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Estimation of mineral, trace element and fatty acid profile of Anatolian water buffalo milk

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Buffaloes are the second-most milk-producing animals in the world and Turkey is the tenth among the top buffalo milk (BM) producers. Although the buffalo population has increased through the world since 1980, a dramatic decrease was observed in the number of Anatolian water buffaloes (AWB) until 2008 (91.8% fall). Thus, there is a growing interest by the Turkish government to encourage production of AWB, BM and output products of BM. However, the nutrient profile of AWB milk (AWBM) has not been determined in details and the data about the biochemical composition of the AWBM are still limited. In this study, chemical, elemental, and fatty acid (FA) compositions of AWBM were determined by FT-120 milk analyzer, ICP-OES, and GC, respectively. Gross chemical composition of AWBM was found as total solids 17.15%, protein 4.67%, and fat 7.66%. Major elements in AWBM were determined as Ca (3,043.00 mg/L), P (1,831.33 mg/L), K (1,797.5 mg/L), and Na (675.66 mg/L) while minor elements were observed as Mg (243.91 mg/L), Zn (9.30 mg/L), Si (3.08 mg/L), Fe (1.59 mg/L), and Mn (0.6 mg/L). Proportions of total FAs were found as saturated FAs (ΣSFA) 66.16%, monounsaturated FAs (ΣMUFA) 29.68%, polyunsaturated FAs (ΣPUFA) 4.16%. In conclusion, the results of this study indicate that the potential nutrient profile of AWBM is of great significance to human nutrition. Furthermore, the results will be useful for the future studies concerning buffalo breeding and dairy processing.

Keywords: Anatolian water buffalo; buffalo milk; minerals; major elements; trace elements; fatty acids

INTRODUCTION

Milk is one of the most important parts of human diet. Buffalo, which is almost universally considered to be an Asian animal, is the second most milk producing animal in the world. The domestic water buffalo (*Bubalus bubalis*) has a great contribution to global milk production as a major milk providing animal in several countries. Food and Agriculture Organization (FAO) reported that there are about 168 million heads of water buffalo (WB) in the world, and more than 95% are found in Asia [1]. Turkey ranked tenth among the top buffalo milk (BM) producers with 54,803 tonnes in 2014 [2]. Turkish WB are commonly known as the Anatolian water buffaloes (AWB) and classified as Mediterranean buffaloes belonging to the River group involving Syrian, Egyptian and Southeast European animals [3, 4]. The AWB has been raised for more than 1,000 years as an antique part of Turkey's livestock resources and there was over 1 million heads just a few decades ago. Although the buffalo population has increased through the world since 1980, a dramatic decrease was observed in the number of the AWB from 1,040,000 heads in 1980 to the observed lowest values of 84,705 heads in

Thus, the fall in the AWB population was 91.8%. Among a number of reasons, intensification of the dairy activities, agricultural mechanization, consumer preferences, and less market demand to buffalo products [5] are shown as main responsibilities for this decrease. By 2011, the AWB population remained in steady state at around 84,726 heads [6]. With the first attempts on the conservation of animal genetics resources and diversities in 2008 and the foundation of the Water Buffalo Breeders Union in 2011, the buffalo number exceeded 100,000 heads and reached to 107,435 heads in 2012 [5, 7]. Notwithstanding the existing discrepancy between the FAO and Turkish Statistical Institute (TUIK) statistics in terms of the buffalo numbers by years, the recent accessible data for the number of AWB were reported by TUIK in 2016 as 133,776 heads in 2015 corresponding to 0.96% of 13,994,071 cattle [8]. From 2008 to 2015, a total of 57.9% increase in the AWB population can be seen as an obvious evidence of the positive impact of the recent legislations and conservation programs arranged by the government to prevent extinction of the AWB in Turkey [5]. Nevertheless, more efforts and promotions by the government are needed to encourage buffalo production and consumption of buffalo dairy products.

