

## CHANGES IN SOME PHYSICO-CHEMICAL CONTENT OF ANATOLIAN BUFFALO MILK ACCORDING TO THE SOME ENVIRONMENTAL FACTORS

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### ABSTRACT

The aim of this study was to determine the some physico-chemical content of raw milk from Anatolian water buffaloes raised under different village conditions in the Tokat province of Northern Turkey. The study materials included 1272 milk samples from 149 water buffaloes raised at 12 separate villages of the Erbaa, Turhal, and Pazar counties in the Tokat Province. Milk samples were collected during the morning milking between the months of February and May 2012 to 2014. The density, freezing point degree, dry matter, nonfat dry matter (or solid non fat), fat, protein, lactose, milk urea nitrogen and casein content of the milk samples were determined. The study results demonstrated that the mean dry matter, nonfat dry matter, fat, protein, lactose, casein content, density, milk urea nitrogen (MUN) and freezing point degree (FPD) of the raw milk samples were 16.99±0.108%, 10.88±0.036%, 5.98±0.107%, 4.85±0.043%, 5.17±0.021%, 3.61±0.036%, 1029±0.056, 21, 22 mg/dl and 0.55°C, respectively. The study data were evaluated according to the water buffaloes' lactation stage, parity, and season by using the SPSS statistical program. It was concluded that the sampling time, parity, village conditions, stage of lactation and calving age had a

significant effect ( $P<0.05$ ) on the density, freezing point degree, dry matter, nonfat dry matter, fat, protein, lactose, and casein content of raw milk from the Anatolian water buffalo.

**Keywords:** physical parameter, chemical parameter, anatolian buffalo, raw milk, lactation number, calving age

### INTRODUCTION

Milk represents an important article in the human diet (Sharif, 2009). Water buffalos are the second most common source of milk source in many countries, and the raising of water buffaloes accounts for nearly 12% of the total worldwide milk production. In the production of dairy products, the quality (and hence the composition) of milk is as important as the quantity produced. Milk composition depends not only on the genotype of the buffalo, but is also affected by various factors such as lactation stage, parity, calving age, and season. The fat, lactose, protein, and dry matter content of water buffalo milk are higher than that of cow milk. Ahmad *et al.* (2008) reported mean fat, protein, and dry matter content values of 7.0%, 4.35% and 17.45%, respectively, for water

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buffalo milk, while Ariota *et al.* (2007), reported protein and fat content values of 8.71% and 3.86%, respectively. Previous studies determined that the feeding regime (Waldner *et al.*, 2002), lactation period (Sethie *et al.*, 1994); Sekerden *et al.*, 1999a), and season (Sekerden *et al.*, 1999b) also affected the fat, protein, and dry matter content of water buffalo milk during dairy production. Furthermore, Foltys *et al.* (1995) reported that the protein and fat content of water buffalo milk was lower in summer in comparison to the winter months.

In addition to the production of milk, water buffaloes are also commonly used as draft animals in rural areas of developing countries. Water buffalo milk and meat products constitute an important source of protein for low-income farmers, and also serve as a significant source of income for rural economies (Borghese, 2005; Yilmaz *et al.*, 2011). The water buffalo population, as well as water buffalo milk production, has gradually decreased in Turkey over the past two decades (Sahin *et al.*, 2011). The number of Anatolian water buffaloes in Turkey was 366,150 in 1991, and 117,591 in 2013 (Anonymous, 2014). There are two general types of water buffalo, which are the swamp water buffalo and the river water buffalo. The river water buffalo is the type that is more suitable for milk production. Water buffaloes in Turkey are known as Anatolian water buffaloes, which are considered as part of the Mediterranean water buffalo breed; the Mediterranean water buffalo, on the other hand, represents a subgroup of the river water buffalo (Soysal *et al.*, 2005).

Anatolian water buffaloes are raised in most rural areas of Turkey, especially in the Northern, Central, Western, Eastern and Southeastern regions of the country Atasever (Erdem, 2008). Anatolian water buffaloes are mainly raised for their milk, and also slaughtered for their meat after their

productive age passes (Sekerden, 2001). Due to their resistance to diseases and relatively lower feed consumption, the Anatolian water buffalo represents a preferred breed in the different regions of Turkey. However, when considering milk quality, dairy operations in Turkey generally take into consideration the genetic background of the water buffaloes, while overlooking the importance and effects of environmental factors in milk production.

