Etlik Vet Mikrobiyol Derg, 2025; **36 (1):** 51-57
doi: https://doi.org/10.35864/evmd.1592580

Original Article
Özgün Araştırma

Blood mineral profile of buffaloes raised in Kızılırmak delta: Ca, P, Mg, Cu, Zn, Fe, Mn analyses

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Geliş Tarihi / Received: 28.11.2024, **Kabul Tarihi** / Accepted: 12.03.2025

Abstract: This study was carried out to determine Ca, P, Mg, Cu, Zn, Fe, Mn, Fe, Mn levels in blood serum samples of buffaloes in Kızılırmak Delta. Atomic Absorption Spectroscopy (AAS) device was used for mineral analysis. The results showed that Ca, Mg and Fe levels were adequate, while P and Cu levels were below the reference range. It was determined that Zn and Mn levels were generally adequate and only 25% of the levels were below the reference value. These findings provide important information in terms of ensuring the mineral balance of buffaloes and developing nutritional strategies and are thought to contribute to the sustainability of buffalo farming in the region.

Keywords: Blood serum, Buffalo, Kızılırmak Delta, Mineral, Samsun

Kızılırmak deltasında yetiştirilen mandaların kan mineral profili: Ca, P, Mg, Cu, Zn, Fe, Mn analizleri

Özet: Bu çalışma, Kızılırmak Deltası'ndaki mandaların kan serum örneklerinde Ca, P, Mg, Cu, Zn, Fe, Mn düzeylerinin belirlenmesi amacıyla yürütülmüştür. Mineral analizi için Atomik Absorpsiyon Spektroskopisi (AAS) cihazı kullanılmıştır. Elde edilen sonuçlar, Ca, Mg ve Fe seviyelerinin yeterli düzeyde olduğunu, P ve Cu seviyelerinin ise referans aralığının altında kaldığını göstermiştir. Zn ve Mn seviyelerinin ise genel olarak yeterli bulunduğunu, yalnızca %25 lik dilimde referans değerin altında kaldığı tespit edilmiştir. Bu bulgular, mandaların mineral dengelerinin sağlanması ve beslenme stratejilerinin geliştirilmesi açısından önemli bilgiler sunmakta ve bölgedeki manda yetiştiriciliğinin sürdürülebilirliğine katkı sağlayacağı düşünülmektedir.

Anahtar kelimeler: Kan serumu, Kızılırmak Deltası, Manda, Mineral, Samsun

Introduction

The Kızılırmak Delta, a UNESCO World Heritage Site, is a nutrient-rich region thanks to its natural features and preserved climatic conditions. For this reason, the milk obtained from buffaloes raised in the region has a high fat and dry matter content, and the cream obtained is also characterised by its high fat content. Bafra Kaymaklı delight was registered as a geographically marked product in 2020 (Değirmencioğlu 2020; Kaya 2023; Turkish Patent and Trademark Office 2023). According to the data of the Turkish Statistical Institute on the number of water buffaloes, Samsun ranks first in Turkey with 23,190 water buffaloes (TÜİK 2023; Kılıç et al. 2023; Yıldırım 2024). More than half of the number of water buffalo in Samsun is located in the Kızılırmak Delta (Bayram and Atasever 2020). Buffalo milk has an important place in the local ice cream, milk-yoghurt, dairy desserts and fresh pastry production sector (Hekimoğlu 2005). Buffaloes utilise rough and poor quality feeds very well, develop at lower costs and also they are compatible with natural life and resistant to diseases (Hasan et al. 2017; Değirmencioğlu 2020; Kılıç et al. 2023).