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In Turkey, not only AWBs have been bred for milk production, but also they are used as household livelihoods with their meat and draught power. In particular, BM outputs are a semi-hard cheese called "peyaz peyneri", ayran [9], buffalo cream from high-fat milk which is favorable as an additive to the traditional Turkish desserts [3]. Efforts on improving milk yields and lactation periods of the AWB from different provinces of Turkey are still continuing. Top three main distribution areas of the AWB are in the West Black Sea (39,738 heads), Central East Anatolia (14,337 heads), Central Anatolia (14,045 heads). Regarding the buffalo production numbers, focal points of the production of Turkish buffaloes are found in the provinces of Afyon (5,183 heads), Bitlis (7,594 heads), Diyarbakır (11,510 heads), Istanbul (11,518 heads), Kayseri (5,313 heads), Mus (6,087 heads), Samsun (17,043 heads), and Tokat (8,839 heads). The Aegean (2,091 heads) and the West Anatolia (1626 heads) have the lowest number of AWB [10]. In comparison to cow milk, it was reported in a comprehensive recent review that BM is richer in almost all main milk nutrients such as fatty acids, proteins, crude protein, calcium, fat, phosphorus and lactose [11]. Considering the high level of nutrients presents in BM and the added economic potential value of the output products of BM such as mozzarella, sweets, cream, it is necessary to better understand the nutrient profiles and composition of BM samples in Turkey. However, little attention has been paid to the detailed characterization of the chemical composition of AWB milk (AWBM). Thus, it is important to enlighten the composition of the AWBM to encourage AWB husbandry, buffalo products, and to conserve this national heritage.

The aim of this study is, therefore, to analyze the milk quality of AWB in terms of chemical, elemental and fatty acid compositions, to create awareness on the potential use of the AWBM, and strengthen its use in dairy production and demand in the market. Total solids, fat, protein, solids-non-fat, lactose, casein, density, urea, total acidity, free fatty acid, citric acid and freezing point depression, elemental levels, and fatty acid composition of AWBM were examined. The results will be useful for the future studies concerning buffalo breeding and dairy processing.

EXPERIMENTAL

Sampling (milk samples)

Milk products used in this study, within General Directorate of Agricultural Research and Policies, were obtained from the incorporated materials in National Community Based Anatolian Water Buffalo Breeding Program (TAGEM/49 MANDA 2012-01). Muş is located in Eastern Anatolia region of Turkey with the latitudes of 38° 44'41" N, 41° 39'14" E and elevation 1,400 meters above sea level (m.a.s.l.). All AWB were multiparous (para 2, lactation stage: 2, body weight, 450-550 kg; milk yield 6.5 ± 2.5 kg/d), and fed on natural pastures of Muş Plain of Turkey. 50 mL milk samples (n=35) were collected directly in sterilized plastic containers in the morning milking session, brought to the laboratory with cold chains, and immediately stored at -20 ºC until required for analysis. No other pretreatments or preservatives were used for the milk samples.

Analytical procedures

Determination of chemical composition. FOSS MilkoScan^{TM} FT-120 (Foss Electric, Denmark), calibrated with appropriate buffalo standards, was used to analyze total solids (%), protein (%), fat (%), casein (%), lactose (%), citric acid (%), density (g/cm^3), urea (mg/dL), total acidity (ºSH), free fatty acids (mmol/10L), and freezing point depression (FPD) (ºC) in raw BM samples. FTIR analytical technology utilized in MilkoScan^{TM} FT-120 is compatible with IDF (International Dairy Federation) principles and AOAC (Association of Official Analytical Chemists) formal procedures [12].

Determination of elements. Milk samples (2 mL) were transferred to DAP60-K PTFE vessels of a microwave digestion system, and 4 mL of HNO_3 (65% w/v) and 1 mL of $HClO_4$ (60% w/v) were added. After digestion, the digested samples were transferred to 20 mL volumetric flasks and made up to final volume with 0.1 M HNO_3. Also, blank samples were prepared and digested in the same way. Final sample solutions were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES) [13].

Determination of fatty acid composition. Milk fat extraction [14] and transformation of the fatty acids to methyl esters [15] were carried out according to literature and are briefly summarized hereafter. 1 mL of BM samples were taken, and homogenized in a hexane/ isopropanol (3:2 v/v) mixture. Lipid extracts were centrifuged at 10,000 rpm for 5 min. Then, solvents were removed at 40 ºC. The fat extracts were stored at -25°C before fatty acid analysis. Three replicate measurements were taken for each sample. Fatty acids in the lipid extract were treated with 2% sulfuric acid (v/v) in methanol and transformed into methyl esters. Fatty acid methyl esters were extracted with hexane. After transforming the fatty acids in the lipid extracts to methyl esters the latter were analyzed by gas chromatography (SHIMADZU GC 2025 with a flame ionization detector) using TR-CN 100 capillary column (60 m × 0.20 mm i. d. with 25 µm film thickness) (Teknokroma, Spain). Calculations were performed using GC Solution program (V.2.42).

Statistical analysis

Descriptive statistics were performed with SPSS statistic software. (SPSS 20.00, Chicago). The data were presented as mean ± standard deviation.