In this context, the aim of this study was to determine the effects of village conditions, parity, calving age, sampling time and lactation stage on the milk some physico-chemical composition of Anatolian water buffaloes.

## MATERIALS AND METHODS

This study was conducted in the Tokat province within the Middle Black Sea Region of Turkey.

### Sample collection

A total of 149 Anatolian water buffaloes raised at 12 different villages of the Turhal, Pazar and Erbaa counties in the Tokat Province were evaluated between February and May 2012 to 2014. Sample collection was performed between February and May 2012 to 2014, and 1272 samples were collected. Lactating water buffaloes were allocated to one of the following three lactation stage groups: the day 30±15, 60±15 and 90±15 group, which was considered as the early (assigned a value of 1); the day 120±15, 150±15 and 180±15 group, which was considered as the middle group (assigned a value of 2) and the 210±15, 240±15 and 270±15 day group, which was considered as the late group (assigned a value of 3).

The water buffaloes were also divided into groups depending on their number of parity. As such, water buffaloes with the same number of parity were allocated to the same group, while all water buffaloes with more than seven parities were included into the group with seven parity. Buffaloes are typically milked once in the morning before being moved to pasture. Therefore raw milk samples (approximately 50 ml) were collected from each udder quarter under aseptic conditions during the morning milking. After milking the raw water buffalo milk into plastic containers composed of 2-bromo-2-nitropropane-1,3-diol (Bronopol), milk samples of 50 mL were prepared by transferring the collected raw milk into clean, aseptic milk bottles. The milk samples were then stored cold inside the sterile bottles.

#### Analysis of milk composition

The dry matter (%w/w), nonfat dry matter (%w/w), fat (%v/v), protein(%w/w), lactose (%w/w), and casein (%w/w) contents, milk urea nitrogen (mg/dl) and density ( $\text{g/cm}^3$ ) and freezing point degree ( $^{\circ}\text{C}$ ) of water buffalo milk samples were determined by using a FOSS Milko Scan<sup>TM</sup> 120 (calibrated with appropriate buffalo standard, Foss electric, Denmark) milk analyzer.

#### Statistical analysis

In this study, lactation stage, parity, village condition and season were evaluated as fixed factors. To determine the environmental effects on milk production, the general linear model (GLM) procedure was used SPSS program (SPSS. IBM Corp Ver. 20.0). Data were analyzed by using a least square analysis of variance in order to identify significant fixed effects.

The utilized model was as follows:

$$Y_{ijklmn} = \mu + a_i + b_j + c_k + d_l + f_m + e_{ijklmn}$$

Where:

- $Y_{ijklmn}$ : Observation value for milk components
- $\mu$ : Population mean
- $a_i$ : The effect of village conditions (i: 1,2,...12)
- $b_j$ : The effect of the parity (j: 1, 2, .....7)
- $c_k$ : The effect of calving age (k =3, 4,5, .....9)
- $d_l$ : The effect of sampling time (l: February, March, April)
- $f_m$ : The effect of the lactation stage (m = 1: early; 2: mid; 3: late)
- $e_{ijklmn}$ : The random residual effect

## RESULTS AND DISCUSSION

#### Chemical composition of the milk samples

According to the study results, the mean dry matter, nonfat dry matter, fat, protein, lactose, casein, density and freezing point degree (FPD) content of the raw milk samples from the Anatolian water buffalo were  $16.99 \pm 0.108\%$ ,  $10.88 \pm 0.036\%$ ,  $5.98 \pm 0.107\%$ ,  $4.85 \pm 0.043\%$ ,  $5.17 \pm 0.021\%$ ,  $3.61 \pm 0.036\%$ ,  $1029 \pm 0.056$  and  $0.55^{\circ}\text{C}$ , respectively. Similar results were obtained by Macedo *et al.* (2001) at São Paulo State (Brazil). A descriptive analysis of the variables evaluated within the content of the current study is provided in Table 1.

