MILK AND FATTY ACID COMPOSITION OF ANATOLIAN WATER BUFFALO (BUBALUS BUBALIS) FROM DIFFERENT PROVINCES

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ABSTRACT

The present study was undertaken (1) to characterize the fatty acid (FA) composition particularly on the concentration of conjugated linoleic acid (2) to investigate of physicochemical properties of Anatolian water buffalo milk, and from six different provinces in Turkey. The fat amount in water buffalo milk samples were in the range of 5.97±0.30% to 9.19±0.57% and the mean fat was 6.96±0.25%. The main individual FA in water buffalo milk were in the order 16:0, 18:1 cis-9, 14:0, and 18:0. The linoleic acid (CLA), bovinic acid (cis-9, trans-11), represented 1.09±0.06 in water buffalo milk. Saturated fatty acids (SFA) were the potent fraction in water buffalo milk fat (70.63±0.7); MUFA and PUFA were 29.37±0.7 and 0.2±0.03, respectively. The data exhibit statistically differences (P≤0.05) in the proportions of individual FA were detected among different provinces in Turkey. Anatolian water buffalos were poor in terms polyunsaturated FA compared to other water buffalos from different countries. Therefore, these results may provide useful information about the nutrient composition of buffalo milk and further studies are warranted to improve the technological and nutritional characteristics of Anatolian buffalo milk.

Keywords: buffalo milk, fatty acids, physicochemical properties, linoleic acid

INTRODUCTION

The principal sources of fat in the human diet are visible fats and oils, red meats, poultry, fish and dairy products. Fats forms a large group of nutrients, making a main component of dairy products, which provide vital components for the human diet. The effects of dietary fats generally reflect the cumulative effects of multiple fatty acids in the diet (Chandrasekharan, 1999). Milk fat contains ~400 different fatty acids (FA), which make it the most complex among natural fats. The milk fatty acids (FA) are produced almost equally from two sources, first in the rumen as a feed and the microbial activity and secondly in the mammary gland as de novo synthesis in animals. For FA in milk, short chain fatty acids (SCFA) (4 to 8 carbons) and medium chain fatty acids (MCFA) (10 to 14 carbons) derived almost exclusively from de novo synthesis, and long chain fatty acids (LCFA) (>16 carbons) are produced from the uptake of circulating lipids, while FA with 16 carbons originate from both sources (Liu et al., 2016). Associations between consumption of fats and the incidence of some chronic diseases such as coronary artery disease, diabetes, cancer and obesity has been shown (Lawrence, 2013).

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An estimated 17.5 million died of cardiovascular disease in 2012 accounting for 46% of all noncommunicable disease deaths (Mendis, 2017). However, other studies prove that consumption of milk may favourably modify the body composition and cardiometabolic risk factors due to milk’s conjugated linoleic acid (CLA) content (Lehnen et al., 2015). Therefore, the production of dairy products from water buffalo milk with enriched mono- and polyunsaturated FA content, especially CLA and omega-3, benefits for high nutritional properties of produced food.

In the last three decades, milk production in the world has incremented by more than 50%, from 500 million tons in 1983 to 811 million tons in 2017 (FAO, 2018). Globally, 16.9% of milk consumed by humans comes from livestock species other than cattle (Faye and Konuspayeva, 2012) and water buffalos (Bubalus bubalis) are the world’s second massive milk producer with 14% behind the dairy cow. There are two main groups of water buffalos: river buffalo and swamp buffalo in the world (Kumar et al., 2007). In Turkey, only one breed, the Anatolian water buffalo is present, and it is classified as Mediterranean river type (Soysal et al., 2010) which represents the best characteristics for milk production. Water buffalo milk products (WBMP), such as mozzarella cheese, curd, yogurt, cream and ice cream, are getting more and more popular due to WBMP’s higher concentrations of protein, fat, lactose, minerals and vitamins in buffalo milk (Han et al., 2012). Water buffalo milk and its derived products could be a good source of CLA for humans, like other food products from ruminants because bacteria present in the rumen may biohydrogenate dietary PUFA to form CLA. Despite its nutritional value, currently, information on chemical composition including CLA content in water buffalo milk is very limited in the world and kind of vague in Turkey. Therefore, the objective of this study was to investigate physicochemical composition and FA content of Anatolian water buffalo milk from six different provinces in Turkey.