Although buffalo meat contains less fat and cholesterol than beef, it contains more protein and mineral substances (Rey and Povea 2012). Buffalo milk is very rich in nutrients, contains high levels of fat and protein, has twice the energy content and lower cholesterol levels than cow's milk (Younas et al. 2013; Değirmencioğlu 2020). Buffalo milk is a natural source of vitamins A, D, C and B6 and minerals. It is very rich in Ca, Mg, P, K, K, Zn and Fe and contains more conjugated linoleic acid (CLA) compared to cow's milk (Abd El-Salam and El-Shibiny 2011). Human and animal organisms are mainly composed of organic and inorganic substances. For the continuation of life, growth, reproduction, etc. functions in living organisms, inorganic substances must be taken in addition to organic structures. Minerals are divided into macro and micro elements

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according to their amounts in tissues (Mert et al. 1999; Kalaycıoğlu et al. 2000).

For living organisms, balanced nutrition is of great importance for the regular maintenance of vital activities. Macrominerals such as calcium (Ca), phosphorus (P), magnesium (Mg), potassium, sodium, chlorine and sulphur and microminerals such as iron (Fe), cobalt, copper (Cu), iodine, manganese (Mn), zinc (Zn) and selenium are required for healthy development and metabolic activities of living organisms (Devlin 1997; Kaneko 1997; Kalaycıoğlu et al. 2000). While these minerals cause serious health problems in case of deficiency, they may have toxic effects when taken in excess. Therefore, it is of great importance to recognise essential minerals and to know their amounts (Mert et al. 1999).

Calcium (Ca) performs many vital functions such as cell division, glycogen metabolism, hormone secretion, muscle contraction, bone mineralisation and coagulation mechanism (Liesegang et al. 2007). Phosphorus (P) plays an important role in energy transfer, bone structure, cell membranes and nucleic acids. It is also essential for rumen microbiota and plays a critical role in maintaining rumen pH and acid-base balance (Schonewille and Beynen 2005). Magnesium (Mg) is the fourth most abundant cation in the body and acts as a cofactor for more than 300 enzymes. Mg deficiency may cause problems such as hypomagnesaemic tetany in calves and lactation tetany in lactating animals (Mert et al. 1999; Kalaycıoğlu et al. 2000). Copper (Cu) is essential for cell respiration, immune system, bone formation, heart function, connective tissue, keratinisation and tissue pigmentation. Deficiency can lead to problems such as anaemia, weakening of the immune system and delayed growth (Mert et al. 1999; Wysocka et al. 2018). Zinc (Zn) deeply affects protein, carbohydrate and nucleic acid metabolism as an integral component of about 300 enzymes. Zn deficiency can cause various metabolic disorders (Mohameden et al. 2023). Iron (Fe) is found in important proteins such as haemoglobin, myoglobin and catalase and increases oxygen carrying capacity. Fe deficiency can lead to problems such as anaemia, weakened immune system and poor circulation (Reeves and Hoffmann 2009; Rolić et al. 2024). Manganese (Mn) is vital for bone development, immune function and general metabolism. Mn prevents cellular damage by supporting the activity of enzymes such as superoxide dismutase (Hilal et al. 2016; Tuncer et al. 2020; Studer et al. 2022). A balanced intake of these minerals is critical for the healthy development, productivity and disease resistance of animals.

Due to the high number of buffaloes in the region and the increasing demand for buffalo milk products in recent years, herd health and productivity in buffaloes are becoming more important. There is no study on mineral levels of buffaloes living in the Kızılırmak Delta. It is important to check the mineral levels in herds at regular intervals in terms of herd health and economy. With this study, it was aimed to investigate the macro and micro mineral levels of buffaloes in the region, to determine the situation, to examine the deficiencies or excesses, to contribute to the literature, to shed light on future studies, to inform the breeders, to take precautions against diseases and especially to raise awareness.

Materials and Methods

Ethical authorisation

Ethics committee permission for the study was obtained from Samsun Veterinary Control Institute Directorate Local Ethics Committee on 02.12.2022 with 19572899/031-73 number and decision number 2022-8.