The results obtained from the preliminary analysis of the mean dry matter, density, freezing point degree, non fat dry matter, protein, fat, milk urea nitrogen and lactose content values for fixed factors are shown in Figure 1, Figure 2, Figure 3, Figure 4 and Figure 5. Based on these analyses, it was determined that sampling time, parity, village conditions, lactation stage and calving age had a significant effect on the dry matter, density, freezing point degree, nonfat dry matter, fat, protein, lactose, and casein content of raw milk from the Anatolian

water buffalo ( $P < 0.05$ ).

In the current study, the lactose content of the milk samples ( $5.17 \pm 0.021\%$ ) was found to be higher than some of the values reported in previous studies (Lopes, 2009; Lingathurai *et al.*, 2009; Han *et al.*, 2012; Gürler *et al.*, 2013). There were also earlier studies that determined lactose content values similar to the ones in the current study (Macedo *et al.*, 2001; Mahmood, 2010; Damé *et al.*, 2010). On the other hand, fat content was identified as the characteristic that demonstrated the highest variability in our sample, with many different factors appearing to affect the fat content of Anatolian water buffalo milk.

The mean dry matter ( $16.99 \pm 0.108\%$  w/w), fat ( $5.98 \pm 0.107\%$  v/v), protein ( $4.85 \pm 0.043\%$  w/w), and lactose ( $5.17 \pm 0.021\%$  w/w) content of this milk samples were higher than the content values reported by Enb *et al.* (2009), and lower than the content values reported by Sekerden, (2008). Furthermore, the fat and dry matter content of this samples were lower than the content values reported by Han *et al.* (2007), while fat content this samples was lower than the values reported by Gürler *et al.* (2013). The mean dry matter content of

this samples was lower than the results reported by certain authors Kök (1996), Macedo *et al.* (2001), Coelho *et al.* (2004), Mahmood, (2010), yet higher than the results reported by Damé *et al.* (2010) and Gürler *et al.* (2013). Moreover, the protein content values of this samples were similar to the content values reported in previous studies on intensive water buffalo farming (Rosati, 2002; Zicarelli, 2004; Cecchinato *et al.*, 2012), and also similar to the statistics published by the National Water Buffalo Breeders Association ANASB (2010).

This research samples' protein content was also higher than the content values identified by certain researchers Lingathurai *et al.* (2009); Damé *et al.* (2010) and Gürler *et al.* (2013). The fat content values identified in the milk samples from this study were lower than the values reported in previous studies (Kök, 1996; Macedo *et al.*, 2001; Rosati, 2002; Zicarelli, 2004; Coelho *et al.*, 2004; Lingathurai *et al.*, 2009; Lopes, 2009; Mahmood, 2010; Cecchinato *et al.*, 2012). An exception to this was Tiezzi *et al.* (2009) study, which identified fat content values similar to our own in two herds in Northeastern Italy. Yet, the fat content value reported by Tiezzi *et al.* (2009) was higher than the

Table 1. Descriptive analysis of the variables studied.

| Parameters                   | N    | Mean  | Se     | Min   | Max    |
|------------------------------|------|-------|--------|-------|--------|
| Milk urea nitrogen (mg/dL)   | 1272 | 21.22 | 0.0001 | 16.80 | 26.60  |
| Non fat dry matter (%w/w)    | 1272 | 10.88 | 0.036  | 8.868 | 19.446 |
| Fat (%v/v)                   | 1272 | 5.98  | 0.107  | 1.01  | 16.829 |
| Protein (%w/w)               | 1272 | 4.85  | 0.043  | 2.146 | 15.643 |
| Lactose (%w/w)               | 1272 | 5.17  | 0.021  | 2.105 | 6.25   |
| Casein (%w/w)                | 1272 | 3.61  | 0.036  | 0.83  | 10.936 |
| Density (g/cm <sup>3</sup> ) | 1272 | 1029  | 0.056  | 1028  | 1033   |
| Freezing Point Degree (°C)   | 1272 | 0.55  | 0.005  | 0.48  | 0.65   |

