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Chemical and microbiological quality of donkey milk

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Abstract: The aim of this study was to investigate the chemical and microbiological quality of raw milk samples collected from clinically healthy appearance donkeys, which raised in two different donkey farm in Balıkesir province of Turkey. In this study, a total of 78 raw milk samples collected from 26 donkeys. The average dry matter, protein, fat and lactose value of raw donkey milk samples in farm 1 and 2 was determined as 8.89%, 8.79%; 1.57% and 1.47%; 0.70%, 0.45%, 6.48% and 6.06%, respectively. The average total aerobic mesophilic bacteria and somatic cell counts in farm 1 and 2 were found as 3.88 and 4.50 log CFU/mL, 3461 and 13000 cells/mL, respectively. The average counts of *Staphylococcus-Micrococcus* spp., coliform bacteria, *Lactobacillus* spp. and *Lactococcus* spp. in farm 1 and 2 were detected as 2.66 and 2.33 log CFU/mL, 1.5 and 1.7 log CFU/mL, 2.16 and 3.30, 4.32 and 5.12 log CFU/mL, respectively. Statistical differences were observed between farms in terms of both fat and lactose values and microbiological parameters ($P<0.05$), except for coliform bacteria. The presence of indicator microorganisms in the raw donkey milk can be indicative of the presence of foodborne pathogens. Raw donkey milk is mostly consumed by cancer patients due to its anticancerogenic effect. Therefore, raw donkey milk may pose a risk for cancer patients and public health. As a result, a solid hygiene policy must be applied in the production of donkey milk, and fresh milk should be stored below the refrigerator temperature.
Keywords: Donkey milk, microbiological quality, somatic cell count.

Eşek sütlerinin kimyasal ve mikrobiyolojik kalitesi

Özet: Bu çalışmanın amacı, Türkiye'nin Balıkesir ilinde bulunan 2 farklı çiftlikte yetiştirilen sağlıklı eşeklerden toplanan çiğ süt örneklerinin kimyasal ve mikrobiyolojik kalitesini araştırmaktır. Çalışma materyali olarak 26 eşekten toplanan toplam 78 çiğ süt örneği kullanıldı. Çiftlik 1 ve 2' deki çiğ eşek sütü örneklerinde ortalama kuru madde, protein, yağ ve laktoz değerleri sırasıyla %8,89, %8,79, %1,57 ve %1,47; %0,7, %0,45, %6,48 ve %6,06 olarak belirlendi. Çiftlik 1 ve 2' deki ortalama TAMB ve somatik hücre sayısı sırasıyla 3,88 ve 4,5 log kob/ml, 3461 ve 13000 hücre/ml olarak tespit edildi. Çiftlik 1 ve 2' deki ortalama *Staphylococcus-Micrococcus* spp., koliform bakteri ile *Lactobacillus* spp. ve *Lactococcus* spp. sayıları sırasıyla 2,66 ve 2,33 log kob/ml, 1,5 ve 1,7 log kob/mL, 2,16 ve 3,30 log kob/mL, 4,32 ve 5,12 log kob/mL düzeyinde tespit edildi. Çiftlikler arasında yağ ve laktoz değerleri ile koliform bakteri sayısı dışındaki mikrobiyolojik parametreler arasında istatistiksel olarak farklılık olduğu gözlendi ($P<0,05$). Çiğ eşek sütlerinde indikatör mikroorganizmaların tespit edilmiş olması gıda kaynaklı patojenlerin varlığına da işaret edebilmektedir. Eşek sütü antikanserojenik etkilerinden dolayı kanser hastaları tarafından genellikle çiğ olarak tüketilmekte ve bu nedenle de kanser hastaları ve halk sağlığı açısından risk teşkil etmektedir. Sonuç olarak, eşek sütü üretiminde iyi hijyenik prosedürler uygulanmalı ve bu sütler buzdolabı sıcaklığının altında saklanmalıdır.
Anahtar sözcükler: Eşek sütü, mikrobiyolojik kalite, somatik hücre sayısı.