Sample Collection

Blood samples from 120 buffaloes (*Bubalus bubalis*) from a breeding facility in the Kızılırmak Delta (coordinates 41.6001° N 35.9363° E) were used. The buffaloes were 4-7 years old, pasture-fed and received no supplementation. The study was conducted in spring and lactating buffaloes. Samples were taken *from jugular veins* using sterile blood collection tubes.

Method

Blood samples were centrifuged and serum was separated. Serum samples were stored at -20°C until analysed. For Ca, P, Mg analysis, the concentration of each mineral was determined by UV-spectrophotometric methods (UV-Vis Spectrophorometer Hitachi) Biosystem spectrophotometric kits were used for Ca, P and Mg analyses. Serum samples were prepared and analysed according to the kit protocols. The results were calculated with the formulae in the protocol.

Cu, Zn, Fe analyses were performed by FL-AAS (Flame Atomic Absorption Spectrophotometer) (GBC Avanta ∑; Australia). Serum samples were directly reconstituted with 1/4 deionised water. The AAS device was set to the appropriate wavelengths for Cu, Zn and Fe. Calibration of the instrument was performed with standard solutions prepared for each mineral. Reconstituted serum samples were analysed by AAS. The concentration of each mineral was calculated using the instrument software. AAS graphite furnace system was used for Mn analysis. Mn wavelength and furnace program were adjusted. Calibration of the device, Mn standard solutions were made in the autosampler. Serum samples were analysed in AAS with graphite furnace. Mn concentration was calculated using the software of the device.

Statistical Analysis

All analyses were performed using Jamovi (Version 2.3.28.0) statistical analysis software. The Kolmogorov-Smirnov test was applied to check the normality assumption of the data. In addition, skewness and kurtosis values were analysed to evaluate the shape of the distribution of the data. According to the results obtained, the data showed normal distribution. Therefore, it was found appropriate to use parametric tests. The mean, standard deviation, minimum, maximum and percentile (25%, 50%, 75%) values of mineral levels were calculated and presented by using descriptive statistical methods. One-Way ANOVA test was used for the comparison of mineral levels between farms. The results of the analyses were evaluated based on **p<0.05** significance level.

Results

In the study, Ca, P, Mg, Cu, Zn, Fe and Mn levels were analysed in 120 buffalo sera. The table below shows the mean values, standard deviations, minimum and maximum values of these elements as well as the percentiles of 25%, 50% and 75%.

Table 1. Ca, P, Mg, Cu, Zn, Fe, Fe and Mn Levels in Blood Serum of Buffaloes and Statistical Differences between Enterprises

	Ca(mg/dl)	P(mg/dl)	Mg(mg/dl)	Cu(µg/dl)	Zn(µg/dl)	Fe(µg/dl)	Mn(µg/dl)
N	120	120	120	120	120	120	120
Mean	9.60	4.72	2.65	50.6	54.3	196	5.01
Standard Deviation	2.30	0.81	0.68	20.2	18.9	68.2	1.13
Minimum	4.32	2.21	1.24	15.3	23.2	65.2	3.28
Maximum	14.3	5.94	4.46	94.2	94.4	356	6.80
25 th	7.96	4.41	2.19	35.1	38.4	156	4.10
50 th	9.33	4.92	2.62	46.2	55.6	194	4.80
75	11.4	5.20	3.16	63.0	68.0	233	5.80
p(farm)	0.755	0.430	0.863	0.043	0.897	0.115	0.001
*Reference range	7.45-13.82	5.71-10.35	2.20-3.93	51.08-151.15	52.27-130.90	60.10-187.49	4.57-8.63

*PetitClerc and Solberg (1987)

The mean Ca level was found to be 9.60±2.30 mg/dl (Table 1). A wide range was observed between Ca levels, with a minimum of 4.32 mg/dl and a maximum of 14.3 mg/dl. Ca levels were 7.96 mg/dl at 25th percentile, 9.33 mg/dl at 50th percentile and 11.4 mg/dl at 75th percentile. This indicates that Ca levels of most buffaloes were within the reference range.