RESULTS AND DISCUSSION

Chemical composition of AWBM

Descriptive statistics of the variables studied for the chemical composition of the AWBM are presented in Table 1. Total milk solids 17.15 ± 1.35%, protein 4.67 ± 0.47%, and fat 7.66 ± 1.12% were in good agreement with the findings of a previous study (total solids: 16.6 ± 1.6%, protein: 4.40 ± 0.51%, and fat: 7.1 ± 1.4%) [16]. In the same way, the solid-non-fat value 10.04 ± 0.51% was aligned with the value of 9.6 ± 0.8% of the same study. While the observed lactose value was 4.94 ± 0.18% of the AWB, the casein value of 3.50 ± 0.48% was similar to the previously reported value of 4.40 ± 0.51 [16]. On the other hand, a good agreement was also observed between the density, urea, total acidity, free fatty acid, citric acid, and FPD values of the AWBM and those presented in a recent study [17] as in the minimum and maximum range of 1.028 to 1.033 g/cm^3, 0.036 to 0.057 mg/dL, 5.96 to 9.94 ºSH, 3.22-6.35 mmol/10L, 0.11 to 0.15%, -0.46 to -0.66°C, respectively. Taken all in consideration, it is noteworthy that the obtained values of the milk chemical composition variables of the AWBM in this study were not only correlated with the reported values in former local studies [16, 17] but also were in the accepted range of composition and properties of BM [11].

Table 2 presents detailed information about the comparison of the elemental levels of BMs from different locations of the world. Major elements in AWB milk were found as Ca (3,043.00 mg/L), P (1,831.33 mg/L), K (1,797.5 mg/L), and Na (675.66 mg/L) while minor elements were observed as Mg (243.91 mg/L), Zn (9.30 mg/L), Si (3.08 mg/L), Fe (1.59 mg/L), and Mn (0.6 mg/L). Five macro elements such as Ca, Mg, K, Na and P were determined in all of our samples. The observed individual mean concentrations of Ca (3,043 mg/L), Mg (243.91 mg/L), K (1,797.5 mg/L), Na (675.66 mg/L), and P (1,831.33 mg/L) in BM samples were the highest compared to the results obtained in earlier studies from Bangladesh [18], Italy [19], Argentina [21], Pakistan [22], and India [25] (Table 3). There are many benefits and crucial roles of Ca in human body such as hormone secretion, enzymatic reaction and relaxation of muscles [26]. Likewise, Mg not only takes role as a cofactor in more than 300 enzymes in the human body but also regulates many diverse biochemical reactions. Moreover, P in milk is found in many derivatives such as ester or inorganic phosphorous and converted into active biological forms thorough intestinal adsorption [27].

Investigation of the obtained results revealed that three essential trace elements were determined in all of our AWBM samples, which were Fe, Mn and Zn (Table 3).

Table 1. AWBM chemical composition (n=35)

Variable	Mean	SD	Minimum	Maximum
Total solids (%)	17.15	1.35	14.59	19.70
Fat (%)	7.66	1.12	6.22	9.48
Protein (%)	4.67	0.47	3.62	5.33
Solids-non-fat (%)	10.04	0.51	9.45	10.96
Lactose (%)	4.94	0.18	4.68	5.31
Casein (%)	3.50	0.48	2.87	4.61
Density (g/cm^3)	1.036	2.36	1.032	1.042
Urea (mg/dL)	0.043	0.013	0.004	0.059
Total acidity (ºSH)	8.63	1.27	7.04	10.53
Free fatty acids (mmol/10L)	4.43	0.97	3.25	6.37
Citric acid (%)	0.18	0.03	0.12	0.22
Freezing point depression (ºC)	-0.63	0.04	-0.55	-0.70

Elemental composition of AWBM

Table 2. Descriptive statistics of data in raw WB milk samples (mg/L)

Elements	Mean	SD	Range	
			Min.	Max.
Ca	3,043.00	329.05	2,929.98	3,156.02
P	1,831.33	404.76	1,692.55	1,970.11
K	1,797.5	315.67	1,688.79	1,905.21
Na	675.66	177.50	614.20	735.8
Mg	243.91	62.00	222.61	265.21
Zn	9.30	2.51	8.44	10.16
Si	3.08	0.75	2.82	3.34
Fe	1.59	0.47	1.43	1.75
Mn	0.60	0.05	0.58	0.62

Table 3. Comparison of the elemental levels of BM samples in this work with previous works[a]