Introduction

Milk is an important source of nutrition especially for babies and children because of lactose, fat, proteins, vitamins and minerals in its composition. In cases where breast milk is not available or inadequate, it is essential for infants to be fed with cow milk or cow milk based formulas. About 3% of children aged 0-3 years who consume cow's milk or cow milk-based formulas have cow's milk protein allergy (CMPA) especially mediated by immunoglobulin E (IgE) (2, 24, 33). Although the milk yield per animal (1 L/day/animal) is low, donkey milk was reported to have a significant advantage over other animals milk due to the fact that high lysozyme and lactoferrin content and mastitis rate in donkeys is low (3,12). Therefore, donkey milk is similar to human milk in terms of lactose, total protein and whey protein content has been reported to be a good alternative in the nutrition of children with CMPA (1, 36). Besides, it is used in the

cosmetic industry for its rejuvenating properties, and its therapeutic effects in the medical field (i.e., antitumor, liver problems, infectious diseases, fever and asthma) (22, 23, 28). Raw donkey milk is mostly consumed as an alternative food by cancer patients. Due to the demand for this purpose, the count of farms that produce raw donkey milk is increasing day by day.

Total aerobic mesophilic bacteria (TAMB) and somatic cell counts (SCC) are important criteria for determining the hygienic quality of raw milk. The migrated leukocytes (neutrophils, macrophages, etc.) and mammary gland epithelial cells are called as somatic cell (14). Particularly SCC is considered to be an important criterion in udder health, milk quality and milk price determination in dairy cows (16). The counts of TAMB and SCC are lower in raw donkey milk when compared with the bovine milk. It is explained by the higher lysozyme content in donkey milk (3750 mg/L) than cow milk (0.09 mg / L) (7). The microbiological quality of raw donkey milk has great importance in terms of public health since both the babies' immune system is not well developed, and some cancer patients are immunosuppressive.

The aim of this study was to reveal the chemical and microbiological characteristics of raw milk samples collected from clinically healthy animals raised in two different commercial farms for donkey milk production.

Materials and Methods

A total of 78 raw milk samples taken from 26 clinically healthy donkeys in lactation period which reared in two different donkey farms (Farm 1: total donkey count 18; milking donkey 13 and Farm 2: total donkey count 21; milking donkey 13) in Marmara region, Turkey, were used for present study. The farms were visited 3 times in 15-day periods between September-October 2018. Before the raw milk samples were taken, the udder lobes were cleaned with 70% methyl alcohol and allowed to dry. After the first few squeezes of milk samples were taken out, approximately 125 ml of milk samples were taken from each udder lobe. After that, samples were taken into sterile plastic containers, carried with cold chain, transferred into the laboratory within 2 hours and analyzed.

Chemical analysis of the samples: The dry matter, fat, protein and lactose analyzes of raw donkey milk samples were performed by using milk analyzer (Bentley Combi FTS 600, USA) which was calibrated with donkey milk.

Microbiological analysis of the samples: 10 ml of raw donkey milk sample was taken from samples, and homogenized with 90 ml Ringer solution (Merck 115525, Darmstadt, Germany) in stomacher for 2 minutes (IUL, Spain). After homogenization period, serial dilutions were prepared and then plated on selective media.

TAMB count was performed using the ISO 4833: 2013 standard method (19). The isolation and enumeration of coagulase-positive staphylococci (*Staphylococcus/ Micrococcus* and *S. aureus)* were performed using the ISO 6888-1:1999 standard method (20). The isolation and enumeration of Coliform bacteria count were performed using the ISO 4832: 2006 standard method (18). The isolation and enumeration of *Lactobacillus* spp**.** and *Lactococcus* spp. were performed according to Maina *et al*. 2004 (29).