Se: standart error

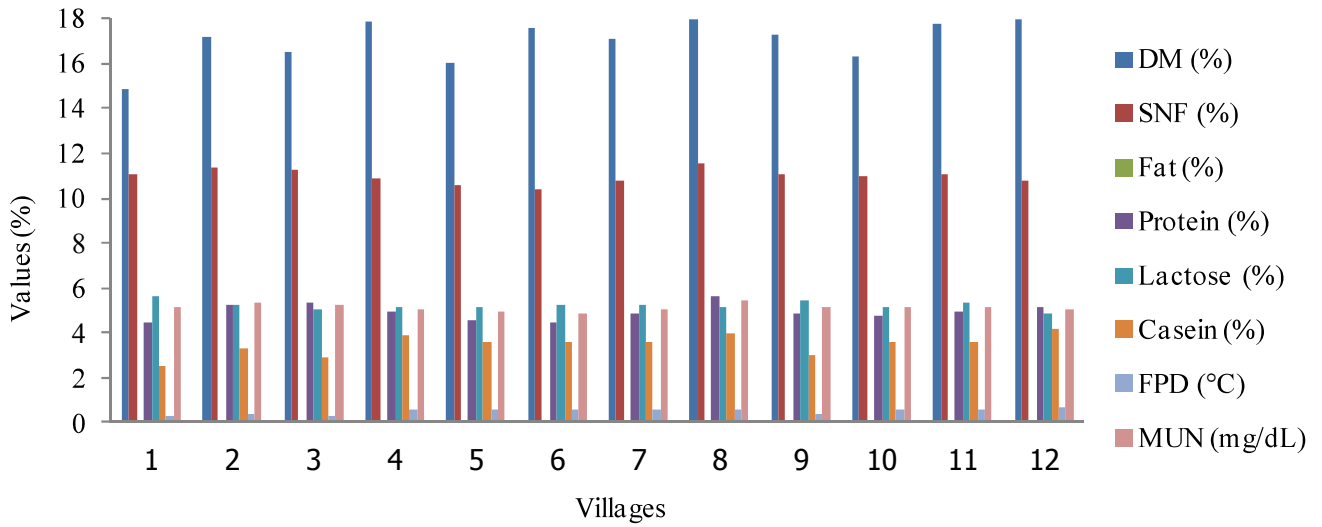


Figure 1. Some physico-chemical composition of buffalo milk according to villages.

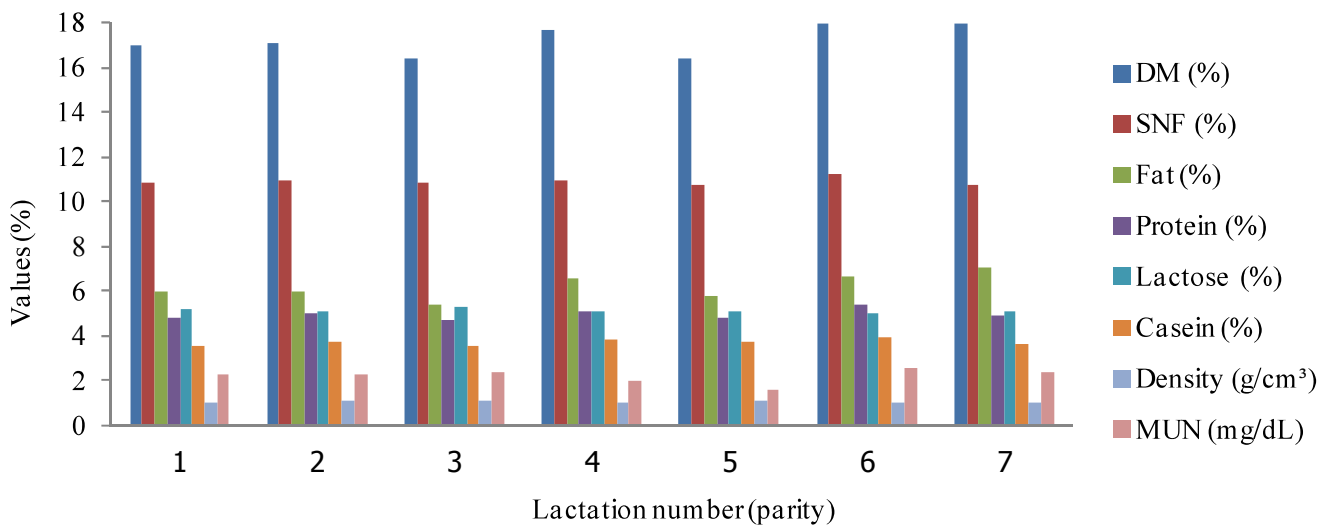


Figure 2. Some physico-chemical composition of buffalo milk according to parity.