**MATERIALS AND METHODS**

**Animals and milk sampling**

Water buffalo milks corresponded to individual milks taken from 57 buffalos of Mediterranean breed of Bubalus bubalis from six different provinces where most intensive water buffalo breeding present in Turkey (Table 1). Provinces were selected according to water buffalo population. All milk samples were collected in same season (April and May, 2018). All animals were grazed in the pasture and swamp area during this period. In Turkey, the distribution of plants in pastures; 15% leguminous, 10% grasses and 75% other plants (belonging to other families) have been reported (Avağ, 2018). In addition, all animals were allowed to consume at least 60% of the roughage during the sampling. Drinking water was always available in pasture. All the water buffalos were milked once a day during the milking period and milk samples were taken from this milking time. The farms were located in Afyonkarahisar (38° 45’ 24.787” N 30° 32’ 19.334” E), Balıkesir (39° 39’ 11.873” N 27° 53’ 25.231” E), Diyarbakır (37° 55’ 29.903” N 40° 12’ 39.539” E), İstanbul (41° 10’ 48.155” N 28° 44’ 17.426” E), Kayseri (38° 40’ 29.572” N 35° 18’ 28.177” E) and Samsun (41° 16’ 46.931” N 36° 20’ 9.841” E) provinces. Forty mL of fresh milk was then drawn and released into 50 mL falcon tube where they were preserved with antibiotic tablet (Bronolab Broad Spectrum Microtabs, 18 mg tablet: comprises 8 mg Bronopol and 0.30 mg Natamycin) and transferred via
insulated foam containers with cooling cassettes. Milk samples were stored 4°C until portions were analyzed the following day. Prior to analysis of FA content, water buffalo milk samples were stored at -20°C.

**Determination of physicochemical and fatty acid composition in the water buffalo milk**

All milk samples were analysed for, fat, protein, lactose and non-fat dry matter by Milkana Multi - Test (Mayasan Gida, İstanbul, Turkey). Milk fat extraction was prepared according to Gerber method (James, 1995). One-step extraction-transesterification process (Sukhija and Palmquist, 1988) was used to prepare fatty acid methyl esters (FAME). The FAME profile for a 0.6-μl sample at a split ratio of 1:50 was generated using a Schimadzu, GC 2010 plus gas chromatography equipped with a flame ionization detector (Schimadzu, Kyoto, Japan), a capillary column (60 m × 0.25 mm ID × 0.250 μm (cat. # 13199)) and H₂ as the carrier gas. The FAMEs were separated using a temperature gradient program (Injection: 2.0 μL split (split ratio 200: 1)), 4 mm inlet liners (cat # 20814), injection temperature: 225°C, carrier gas: hydrogen, flow rate: 1.2 ml / min, oven temperature: 100°C (4 minutes) to 240°C (10 minutes) 3°C / minute and the peaks were identified based on comparison of retention times with authentic standard (Supelco #37, Supelco Inc., Bellefonte, PA, USA; L8404 and O5632, Sigma Aldrich, St. Louis, MO, USA). Milk fat CLA level was identified based on comparison of retention times with CLA standard (cat # 16413 Sigma-Aldrich, St. Louis, MO, USA).