Somatic cell count*:* SCC was analyzed according to Bacto Count IBC Series (US) and ISO 13366/IDF 148-2: 2006 (17).

Statistical analysis: Data were analyzed using SPSS version 22.0 software (SPSS, Inc., Chicago, IL, USA) using independent samples t tests to compare within groups. $P< 0.05$ was considered statistically significant.

Results

The average dry matter, protein, fat and lactose values of the raw donkey milk samples were 8.89%, 1.57%, 0.7%, 6.48% in farm 1 and 8.79%, 1.47%, 0.45%, 6.06% in farm 2, respectively. A statistically significant difference was observed amounts of fat and lactose between the farms ($P <0.05$) (Table 1).

The average of TAMB count was 3.88 log CFU/mL and SCC was 3461 cells/mL in farm 1. The highest of TAMB count was 4.5 log CFU/mL and SCC was 13000 cells/mL in this farm. There was a statistically significant difference between two farms in terms of TAMB and SCC ($P <0.05$) (Table 2).

Table 1. Chemical analysis of raw donkey milk samples in farms

	Farm 1 Min-Max	Farm 1 Mean± SEM	Farm 2 Min-Max	Farm 2 Mean± SEM
Dry substance (%)	8.37 - 9.35	8.89 ± 0.43^{a}	7.65 - 9.36	8.79 ± 0.42^{a}
Protein (%)	1.09 - 2.01	1.57 ± 0.71^{a}	1.18 - 2.63	1.47 ± 0.13^{a}
Fat (%)	0.26 - 1.0	0.7 ± 0.05^{a}	0.25 - 0.66	0.45 ± 0.03^{b}
Lactose (%)	6.25 - 6.68	6.48 ± 0.03^{a}	5.84 - 6.36	6.06 ± 0.05^{b}

[a,b:] $P < 0.05$ was considered statistically significant in the same line.

Table 2. Microbiological analysis and somatic cell count results in raw donkey milk samples

	Farm 1 Mean± SEM	**Farm 2 Mean± SEM**
TAMB (log CFU/mL)	3.88 ± 0.03^{b}	6.06 ± 0.43^{a}
Somatic cell (cell/mL)	3461 ± 924.14^{b}	25153 ± 4688.83^{a}
Lactobacillus (log CFU/mL)	2.16 ± 0.16^{b}	3.30 ± 0.13^{a}
Lactococcus (log CFU/mL)	4.32 ± 0.23^{b}	5.12 ± 0.15^{a}
Staphylococ / micrococ (log CFU/mL)	0.38 ± 0.21^{b}	2.25 ± 0.07^{a}
Coliform (log CFU/mL)	1.01 ± 0.5^{a}	1.74 ± 0.02^{a}

[a,b:] $P < 0.05$ was considered statistically significant in the same line.

Although, the average count of *Staphylococcus-Micrococcus* spp. was 2.66 log CFU/mL and the count of coliform bacteria was 1.5 log CFU/mL in the milk samples of the farm 1, coagulase-positive staphylococcus was not determined. In the case of raw donkey milk in farm 2, the average count of *Staphylococcus-Micrococcus* spp. was 2.33 log CFU/mL and the count of coliform bacteria was 1.7 log CFU/mL. Interestingly, coagulase-positive staphylococcus was detected only in one sample (2 log CFU/mL) but *S. aureus* could not be determined in this farm. A statistically significant difference was observed in terms of *Staphylococcus-Micrococcus* spp. count between two farms ($P < 0.05$) (Table 2).

In milk samples of the farm 1, the average count of *Lactobacillus* spp and *Lactococcus* spp. were 2.16 and 4.32 log CFU/mL while the average counts of *Lactobacillus* spp and *Lactococcus* spp. were 3.30 and 5.12 log CFU/mL in milk samples of farm 2, respectively. The difference was statistically significant in terms of the counts of *Lactobacillus* spp. and *Lactococcus* spp. between two farms ($P < 0.05$) (Table 2).