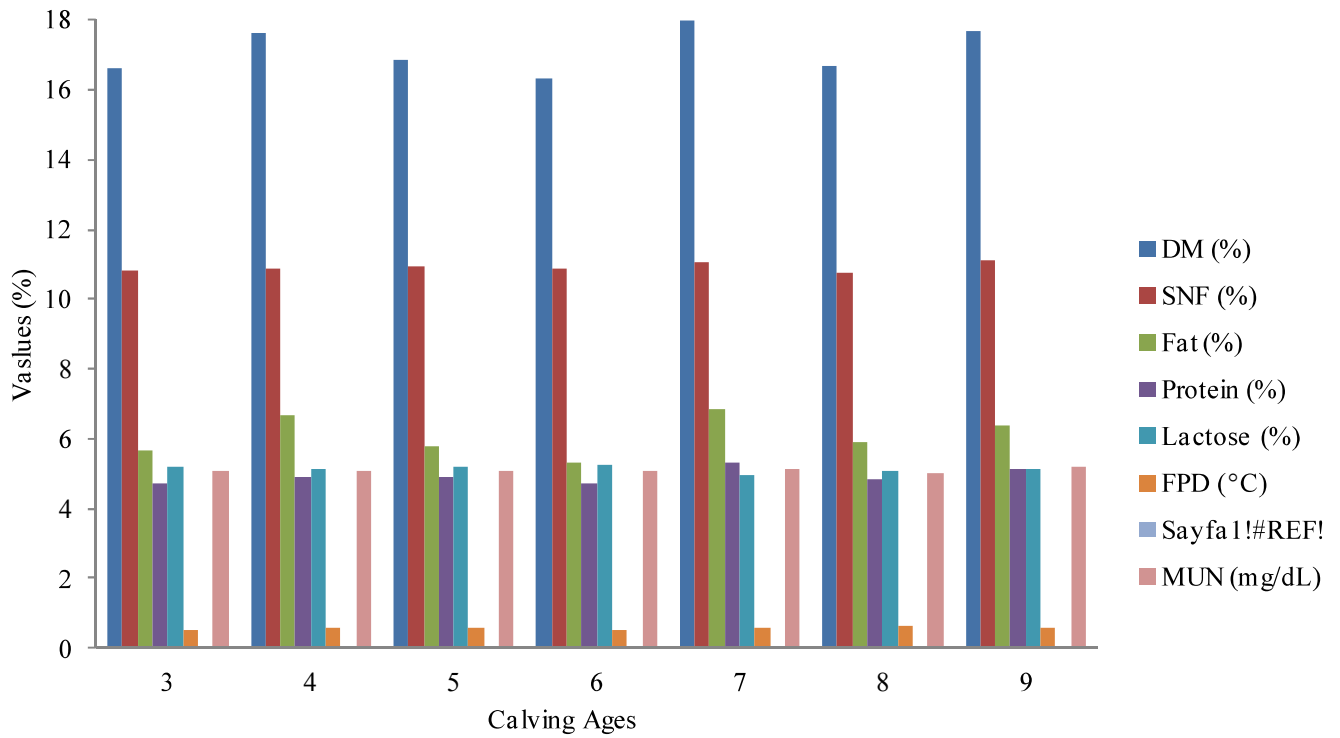


Figure 3. Some physico-chemical composition of buffalo milk according to calving ages.