**Statistical analyses and calculations**

One-way ANOVA was done for assessment of the differences in the FA composition among provinces. The means were determined by using Duncan’s multiple range tests. The results of the statistical analysis were presented as mean values and standard error of the means (SEM). Level of significance was considered as P<0.05. ANOVA was performed in IBM SPSS Statistics for Windows, version 17 (IBM Corp., Armonk, N.Y., USA). In order to analyze the influence of the whole composition of the measured FAMEs on provinces a principal component analysis (PCA) was used. PCA is a multivariate statistical method that are widely used in situations when the effect of many possibly correlated predictor variables into a set of values of linearly uncorrelated variables. PCA analysis was performed in paleontological statistics software package (PAST) version 3.20 (Hammer, Harper and Ryan, 2001).

Atherogenicity indices were calculated as the content ratio of SFA / unsaturated FA using the following formula proposed by (Ulbricht and Southgate, 1991):

\[
\text{Atherogenicity index (AI)} = \frac{[C12:0 + 4(C14:0) + C16:0]}{\Sigma (MUFA + PUFA)}
\]

The Δ⁹ desaturase ratio were used as an indicator of the Δ⁹ desaturase activity using FA that are substrates and products for Δ⁹ desaturase and calculated using the following model proposed by (Lock and Garnsworthy, 2003):

\[
\text{Desaturase index (DI)} = \frac{C14:1}{C14:0}
\]

**RESULTS**

The physical characteristics such as fat, non-fat dry matter, lactose, protein and pH, are crucial parameters for the physicochemical properties and nutritional attitude of milk.
Different physical properties of the different milk samples from 6 provinces in Turkey belongs Anatolian water buffalo were shown in Table 1. The fat amount in water buffalo milk samples were in the range of 5.97±0.30% to 9.19±0.57% and the mean fat was 6.96±0.25%. In the present experiment, the lowest fat content was identified for the P5 province water buffalo milk while for P2 province showed the highest value (Table 1). Among the water buffalo milk samples, the lowest value was identified for non-fat dry matter in P2 province, followed by P5 province milk and the highest was demonstrated in P1 province milk samples (Table 1). For the protein, the lowest value was recorded for P2 province 3.12±0.06% and the highest was for the P3 province milk samples 3.77±0.07%. In general pH is the measure of sample acidity and alkalinity and small differences were detected for the pH measurement in all the milk samples. The pH values for the milk samples were in between 5.20 and 5.26. P3 milk samples showed the highest pH among all investigated milk samples (Table 1). Lactose which is known as milk sugar and is consisted of galactose and glucose. In all investigated milk samples, P3 province water buffalo milk contained the highest and P2 province milk samples lowest amount of lactose (Table 1).

Milk fat concentration, mean concentration of some individual FA and the sum of FA in different provinces are summarized in Table 2. Milk FA was quantified by gas chromatography fitted with a flame-ionization detector and a FA standard mix (contain 37 FA). Nineteen FA could not be detected with enough accuracy thus they were excluded in the statistical analysis. Those FA have been indicated as unknown followed by their retention time (in minutes) in the chromatographic run. In present study, the major FA (expressed as mean ± SEM in g / 100 g fat) were C16:0 (palmitic acid, 34.90±0.5), cis-9 C18:1 (oleic acid, 25.97±0.74), C18:0 (stearic acid, 14.33±0.48), and C14:0 (myristic acid, 11.13±0.27) accounting for approximately 86% of the total milk fat. C4:0 (butyric acid, 2.66±0.08), C12:0 (lauric acid, 2.09±0.07), C15:0 (pentadecanoic acid, 1.54±0.13) and C10:0 (capric acid, 1.52±0.07) were also relatively abundant in water buffalo milk samples which was approximately 7.5% of the total milk FA (Table 2). The main CLA, bovinic acid (cis-9, trans-11), represented 1.09±0.06 in water buffalo milk (Table 2).