Discussion and Conclusion

There are limited studies on the chemical and microbiological quality of donkey milk. In this study, chemical and microbiological qualities of the raw donkey milk samples from two different donkey farms in the Marmara region, which has the significant rate of production potential and intense human population in Turkey, were investigated. One of the most important indicators of raw milk quality is chemical composition. Although there were a difference between the amounts of fat and lactose in milk samples, there was no difference in the amount of dry matter and protein between the farms ($P < 0.05$). The average dry matter and protein values obtained from this study were similar with previous studies (8, 9, 21, 34, 35) but these values are lower than the values of dry matter (9.5%) which reported by Martini *et al.* 2014. In this study, mean fat value obtained from both farms (0.45-0.70%) was higher than the fat values (0.09%) determined by Claeys et al. (8). Besides, fat values of the samples in the farm 1 were higher than the results of some researchers (12, 21, 26, 36). The lactose values of the milk in both farms were similar with the findings of some researchers (21, 36), whereas it was found lower than different study findings (26, 28). Csapo et al. (10) reported that the differences in fat values between farms (0-7%) would be resulted from genetic structure and environmental factors (i.e., nutritional conditions, lactation period). Milk yield and the chemical composition of milk vary depending on genetic, physiological and environmental factors (11, 21, 40). Although, yield and chemical composition of the cow milk was determined in the previous studies, donkey milk yield and chemical composition included high variability. This variability was mostly depending on different pasture conditions in donkey breeding (8). Besides, it can be affected from many donkey breeds in the world.

In the diagnosis of subclinical and clinical mastitis in dairy cows, TAMB and SCC are accepted as the gold standard in raw milk samples (15). However, national and international levels have not been determined in the count of TAMB and SCC in donkey milk. There are some international studies about to establish standardization of donkey milk for these two parameters (15). In this regard; Ivankovic et al. (21) found the average of TAMB count as 3.58 log CFU/mL and SCC 12500 cells / mL during the lactation period (150 days) in the milk samples taken from fourteen donkeys. Sarno et al. (38) reported the average of TAMB count between the range of 2.84-3.92 log CFU/mL, and SCC below 50000 cells/mL in raw milk samples taken from eight healthy donkeys. In another study, Pilla et al. (32) found that the count of TAMB in the donkey milk samples was very low (<250 CFU/mL) and SCC was below 50.000 cells/mL. Malissiova et al. (26) reported that TAMB and SCC were 3.04-4.79 log CFU/mL and 5000 -13000 cells/mL in 90 raw donkey milk samples, respectively. In this study, results of TAMB and SCC of the milk samples taken from farm 1 were observed to be similar to the previous studies mentioned above (21, 26, 38). Conversely, milk samples of farm 2 were found higher than the data obtained from both farm 1 and previously mentioned studies (21, 26, 33, 38). Except for one of the milk samples in farm 2, TAMB count

in both farms was identified below the raw milk standard determined for other mammals (less than $1.5x10^6$ CFU/mL at 30 °C) (EC853/2004). The difference in the counts of TAMB and somatic cells showed that udder health and hygiene were important criteria for safe raw milk production. In addition, samples with the high count of somatic cells as well as having high TAMB counts in farms 1 and 2 indicates that these two parameters can be related to udder health in donkeys as in cows (30). Differences in SCC can be caused by many individual and environmental factors such as animal species, milk yield, lactation period, milking technique as well as udder health (25).