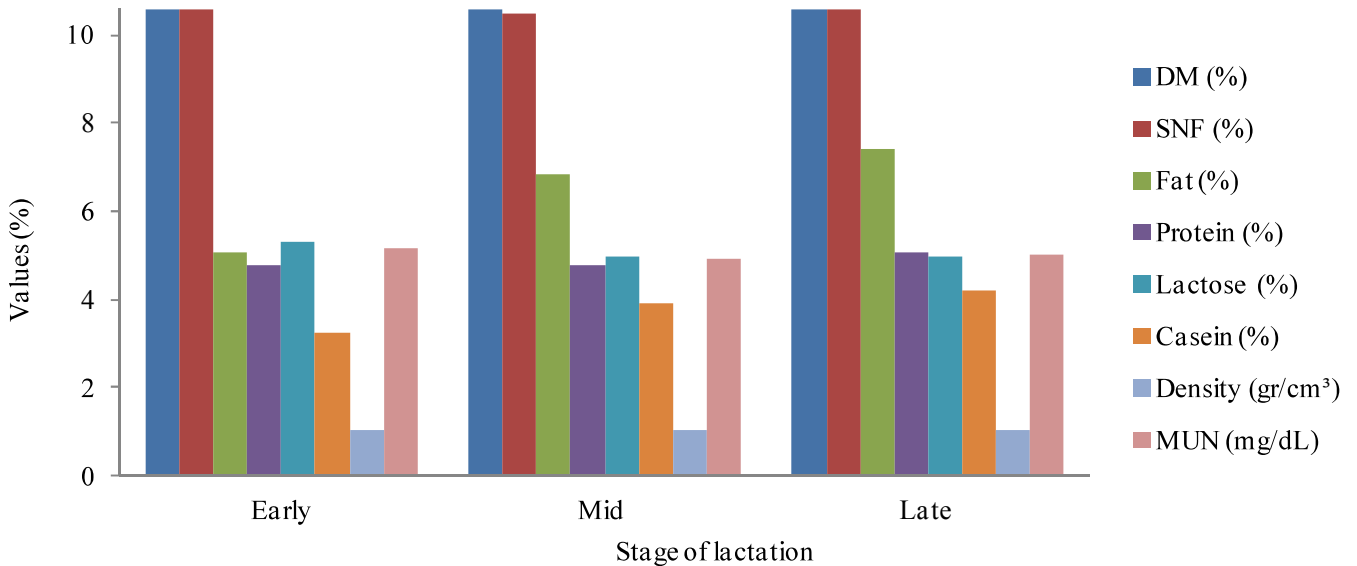


Figure 4. Some physico-chemical composition of buffalo milk according to stage of lactation.

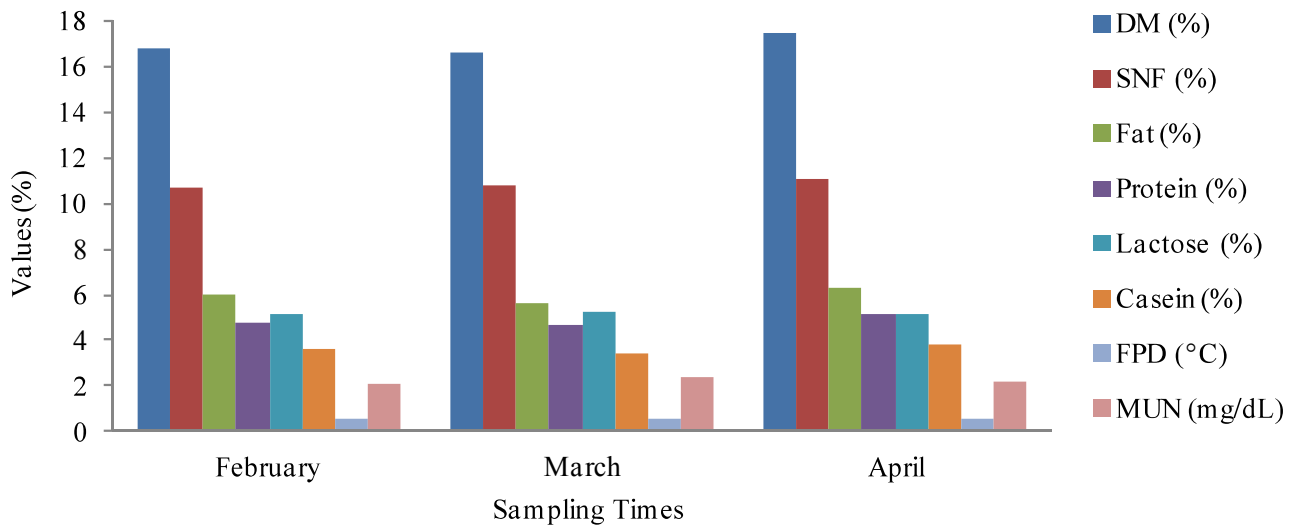


Figure 5. Some physico-chemical composition of buffalo milk according to sampling times.

value reported by Damé *et al.* (2010) for Murrah and Mediterranean water buffaloes. The casein content of this samples was very similar to the content values (3.86%w/w) reported by Ariota *et al.* (2007) and Cecchinato *et al.* (2012).