Saturated fatty acids (SFA) were the potent fraction in water buffalo milk fat (70.63±0.7); MUFA and PUFA were 29.37±0.7 and 0.2±0.03, respectively. Considering the classification based of the carbon chain length: medium-chain fatty acids (MCFA) exhibited the greater part (51.77%) of the water buffalo milk fatty acid fraction, whereas long chain fatty acids (LCFA) and short chain fatty acids (SCFA) represented 41.81% and 6.21%, respectively. Among the unsaturation index and ratios, the atherogenicity, had the highest mean percentage 2.07±0.07. In the study, three ratios (DR14, DR16 and DR18) were calculated to estimate FA acid desaturase activity and get an indication of the syntheses of unsaturated FAs. Ratios given in the present study represent the product / substrate relationship for desaturase. Highest desaturase, desaturase ratio was found for DI14, DI16 and DI18; 0.08±0.00, 0.02±0.00 and 0.64±0.01 respectively.

The results are shown in Table 2 by applying one-way ANOVA which FA differences according to the provinces. However, potential coherences of different FAs are not considered in this analysis. Therefore, the principal component analysis (PCA) was used in order to determine principal components (PC) based on the whole FA
composition. The data consisted of 57 rows (milk samples) and 18 FA measurement including fat and protein. Loadings and PCA scores plot of the first and the third PC of the auto-scaled data showed in Figure 1. The data matrix explained approximately 91.3% of the total variance. The difference between the samples from six provinces is clear. P5 province lay mainly in the NE quadrant with positive scores on the first PC and positive scores on the third PC, whereas P3 occupied the SE quadrant with negative scores on the first PC and positive scores on the third PC. West Anatolian samples lay mainly in the NW quadrant with positive scores on the first PC and negative scores on the third PC (Figure 1).

DISCUSSION

The milk quality is greatly determined by its chemical composition such as protein, lactose and fat and FA contents due to its direct relation to human health, and the organoleptic characteristic. The level of physicochemical compositions in the measured milk samples were also compared with the previously reported literature from Anatolian water buffalo (Ermetin, 2017) and as well as from different countries (Khan, Islam and Siddiki, 2007; Imran et al., 2008; Kashwa, 2016). Similar milk fat percentages were described for Anatolian water buffalos (Şekerden, 1999), although sometimes lower values were reported (Tonhati et al., 2011), likely reflecting the effect of different management, feeding, and environmental conditions. The results show that values for the fat in all the milk samples are in good agreement with the reported literature. In the current milk samples, lactose was in the range of 4.38±0.27% to 5.44±0.59% (Table 1). Tufarelli, Dario and Laudadio (2008); Imran et al. (2008); Mihaylova and Peeva (2007) reported that the lactose in the milk was from 4.6% to 4.85% which is slightly lower than found in the current study. Not relevant differences seemed to be detected in total SFA and UFA.

This is the first comprehensive data on the FA content of Anatolian water buffalos. Although data about physical properties were reviewed in literature no data was available for the FA content of Anatolian water buffalo (Abed El-Salam and El-Shibiny, 2011). Main FA profile in the present study coincided with that reported for Mediterranean buffalos in (Pegolo et al., 2017) in which the major individual FA in water
buffalo milk were 16:0, 18:1 cis-9, 18:0, and 14:0. Similarly, SFA were the predominant fraction in Mediterranean water buffalo milk fat (70.49%); and monounsaturated, PUFA were at 25.95 and 3.54%, respectively (Varricchio et al., 2010; Pegolo et al., 2017). However, we identified lower mean PUFA for Anatolian water buffalos (0.20, Table 2) compared to Mediterranean buffalos, reared in Italy (Varricchio et al., 2010; Pegolo et al., 2017). The same FA pattern was also observed in Nili-Ravi buffalos in Pakistan, but they also identified PUFA as 4.91 (Qureshi et al., 2010) which is higher than that of which was identified in the current study. PUFAs play a crucial role in cells’ physiological and biochemical processes and decreasing the risk of many diseases via resolving their inherent inflammation condition (Zárate et al., 2017). Various studies showed that the FA profile of milk and its dairy products can be changed due to feeding system and stage of lactation in sheep (Cividini et al., 2018), dairy cattle (Odongo et al., 2007) as well as in Mediterranean water buffalo (Pegolo et al., 2017). Chilliard et al. (2000; 2001) showed that feeding animals with different types of forages and animal fat or marine oils had potential effects on polyunsaturated FA (Chilliard et al., 2000).