Determining the count of hygiene indicator microorganisms such as *Staphylococcus /Micrococcus* spp. and coliform bacteria is an important for microbiological quality of raw milk. Sahinturk and Oner (34) defined the counts of *Staphylococcus-Micrococcus* spp. between 3.53-6.31 log CFU/mL in 13 raw donkey milk samples. In another study, the count of *Staphylococcus* spp. was determined to range from $5x10^1$ to $3.6x10^3$ CFU/mL in 41 raw donkey milk samples (26). Results of the present study were lower than the results of studies mentioned before (26, 34). *S. aureus* could not be detected from the raw milk samples in present study. Similar to our study, some researchers reported that they could not detect *S.aureus* in any of the raw donkey milk samples (6, 34, 37). In contrary, *S. aureus* was identified *in* 5 of 101 raw donkey milk samples by Pilla et al. (32). In addition, Chandrashekar et al. (6) reported that *S. aureus* (10^3 CFU / mL) reduced below one log/CFU levels end of 5 hours at +4 C in their experimental studies. The absence of *S.aureus* in donkey milk may be due to the antibacterial effect of lysozyme and lactoferrin in donkey milk (6, 37). On the other hand, Sahinturk and Oner (34) and Zhang et al. (41) found that the number of coliform bacteria in donkey milk was higher than our study. Saric et al. (37) also reported that they could not detect coliform bacteria in donkey milk on day 0 but they detect on the 5th day of storage at +4 ° C. The difference in the number of coliform bacteria in donkey milk indicates the level of contamination caused by farm and milking hygiene and feces.

Lactic acid bacteria can be isolated from many sources such as milk, plants, fermented foods and gastrointestinal tract of mammals. Although, it is known the inhibitory effects of lactic acid bacteria against pathogenic microorganisms on the production of fermented foods, some of them play a role in the spoilage of foods (39). In our study, the count of *Lactobacillus* spp. and *Lactococcus* spp. were detected as 2.16-3.30 log CFU/mL and 4.32-5.12 log CFU/mL respectively. Carminati et al. (4) reported that count of *Lactobacillus* spp. in donkey milk samples was 3.57 log CFU/mL on average, while the count of *Lactococcus* spp. was 3.97 log CFU/mL. Sahinturk and Oner (34) also reported the average count of *Lactobacillus* spp. as 5.08 log CFU/mL and the count of *Lactococcus* spp. as 5.84 log CFU/mL in the raw donkey milk samples. Saric et al. (37) determined the count of lactic acid bacteria as 2.31 log CFU/ml in donkey milk samples. There are some studies on the presence of lactic acid bacteria that cause microbial spoilage in different type's raw milk. Franciosi et al. (13) determined the count of *Lactobacillus* spp. between 3.4-6.2 log CFU/mL and *Lactococcus* spp. 4.2-6.8 log CFU/mL in raw cow milk, respectively. Picon et al. (31) found the count of *Lactobacillus* spp. in the range of 3.47-3.88 log CFU/mL and *Lactococcus* spp. the range of 5.37-5.99 log CFU/mL in the raw goat's milk. It was determined that lactic acid bacteria were predominant in raw donkey milk microflora. All raw milk hygiene indicators (*Staphylococcoccus/Micrococcus* spp., Coliform bacteria count) and the differences in the count of *Lactobacillus* spp. and *Lactococcus* spp. between the farms can be changed due to maintenance, feeding and hygienic milking applications. Besides, it can be affected from different levels of lysozyme enzymes which obtained from different lactation period. This situation may be caused by the antibacterial effect of lysozymal enzymes rich donkey milk on many Gram-positive and Gram-negative bacteria including lactic acid bacteria (5). As a result of this effect, donkey milk has a prolonged shelf life and may distributed as raw milk.

In recent years, donkey milk has been playing an important role in the nutrition of cancer patients, infants and children with CMPA. However, the presence of indicator microorganisms in raw donkey milk may indicate the presence of food pathogens In addition, infected raw donkey milk can be dangerous for human health depends on possible donkey related zoonotic diseases. In conclusion, considering the statistical significances between chemical and microbiological findings of two different farms, related to not only the maintenance and feeding but also product standardization is required in donkey milk production.

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Ethical Statement

This study does not present any ethical concerns.

Conflict of Interest

The authors declared that there is no conflict of interest.

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