The density of all the raw milk samples were found to be  $1029 \pm 0.056$  g/cm<sup>3</sup>. Small variations were found for this parameters in all the milk samples. The density is mainly due to the water content present in the sample. Furthermore, it is a measure that provides information about the purity of the raw milk. The current research produced results that support the findings of a great deal of the previous work in this field.

The density of buffalo milk were reported to be 1033 g/cm<sup>3</sup> by Mahmood (2010). Kanwal *et al.* (2004) stated that buffalo milk density was 1020 g/cm<sup>3</sup>. Furthermore, Ahmad *et al.* (2005) reported that buffalo milk density was 1032 gr/cm<sup>3</sup>. The density value was lower than the findings of some previous research results (Padghan *et al.*, 2008; Braun and Preuss, 2008). Buffalo milk densities were 1034, 1032, 1032, and 1033 gr/cm<sup>3</sup>, for winter, spring, summer, and autumn seasons, respectively

(Aurelia *et al.*, 2009). Turkish Food Regulations report that the density of raw buffalo milk is 1028 g/cm<sup>3</sup> (Anonymous, 2000). The present results are similar to this standard. This value ( $1029 \pm 0.056$  g/cm<sup>3</sup>) is lower than findings of Franciscis *et al.* (1988) and similar to the results of Zaman *et al.* (2007) and Sahin *et al.* (2014). The results of the present research are consistent with those of Khan *et al.* (2007), who found that the density was 1032 g/cm<sup>3</sup> for swamp buffaloes, and 1032 g/cm<sup>3</sup> for water buffaloes.

The freezing point of raw milk is an important feature to determine the amount of water added (Aydın *et al.*, 2010). In this experiment, the average freezing point was determined as 0.55°C in milk samples. Similarly Rosenman, (2010) and Sahin *et al.* (2014) reported that the buffalo milk freezing point was 0.52°C, 0.56°C, respectively. The freezing point of buffalo milk in Germany ranged from -0.55°C to -0.51°C (Braun, 2008). Filik *et al.* (2011) and Ayaşan *et al.* (2012) reported that the freezing point of Holstein cattle milk is 0.51°C and 0.52°C.

In this study, the value of milk urea nitrogen

(MUN) was  $21.22 \pm 0.0001$  mg/dl. El Shewy *et al.* (2010) reported that MUN was 19.60 and 28.03 mg/dl for buffaloes in the winter and summer seasons, respectively. Furthermore, Roy *et al.* (2005) and Sharma *et al.* (2009) reported milk urea nitrogen concentrations between 40.10 to 49.15 mg/100 ml and 38.94 mg/100 ml in buffaloes, which were much higher than the present investigation. The protein/energy ratio of animal feed had an effect on milk urea concentration (Baker *et al.*, 1995; Ayasan, 2009).

Milk urea levels may change depending on a number of factors. Milk composition, breed, season, time of feeding, somatic cell count, feeding regime, feeding method, and water and dry matter consumption are among the most important of these factors (Nourozi *et al.*, 2010; Roy *et al.*, 2011).

These findings further support the results of the study of Roy *et al.* (2005) who reported that feeding regimes had a significant effect on raw milk urea concentration. Also, same researchers revealed that this effect might be due to the difference in the quality and type of protein between the diets and feeding strategy of the research. The composition of milk free fatty acids is dependent on various factors, such as stage of lactation, genetic variation, breed, calving age, animal health, and feed composition (Garnsworthy *et al.*, 2006; Qureshi *et al.*, 2010).

### **Environmental effects**

The ratio of milk components (fat, protein, lactose, and total solids) can vary according to nutrition, the season of the year, and other factors such as the age, breed, and lactation stage of the animal (Amaral *et al.*, 2005; Damé *et al.*, 2010). In this study, the effect of lactation stage on water buffalo milk fat content was identified as significant ( $P > 0.05$ ). Sekerden, Avsar (2008) had similarly reported that lactation stage significantly

affected the fat content of water buffalo milk ( $P < 0.05$ ), while the effect of the village conditions on the protein content was not significant ( $P > 0.05$ ). Patel *et al.* (1991) and Sethi *et al.* (1994) had similarly determined that the mean fat content of water buffalo milk was significantly affected by the lactation stage.