The mean P level was calculated as 4.72±0.81 mg/dl (Table 1). P values ranged between 2.21 mg/dl and 5.94 mg/dl. Phosphorus levels were 4.41 mg/dl at the 25th percentile, 4.92 mg/dl at the 50th percentile and 5.20 mg/dl at the 75th percentile. These values indicate inadequate P intake with mean P levels below the reference range.

The mean Mg level was found to be 2.65± 0.68 mg/dl (Table 1). Mg values ranged between 1.24 mg/dl and 4.46 mg/dl. Mg levels were 2.19 mg/dl at 25th percentile, 2.62 mg/dl at 50th percentile and 3.16 mg/dl at 75th percentile. These values show that Mg levels in the 25th percentile are at the lower limit of the reference range.

The mean Cu level was found to be 50.6±50.2µg/dl. Cu levels ranged between 15.3 µg/dl and 94.2 µg/dl (Table 1). Cu levels were 35.1 µg/dl at 25th percentile, 46.2 µg/dl at 50th percentile and 63.0 µg/dl at 75th percentile. These values show that the mean Cu level is below the reference range.

The mean Zn level was 54.3±18.9 µg/dl. Zn levels varied between 23.2 µg/dl and 94.4 µg/dl (Table

1). Zn levels were 38.4 µg/dl at the 25th percentile, 55.6 µg/dl at the 50th percentile and 68.0 µg/dl at the 75th percentile. These values indicate that Zn levels were within the reference range, but the Zn levels of buffaloes in the 25th percentile were below the reference value.

Fe values ranged between 65.2 µg/dl and 356 µg/dl (Table 1). The mean Fe level was found to be 196±68.2 µg/dl. Fe levels were 156 µg/dl at 25th percentile, 194 µg/dl at 50th percentile and 233 µg/dl at 75th percentile. These values show that Fe levels are above the reference range and Fe intake of buffaloes is sufficient.

The mean Mn level was found to be 5.01±1.13 µg/dl (Table 1). Manganese levels varied between 3.28 µg/dl and 6.80 µg/dl. Manganese levels were 4.10 µg/dl at 25th percentile, 4.80 µg/dl at 50th percentile and 5.80 µg/dl at 75th percentile. These values show that Mn levels are close to the lower reference range. However, the Mn level of buffaloes in the 25th percentile was found below the reference value. There was a significant difference between the farms in terms of Cu ($p<0.05$) and Mn ($p<0.001$), but no difference was found in terms of other minerals ($p>0.05$).

Discussion and Conclusion

In buffaloes, Ca is an essential mineral that supports bone health and is involved in important physiological processes such as milk productivity, muscle contractions, nerve transmission, enzyme activation and blood clotting (Hagawane et al. 2009; Kadhim and Al-Dulaimi, 2015; Runa et al. 2022). The reference range of serum Ca in buffaloes was reported as 7.45-13.82 (PetitClerc and Solberg 1987). The mean serum Ca value obtained in our study was 9.60 ± 0.21 mg/dl (Table 1). The serum Ca levels of buffaloes are within the reference range. In the study of Chabra et al. (2015), Ca levels were found to be similar to our study. In the study of Hagawane et al. (2009), the mean Ca values of healthy lactating buffaloes were found to be higher. In two different studies, researchers found Ca values lower than our values (Runa et al. 2022; Demir et al. 2023). Kadhim and Al-Dulaimi (2015) examined the effect of seasons and found Ca values lower than our values in spring season, higher in summer season and similar in autumn and winter seasons. The differences in these studies may be due to seasonal differences, genetic differences, geographical differences and different pasture plants. In our study, the serum Ca levels in buffaloes were found to be adequate.

