds and genetics factors, the nutrition, the environment and the milking management practice, might have important effects on milk composition and quality. The non significant differences for SNF of cow milk from the different sources (Table 2) agreed with Suman (2009). Whereas, Bhoite and Padekar (2002) and Hossen *et al.* (2012) reported significantly higher fat and SNF content in different sources and breeds. The SNF content was significantly (P≤0.05) influenced by both stage of lactation and parity order in milk of local cows and crossbred cows (Shuiep *et al.*, 2016).

Density of milk samples collected during different seasons revealed non significant variations (Table 1 and 2). However the milk samples from different sources indicated significant variations (Table 2 and 3). This result was similar to that reported by Abdel Rahman *et al.* (2009) and Bashir and El Zubeir (2013) who found the density of milk was 1.031g/cm3. Moreover Abdel Rahman *et al.* (2009) attributed the differences in milk composition to initial raw milk used and the procedure of processing.

The acidity of milk samples collected from different sources during different seasons (Table 1, 2 and 3) revealed highly significant variation. The values found were similar to that reported in earlier by Ahmed and El Zubeir (2007) who found that acidity of milk samples was 0.193% and 0.164% during summer and winter, respectively. This study also supported Al-Zenki *et al.* (2007) who stated that the mean value of total titratable acidity was 0.18%. The higher temperature during summer causes the higher bacterial load in milk, which supported Mohamed and El Zubeir (2007) who reported that the acidity of cow milk samples was 0.154±0.012 during winter season, while it was 0.2±0.033 during summer season. Mohamed *et al.* (2016) demonstrated that during storage, the titratable acidity of LPS treated milk was lower than that of control milk samples, though they had the same initial acidity This effect being more pronounced upon storage at 8°C than 30°C.

The total bacterial count of raw cow milk samples collected during summer season was higher during winter season (Table 4). The results were in the range stated by standard quality on low total bacterial contamination (less than 5 log cfu/ml), which agreed with Wasiksiri *et al.* (2010) who found that log TBC in milk samples was log 3.720± 0.614. This result supported Mohamed and El Zubeir (2007) who found that total bacteria counts of market milk in Sudan during summer season (log 6.895±0.678) was higher than winter season (log 5.563±0.572). On the other hand, Elmoslemany *et al.* (2009) reported that season is a significant predictor for all bacterial counts with the lowest counts tending to occur in winter. However, the results disagreed with Gouranga *et al* (2008) who found that highest occurrence of total bacteria counts (5.64×10^6 cfu/ml) was during winter season, whereas the lowest (3.78×10^6 cfu/ml) was during summer. The non significant differences of total bacteria count between different sources (Table 5 and Table 6) might be because that collection points supplies the sale points. Karmen and Slauia (2008) investigated the quality of raw milk after every two days and found that the total bacterial count was higher than 100,000 cfu/ml in 48 (23.6%) out of all tested samples. Similarly Addo *et al.* (2011) indicated that, in

Ghana, the total plate count was < 10^5 cfu/ml in about 45.2% of the milk samples. On the other hand, the higher mean of total bacterial count of milk from small-scale farms obtained in this study supported Jayarao *et al.* (2004) who reported that the herd size and farm management practice influence the somatic cell and bacterial count in bulk tank milk. In

Table 3: Comparison of physicochemical characteristics of cow milk samples collected during winter and summer season from different sources in Khartoum State

Measurements	Sources		Seasons		Sources × Seasons	
	Mean square	Significant level	Mean square	Significant level	Mean square	Significant level
Fat (%)	6.15	0.001***	32.09	0.001**	0.858	0.186^{NS}
Protein (%)	79.6	0.392^{NS}	234.4	0.28^{NS}	78.6	0.68^{NS}
Lactose (%)	0.38	0.001***	0.76	0.007**	0.37	0.30^{NS}
Solid not fat (%)	75718	0. 001*	83716	0.001***	57868	0.001***
Density (g/cm^3)	2.65×10^{-5}	0.001***	3.32×10^{-6}	0.109^{NS}	2.97×10^{-6}	0.101^{NS}
Acidity (%)	0.009	0.001***	0.14	0.001***	0.001	0.46^{NS}

NS = Non significant at P≥0.05
*** = Significant at P≤0.05**
**** = Highly significant P≤0.01**

Table 4: Comparison of hygienic quality of cow milk samples collected during winter and summer seasons in Khartoum State

Bacterial loads	Seasons	Mean ± SD	Minimum	Maximum
Log total bacterial count	Winter	5.99± 0.17	5.6	6.3
	Summer	7.58±0.14	5.2	7.3
	Total	6.78±0.15	5.6	7.2
Log coliform count	Winter	3.42±0.17	3.0	3.7
	Summer	5.54±0.13	5.3	5.8
	Total	4.48+0.15	5.4	5.8
Log psychrotrophic count	Winter	2.45±0.15	2.1	2.7
	Summer	2.60±0.12	2.3	2.8
	Total	2.52+0.13	2.2	2.8