Palmitic acid (PA) (C16:0) was found as the major SFA in the current study (Table 2) and almost same mean values were detected in Mediterranean water buffalos (Bergamo et al., 2003; Ménard et al., 2010; Varricchio et al., 2010; Pegolo et al., 2017) and silage fed water buffalos in Bulgaria (Penchev et al., 2016) by various researchers. However, compared to Anatolian water buffalos in the present study, PA acid content in Murrah buffalos found lower (Fernandes et al., 2007; Shelke and Thakur, 2011). Although PA is known for putative detrimental health effects, it has multiple crucial physiological activities in order to maintain membrane phospholipids balance (Carta et al., 2017). However, more than palmitic acid’ uptake, balance of dietary PA / PUFA ratio is crucial to avoid unwanted physio-pathological conditions (Carta et al., 2017). The relative high content of lauric acid (C12:0) among detected MCFA in the present study which may display antibacterial activity against various Gram-positive strains (Batovska et al., 2009).

Concerning the unsaturated fatty acids (UFAs), substantial proportions of oleic acid (OA) (C18:1ω-9) and conjugated linoleic acid (CLA) isomer, C18:2 cis-9 trans-11 (rumenic acid) acids were identified in raw milk lipids. Evidences in the last years have showed the effects of OA in human health and disease in which OA may improve the immune response associated with elimination of pathogens such as bacteria and fungi (Sales-Campos et al., 2013). CLA is attracting interest because of its expressed effects on body composition specifically a reduction in body fat mass together with anticarcinogenic, antiatherogenic, antidiabetogenic, and immune modulating properties (Rainer and Heiss, 2004). The mean CLA in Anatolian water buffalos (Table 2) were higher compared water buffalos reared in Italy. Pegolo et al. (2017) found CLA cis-9, trans-11 isomer as 0.45 and Menard et al. (2010) found the same isomer as 0.90 more similar to findings of the current study. Murrah buffalos in Brazil showed higher CLA contents (1.242) as compared to the results obtained in the present study. The concentration of CLA in milk fat can be enhanced by changes in the livestock nutrition via ruminal biohydrogenation (Chilliard, Ferlay and Doreau, 2001; Daley et al., 2010). However, endogenously production of CLA from adipose tissue and the mammary gland in lactating dairy
Table 1. Least squares means and standard errors for the physicochemical parameters in water buffalo milk from different provinces.

<table>
<thead>
<tr>
<th>Traits</th>
<th>Provinces</th>
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<td>P5</td>
<td>P6</td>
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<td>11</td>
<td>10</td>
<td>11</td>
<td>8</td>
<td>10</td>
<td>57</td>
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<tr>
<td>Fat, %</td>
<td>8.14ab</td>
<td>9.19a</td>
<td>6.81bc</td>
<td>7.66abc</td>
<td>5.97c</td>
<td>6.83bc</td>
<td>6.96</td>
<td>0.256</td>
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<tr>
<td>Non-fat dry matter, %</td>
<td>9.58ab</td>
<td>8.12d</td>
<td>9.96a</td>
<td>9.32abc</td>
<td>8.87c</td>
<td>9.04bc</td>
<td>9.14</td>
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<td>Lactose, %</td>
<td>5.20ab</td>
<td>4.38c</td>
<td>5.44a</td>
<td>5.06ab</td>
<td>4.83b</td>
<td>4.92b</td>
<td>4.97</td>
<td>0.066</td>
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<td>Protein, %</td>
<td>3.65ab</td>
<td>3.12d</td>
<td>3.77a</td>
<td>3.55abc</td>
<td>3.37cd</td>
<td>3.44bc</td>
<td>3.48</td>
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<tr>
<td>pH</td>
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<td>5.21b</td>
<td>5.26a</td>
<td>5.20c</td>
<td>5.21bc</td>
<td>5.21b</td>
<td>5.21</td>
<td>0.003</td>
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</table>

abcd Means in the same row for each type of capsule without common letter differ significantly (P≤0.05); n = number of samples. SEM: standard error of means; NS P>0.05; *P≤0.05; **P≤0.01; *** P≤0.001.