In buffaloes, P and Ca together are the main components of bone structure and support healthy bone development (Hagawane et al. 2009). It also increases cellular energy production by being involved in the structure of energy carrier molecules (Kadhim and Al-Dulaimi 2015). The reference range of blood serum P in buffaloes was reported as 5.71-10.35 (PetitClerc and Solberg 1987). The mean P value obtained in our study was 4.72±0.81 mg/dl, which is below the reference range (Table 1). The P level in the study conducted by Chhabra et al. (2015), Jadhav et al. (2018) is very close to the P level in our study and shows a similar situation. The P levels obtained are below the reference range, indicating that the P intake of buffaloes is inadequate. P deficiency may have negative effects on reproductive performance, bone development, energy metabolism and milk yield. Therefore, it is important to maintain adequate levels of P sources in buffalo diets and to supplement when necessary.

In buffaloes, Mg is critical for bone health, nerve and muscle functions and energy metabolism (Richardson 2015). In our study, the mean magnesium level was found to be 2.65 mg/dl. This value is at the lower limit of the reference range of 2.20-3.93 mg/dl. The 25th percentile value is 2.19 mg/dl, the 50th percentile value is 2.62 mg/dl and the 75th percentile value is 3.16 mg/dl. In the study of Jadhav et al. (2018), magnesium level was found to be 2.27 mg/dl and this value is at the lower limit of the reference range. In addition, in a study conducted in Anatolian buffaloes, magnesium level was found to be considerably lower than our values (Demir et al. 2023). The buffaloes in the region were found sufficient in terms of Mg. However, since the 25th percentile is also at the lower limit, attention during the periods when the requirements of the animals increase may prevent possible deficiency.

Cu plays a critical role in energy and iron metabolism in buffaloes and improves general health status by increasing the functionality of the immune system (Jomova 2022). In our study, the mean Cu level was determined as 50.6 µg/dl (Table 1). The reference range in the literature varies between 51.08-151.15 µg/dl, which shows that the value in our study is below the reference range. According to the percentile values, the 25th percentile value was 35.1 µg/dl, the 50th percentile value was 46.2 µg/dl and the 75th percentile value was 63.0 µg/dl. These values show that 25% of the buffaloes were below the reference value with 35.1 µg/dl and 50% of the buffaloes were below the reference value with 46.2 µg/dl and the general copper intake was insufficient. In a study conducted in Anatolian buffaloes in Bitlis province, Cu values were found to be considerably lower than our values (Demir et al. 2023). In other studies, researchers found Cu levels higher than our values (Chhabra et al. 2015; Sherasia et al. 2016; Jadhav et al. 2018). Copper deficiency is a critical mineral for growth and reproductive functions (Spears et al. 2022). Therefore, necessary measures should be taken against Cu deficiency in the region.

In buffaloes, Zn is one of the trace elements involved in many important biological functions such as immune system, cell division, protein synthesis and DNA synthesis. The reference range was determined as 52.27-130.90 µg/dl (PetitClerc and Solberg (1987). In our study, the mean Zn level was found to be 54.3 µg/dl (Table 1). When the obtained data were analysed, the 25th percentile value was calculated as 38.4 µg/dl. This shows that 25% of the population has a zinc level below 38.4 µg/dl. This indicates that some individuals have zinc levels below the reference range and are at risk of potential deficiency. In the study of Demir et al. (2023), Zn levels were found to be lower than our data. In other studies in the literature, Zn levels of lactating buffaloes were found higher than our data (Sherasia et al. 2016; Jadhav et al. 2018; Naliyapara et al. 2023). These results reveal that zinc levels of buffaloes vary significantly depending on geographical regions and feeding habits. Although buffaloes in the Kızılırmak Delta are generally adequate in terms of Zn, caution should be exercised against Zn deficiency as 25% of the buffalo population is below the reference value.