The coliform count of milk samples collected during different seasons revealed significant ($P<0.01$) differences with higher counts during winter (Table 4 and Table 6). Similarly Salman and Hamad (2011) found that 60.1% of milk samples were of coliform count between 0<100 cfu/ml in Khartoum State, with high percentages (76.9%) during winter compared to those found during summer (53.6%). This might be due to the traditional methods of distribution and transportation of milk as was reported by Elmagli and El Zubeir (2006). Maddalena *et al.* (2011) indicated that coliform count and somatic cell count, expressed in milk were significantly increased during hot season compared with cold season. Similarly, Gillespie *et al.* (2012) reported that coliform count was significantly higher during summer season than winter season. The value of coliform count in milk samples collected from the sales points was higher than the milk samples obtained from collection points (Table 5). This might be due to contamination during transportation and storage as vendors take some hours to transport milk from farms or collection centers to the sale points or might be due to the presence of diseases in the herd. Addo *et al.* (2011) showed that the coliforms exceeded 10^3 cfu/ml in 66.0% and that *E. coli* was detected in 11.2% in Ghana. Sanderson *et al.* (2005) reported that coliforms are important mastitis pathogens, widely distributed in the farm environment. This supported Garedew *et al.* (2012) who reported that the presence of high coliform isolates from critical control point might be attributed to high prevalence of subclinical coliform mastitis, unclean dairy houses, improper milking procedure and udder preparation. Coliform count (CC) is a known regulated test that has been used historically to assess milk production and practices such as milk refrigeration, milking machine sanitation, and pre milking udder hygiene (Murphy and Boor, 2000). The presence of coliform bacteria in milk products indicated that unsanitary production or improper handling of either milk or milk utensils (El Zubeir and Ahmed, 2007).

It is probable that the high count of psychrotrophic bacteria of raw cow milk obtained in the present study was due to the high temperature and unavailability of cooling during transportation and storage of milk. Pyschrotrophic bacteria revealed non-significant differences during seasons (Table 4), while the milk sources (Table 5) revealed significant differences (Table 6). These results also supported Foltys and Kirchnerová (2011) who reported that the season was not affecting psychrotrophic bacteria. Similarly, Lukášová *et al.* (2007) observed no seasonal variations effect on psychrotrophic bacterial count of milk. The longer time during refrigerated storage, the greater the chance the psychrotrophic bacteria to increase in number (Murphy and Boor, 2000).

Table 5: Comparison of hygienic quality of cow milk samples collected from milk supply chain in Khartoum State

Bacterial loads	Measurements	Sale points	Collection points	Total
Log total bacterial count	**Mean ±SD**	6.81±0.1	6.71±0.1	76.76±0.1
	Minimum	5.7	6.6	5.7
	Maximum	6.9	7.9	7.9
Log coliform count	**Mean ±SD**	4.94±0.1	5.17±0.1	5.05±0.1
	Minimum	4.8	5.0	4.8
	Maximum	5.0	5.8	5.8
Log psychrotrophic count	**Mean ±SD**	1.63±0.11	2.30±0.12	1.96±0.1
	Minimum	1.3	2.1	1.3
	Maximum	1.8	2.5	2.5

Table 6: Comparison of hygienic quality of cow milk samples from different sources during different seasons in Khartoum State

Measurements	Sources		Seasons		Sources× Seasons	
	Mean square	Significant level	Mean square	Significant level	Mean square	Significant level
Log total bacterial count	1.05	0.06^{NS}	45.5	0.001***	0.376	0.37^{NS}
Log coliform count	2.95	0.002***	34.4	0.000**	0.281	0.54^{NS}
Log psychrotrophic bacterial count	5.47	0.001***	1.55	0.37^{NS}	3.70	0.001***

NS = Non significant at P≥0.05
***= Highly significant at P≤0.01
** = Significant at P≤0.001

Conclusion

The bacteriological quality observed in the present study requires further investigation of the status of the animals' health, and the significance of the effect of milking utensils and their contribution on microbial quality. Also milk should be cooled immediately after milking, during transportation and storage to eliminate the growth and multiplication of microorganisms. Introduction of proper collection centers and milk pasteurization factories to ensure the safe distribution of the products to the consumers have to be encouraged. In addition extension services among dairy farmers, labours and milkers is needed on good dairy farming practices such as housing, milking and hygiene, proper sanitary practices, cleaning program, biosecurity and diseases prevention

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