Element	Turkey This work (mg/L) (n=35)	Bangladesh [18] (mg/kg) (n=9)	Italy [19] (mg/kg) (n=6)	Campania, Italy [20] (mg/kg) (n=68)	Argentine [21] (mg/kg) (n=105)	Pakistan [22] (mg/L)[b]	West Bengal [23] (mg/kg) (n=10)	Egypt [24] (mg/kg) (n=60)	India [25] (mg/kg) n=496)
Ca	3,043	1.480	1.740		1.120	702			
Fe	1.59		0.3		1.61		3.50	0.980	3.05
K	1,797.5	860	641		920	145			
Mg	243.91	140			80	193			
Mn	0.60	0.07	0.0024	0.0493	0.27		1.74	0.076	0.56
Na	675.66	370			350	16			
P	1,831.33	1.070	1.190		990				
Zn	9.30	4.58	6.49	5.74	4.1		3.75	4.37	3.57

[a]Data are reported as mean values
[b]Number of samples is not available

In particular, Zn was the most abundant of the trace elements (9.30 mg/L) which was followed by Fe (1.59 mg/L) and Mn (0.60 mg/L). Zn content was above the values obtained in all of the similar studies given in Table 3. The mean Fe content of our AWBM samples was more or less different from those reported in the literature. It was higher than for Egypt (0.980 mg/kg) [24], and Italy (0.3 mg/kg) [19] while lower than for Argentina (1.61 mg/kg) [21], West Bengal (3.50 mg/kg) [23] and India (3.05 mg/kg) [25]. The mean concentration of Mn was observed as 0.60 mg/mL, which was very close to the reported value for India (0.56 mg/kg) [25] and the second highest value after West Bengal (1.74 mg/kg) [23]. In contrast, the level of Mn was much higher than the reported values for Bangladesh (0.07 mg/kg) [18], Italy (0.024 mg/kg) [19], Campania, Italy (0.0493 mg/kg) [20], Argentine (0.27 mg/kg) [21] and Egypt (0.076 mg/kg) [24]. On the other hand, the mean concentration of Si was found as 3.08 mg/ L which is very close to the value of 3.26 mg/L for BM reported in the study of Nirgude *et al.* [28] and lower than in goat milk (10.59 mg/mL) of local goat breeds collected from Üzümdallı village in Hatay province of Turkey [29]. Si plays a role in the normal metabolism of higher animals as connective tissue, especially in bone and cartilage and forms important relations with other elements [28].

The fatty acid content of AWBM

Chemical composition of milk can be influenced by several factors such as animal species, genetics, environmental conditions, lactation stage, and nutritional status [30-35]. Among these factors, significant differences were found in BM fat composition due to variations in animal nutrition related with the buffaloes' feeding management with different herds [30]. On the other hand, the milk fatty acid quality was reported as better in younger buffaloes at early lactation stage depending on the effect of age and lactation on milk fatty acid profile in dairy buffaloes [32]. Apart from these factors, it was found that WB milk has lots of biochemical materials such as amino acids, fatty acids, minerals, enzymes and vitamins, which are very important for human metabolism, especially for the skeletal system [36]. No information is available for the fatty acid composition of milk samples from buffalo in Turkey. Milk fatty acid (FA) compositions of the AWBMs are presented in Table 4. ΣSFA fraction rate was found to be 66.16%. The major FAs in all AWBM samples were palmitic acid (C16:0) 33.90%, myristic acid (C14:0) 10.09%, stearic acid (C18:0) 12.82%. ΣUFA accounted for 33.84% while the percentage of monounsaturated fatty acids (ΣMUFA) and total polyunsaturated fatty acids (ΣPUFA) constituted 29.68% and 4.16%, respectively. On the other hand, the levels of important PUFAs were linoleic acid (C18:2n6c) 1.76%, linolelaidic acid (C18:2n6t) 0.25%, gamma linolenic acid (C18:3n6) 1.01%, and docosahexaenoic acid (C22:6n3) 0.28%. The largest MUFA was oleic acid (C18:1n9c) 22.33%, and the others were myristoleic acid (C14:1) 1.07% and elaidic acid (C18:1n9t) 1.59%.

Table 4. Fatty acid content of Turkish WB milk (%)[a]