Fe is an essential mineral that provides oxygen transport and storage through haemoglobin and myoglobin synthesis in buffaloes and plays an important function in enzymatic activities (Reeves and Hoffmann 2009). Fe levels were found to be 196 µg/dL on average. These values are above the reference range of 60.10-187.49 µg/dL, indicating that buffaloes have high iron intake. Naliyapara et al. (2023) found Fe levels higher than our results in their study, while other researchers found low levels (Jadhav et al. 2018; Demir et al. 2023). Although the iron levels of buffaloes varied significantly depending on geographical regions and feeding habits, they were found to be sufficient for our region. Mn in buffaloes is a vital trace element for growth, reproduction, bone development and antioxidant defence (Hilal et al. 2016; Studer et al. 2022). The reference range was determined as 4.57-8.63 µg/dl and the mean manganese level was calculated as 5.01 µg/dl. This value is at the lower limit of the reference range and indicates a generally healthy level. However, when the data are analysed, it is seen that 25% of the population has Mn levels below 4.10 µg/dl, i.e. insufficient. In 50% of the data, the Mn level was found to be close to the lower reference value range with 4.80 µg/dl. Our Mn data are similar to some studies of Sherasia et al. (2016) and higher than other studies (Jadhav et al. 2018; Demir et al. 2023). Since inadequate Mn intake can lead to health problems such as growth retardation, reproductive problems and weakening of the immune system, Maurya et al. (2021), it is of great importance to keep Mn levels in balance in buffalo feeding programmes.

A statistically significant difference was found between farms in Cu ($p < 0.05$) and Mn ($p < 0.001$) values. These differences may be attributed to variations in soil composition, vegetation, and mineral content in water sources, despite the buffaloes grazing in the same pasture and receiving no supplementation. The gastrointestinal absorption of minerals, their utilization by animal tissues, the animal's age, required mineral levels, the chemical form of the mineral, interactions between minerals, and the composition and proportions of feed ingredients also significantly influence mineral metabolism (Hilal et al. 2016; Weiss and Hansen 2024). These factors may have affected Cu and Mn absorption and metabolism, leading to differences in values.

The findings obtained in this study provide important information about the general health status of buffaloes serum mineral levels. Ca was found to be within the reference range and adequate for most buffaloes. Although buffalo Mg levels were generally normal, 25% of the buffaloes were below the lower limit of the reference value, which warrants further attention. However, mean P and Cu levels were below the reference range, indicating that buffaloes are deficient in P and Cu minerals. This deficiency may adversely affect the overall health status of buffaloes; therefore, P and Cu intake needs to be increased. Although Zn and Mn levels were generally within the reference range, 25% were below the reference range. Therefore, increasing Zn and Mn intake would be beneficial for animal health. Iron (Fe) levels were found to be adequate and it was observed that buffaloes had a healthy Fe intake. In this context, it is recommended that feeding programmes should be carefully planned, monitored and supplemented when necessary to maintain the mineral balance of buffaloes. This approach has the potential to improve the overall health status of buffaloes and increase their productivity.

* "The views and opinions expressed are solely those of the author(s) and do not necessarily reflect the views of the Republic of Turkey Ministry of Agriculture and Forestry. The Ministry of Agriculture and Forestry of the Republic of Turkey cannot be held responsible". This text is included in line with the recommendation of our Ministry.

Ethical Statement: This study was conducted with permission from the Local Ethics Committee for Animal Experiments of the Samsun Veterinary Control Institute Local Ethics Committee on 02.12.2022 with the number 19572899/031-73 and decision number 2022-8.

Acknowledgements: I would like to sincerely thank our Institute Director Dr. Hamza KADI, our former director İsmail AYDIN, our veterinary colleagues and farmers working in Provincial/District Directorates of Agriculture and Forestry, Dr.Yunus KILIÇOĞLU, Rıfat PAK and Adnan VAHAPOĞLU for their contributions to this project.

Financial Support: No person/organisation financially supported the study.

Author Contributions: Neslihan Ormancı designed and conducted the study, analysed the data and wrote the manuscript.

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