In this context, a considerable variation was observed in the fat, dry matter, and casein content of this milk samples depending on the lactation stage of the animal. This observation was in parallel with the findings from the studies of Patel *et al.* (1991); Darshan *et al.* (1991) and Sethi *et al.* (1994).

Previous studies have also determined that nutrition (Waldner *et al.*, 2002) and lactation stage (Sethi *et al.*, 1994; Sekerden *et al.*, 1999a) can considerably affect the fat, protein, and dry matter content of water buffalo milk.

The effect of sampling time on the fat content of this milk samples was identified as significant. On the other hand, another study conducted with Anatolian water buffaloes demonstrated that the effect of sampling time on fat content was not significant ( $P > 0.05$ ) (Sekerden, 2008). The effect of sampling time of the protein content of this milk samples was identified as significant. In a similar study conducted in Turkey, Sekerden, (2008) also reported that the effect of sampling time on the protein content of water buffalo milk was significant ( $P < 0.05$ ). In the current study, we observed that the lactation stage had a significant effect on both the dry matter and nonfat dry matter of the milk samples ( $P > 0.05$ ).

This observation was in parallel with the results of previous studies (Sekerden *et al.*, 1999; Sekerden, 2008). Furthermore, Sethi *et al.* (1994) also described that the mean dry matter and nonfat dry matter content of water buffalo

milk were both significantly affected by the stage of lactation. The protein and fat content of this samples were significantly affected by the lactation stage. Sekerden (2008) have also determined that the lactation stage had a significant effect on the protein and fat content ( $P < 0.05$ ).

Lactation stage had a significant effect on the casein content of this samples ( $P < 0.05$ ). Similar results pertaining to the lactation stage were obtained in the study of Sekerden *et al.* (1999a), which also determined that the lactation stage had a significant effect on the dry matter and fat content. This observation was consistent with the result of this study, and also the results of the study of Sethi *et al.* (1994). Darshan *et al.* (1991) have reported that the effect of lactation stage on the dry matter content was insignificant ( $P > 0.05$ ). According to previous studies, the fat content of water buffalo milk was affected by both the number of lactation (Patel *et al.*, 1991; Sethi *et al.*, 1994; Sekerden *et al.*, 1999a), and the lactation stage (Sekerden *et al.*, 1999a).

The analyses indicated that the effects of parity, calving ages, villages, stage of lactation, and sampling time of all traits were statistically significant on density and freezing point degree ( $P < 0.05$ ). The results of the present investigation are in agreement with the findings of some researchers (Sahin *et al.*, 2014). However, Zaman *et al.* (2007) reported that the stage of lactation and parity of buffalo milk density was insignificant. The some physicochemical compositions of Tokat Anatolian buffalo raw milk determined in this study were in agreement with other research results. It was determined that the density, and freezing point of Anatolian buffalo milk were affected by different environmental factors. Additionally, the quality and chemical compositions of the milk are of great importance to the dairy sector and human

health because milk composition is related to milk products.

The results indicated that the effects of parity, calving ages, villages, stage of lactation and sampling time were statistically significant ( $P < 0.05$ ). Similarly Sahin *et al.* (2014) was reported that lactation stage, parity, calving age, villages and sampling times on milk urea nitrogen were significantly important ( $P < 0.05$ ).

The chemical composition of water buffalo milk is rich in nutrients; it thus offers considerable opportunities for the expansion of local dairy production, and also for meeting the increasing demand for milk. The composition that was indentified within the context of this study for raw milk from the Anatolian water buffalo was in agreement with the results of previous studies. The chemical composition of these milk samples also met the requirements of the Turkish Food Codex. In the current study, it was also determined that the dry matter, nonfat dry matter, fat, protein, lactose, and casein content of raw milk from the Anatolian water buffalo were affected by environmental factors.

The quality and composition of milk are of great importance for the dairy industry, since the composition of milk is also directly associated with the milk yield. The most significant finding of this study was the observation that different village conditions resulted in different dry matter, nonfat dry matter, fat, protein, lactose, and casein content values. Additional studies need to be conducted in Turkey to further elucidate the effects of environmental factors on the composition water buffalo raw milk.

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