SFA	Mean ± SD	PUFA	Mean ± SD	MUFA	Mean ± SD
C4:0	0.26 ± 0.05	18:2n6c	1.76 ± 0.22	C14:1	1.07 ± 0.25
C6:0	1.04 ± 0.16	18:2n6t	0.24 ± 0.04	C15:1	0.57 ± 0.09
C8:0	0.59 ± 0.09	18:3n6	1.02 ± 0.18	C16:1	2.21 ± 0.32
C10:0	1.12 ± 0.16	18:3n3	0.17 ± 0.03	C17:1	0.46 ± 0.11
C11:0	0.06 ± 0.02	20:3n6	0.21 ± 0.09	C18:1n9t	1.60 ± 0.25
C12:0	1.68 ± 0.18	C20:2	0.07 ± 0.01	C18:1n9c	22.34 ± 2.93
C13:0	0.09 ± 0.01	20:3n3	0.04 ± 0.01	C20:1	1.00 ± 0.14
C14:0	10.09 ± 1.50	20:4n6	0.17 ± 0.04	C22:1n9	0.24 ± 0.05
C15:0	1.95 ± 0.28	C22:2	0.13 ± 0.02	C24:1	0.22 ± 0.06
C16:0	33.90 ± 2.47	C20:5	0.07 ± 0.01	ΣMUFA	29.68 ± 2.37
C17:0	1.37 ± 0.17	C22:6	0.28 ± 0.04	ΣUFA	33.84 ± 2.70
C18:0	12.82 ± 1.77	ΣPUFA	4.16 ± 0.52		
C20:0	0.40 ± 0.14				
C21:0	0.08 ± 0.02				
C22:0	0.38 ± 0.09				
C24:0	0.34 ± 0.08				
ΣSFA	66.16 ± 4.62				

[a]SFA, saturated fatty acids, MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA, unsaturated fatty acids. Results are presented as means of 35 different samples with three replicate measurements ± standard deviation (SD).

Taken into consideration the results of our study, similar levels of ΣFAs in milk fat have been reported in the literature. Mihaylova *et al.* [37] found the ratio of FAs in BM as 72.15% (ΣSFA), 24.70% (ΣMUFA), 3.15% (ΣPUFA) while Fernandes *et al.* [38] reported these ratios as 65.04%, 31.68% and 3.28%, respectively. In another study, Talpur *et al.* [39] compared milk FA levels of Nili-Ravi and Kundi. Results of their study revealed that ΣSFA, ΣMUFA, ΣPUFA values of Nili-Ravi were 69.09%, 25.20%, and 2.76% while those of Kundi were 66.96%, 27.62%, and 2.77, respectively. It is well known that long-chain PUFAs have an important role in many aspects of children's health, notably in neuro developmental and psychiatric conditions [40]. Moreover, it was reported that diets with high amounts of MUFAs and PUFAs decrease the risk for coronary artery disease [41]. Considering the results of the above mentioned studies, it is noteworthy that ΣPUFA levels were found in our study (4.16%, Table 4). PUFAs consist of many long-chain FAs. Some of the members of long-chain omega-3 FAs are eicosapentaenoic acid (C20:5n3) and docosahexaenoic acid (C22:6n3). They diminish the risks of cardiovascular incidence [42]. In the study on the long-chain FA profiles in BMs we found linolelaidic acid (C18:2n6t), gamma-linolenic acid (C18:3n6), cis-8,11,14-eicosatrienoic acid

(C20:3n6), cis-11,14-icosadienoic acid (20:2n3), cis-11,14,17-eicosatrienoic acid (C20:3n3), erucic acid (C22:1n9) and nevronic acid (C24:1). Furthermore, linoleic (C18:2n6c), linolenic (C18:3n3) and oleic (C18:1n9c) acids are known as to be cardio protective fatty acids [43]. In our study, the total of these FAs were 24.27% of total FAs whereas Qureshi *et al.* [44] and Mihaylova *et al*. [37] reported them as 34.38% and 20.81%, respectively. On the other hand, the total fraction of lauric acid (C12:0), myristic acid (C14:0) and palmitic acid (C16:0) is called hypercholesterolemic FAs (HCFA), over intake of which from diet causes cardiovascular diseases [45]. In our study, the HCFA ratio was determined as 45.67% similar to Pakistan buffalo breed Nili-Ravi buffaloes having 43.33% [44] and Bulgarian Murrah buffaloes having 43.62 % [37]. Moreover, Talpur *et al*. found HCFA ratios of BM as 45.48% (Kundi) and 46.54% (Nili-Ravi) [39]. BM has a high level of nutrients and therefore an economic potential value. Production of yogurt, cheese, ice cream, and sweets from Water BMs is not common in Turkey as in Italy that has its own brand like mozzarella cheese and wide WB milk products. In summary, AWBM deserves much more attention in diets of children and older people in Turkey.

CONCLUSION

In this study, chemical, elemental and fatty acid compositions of milk samples from AWB were extensively analyzed. The results obtained from these biochemical parameters showed that the AWBM is rich in terms of various elements, PUFA and MUFA FAs. This study is important since it reveals the potential nutrient profile of AWBM for human nutrition and creates awareness on the output products of AWBM. Additionally, the results will be useful for the future studies concerning buffalo breeding and dairy processing.

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