P1: Afyonkarahisar; P2: Balıkesir; P3: Diyarbakır; P4: İstanbul; P5: Kayseri; P6: Samsun.

Figure 1. Principal component analysis (scores and loading plot) of Anatolian water buffalo milk FA profile. Loading plot describing the relationship among milk FA derived from a principal component analysis based on proportions (% of total FA) in milk from six provinces (n = 57). Three clusters of FA were distinguished. Conjugated linoleic acid (CLA) and milk protein percentage were loaded opposite (negatively related) to milk fat percentage whereas saturated palmitic and unsaturated oleic acid were loaded with milk fat percentage.
Table 2. Least squares means and standard errors for FA content (g/100 g fat) in water buffalo milk from different provinces.

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Provinces</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>P5</th>
<th>P6</th>
<th>Mean</th>
<th>SEM</th>
<th>P-value</th>
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<td>C4:0</td>
<td></td>
<td>2.67&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.71&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.03&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>2.66</td>
<td>0.08</td>
<td>*</td>
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<tr>
<td>C6:0</td>
<td></td>
<td>0.59&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.67&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>1.25</td>
<td>0.14</td>
<td>*</td>
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<td>0.51&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.88&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.06&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>1.52</td>
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<td>12.02&lt;sup&gt;b&lt;/sup&gt;</td>
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SCFA: short-chain fatty acids; MCFA: medium-chain fatty acids; LCFA: long-chain fatty acids; CLA: conjugated linoleic acid; SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids, PUFA: Polysaturated fatty acids; <sup>abcd</sup> Means in the same raw for each type of capsule without common letter differ significantly (P≤0.05). SEM: standard error of means; AI: atherogenicity index; DI: desaturase index; NS P>0.05; *P≤0.05; **P≤0.01; *** P≤0.001. P1: Afyonkarahisar; P2: Balıkesir; P3: Diyarbakır; P4: İstanbul; P5: Kayseri; P6: Samsun.
cows by Δ⁹-desaturase has been also demonstrated (Griinari et al., 2000). Therefore, to assess the nutritional quality of lipids desaturase and atherogenicity indexes (AI) are calculated. The AI showing the inhibition of the aggregation of plaque and diminishing the levels of esterified FA, cholesterol, and phospholipids, thereby preventing the appearance of micro- and macro-coronary diseases (Ulbricht and Southgate, 1991). Among unsaturation indexes, Varricchio et al. (2010) stated that Mediterranean buffalos reared in Italy exhibits a range between 2.15 and 2.61% which is lower than the samples measured in the current experiment. Higher AI value implies a lower PUFA / SFA ratio and this is in accordance with the present findings that Anatolian water buffalos showed lower PUFA compared to Mediterranean buffalos (Varricchio et al., 2010; Pegolo et al., 2017).

CONCLUSIONS

Taken together this study presents a detailed analysis of FA profile of Anatolian buffalo milk, including FA present in small concentrations those may have a positive effect on human health. We confirmed Anatolian water buffalo milk samples showed variation in the FA profile due to the specific FA origin and metabolic pathway most probably related with their environmental conditions. Interestingly, East Anatolian water buffalos exhibit higher CLA content compared to West Anatolian ones and milk fat vice versa. Generally, Anatolian water buffalos were poor in terms polyunsaturated FA compared to other water buffalos from different countries. Therefore, these results may provide useful information about the nutrient composition of buffalo milk and further studies are warranted to improve the technological and nutritional characteristics of Anatolian buffalo milk.

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