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Research Article

The Determination of Minerals in Soil, Water, Fodder, and Serum and their Effects on Reproductive Efficiency of Nili Ravi Buffaloes

Muhammad Binyameen[1,*], Nasim Ahmad[2], Saba Anwar[1,*], Khalid Javed[3], and Abdul Sattar[2]

[1]Buffalo Research Institute, Pattoki District Kasur 55300, Pakistan
[2]Department of Theriogenology, University of Veterinary and Animal Sciences, Lahore 54100, Pakistan
[3]Department of Livestock Production, University of Veterinary and Animal Sciences, Lahore 54100, Pakistan

* ***Corresponding author:*** Muhammad Binyameen, Reproduction Division, Buffalo Research Institute, Pattoki, Kasur,55300, Pakistan. Email: drbinyameen@yahoo.com; Saba Anwar, Buffalo Research Institute, Pattoki District Kasur 55300, Pakistan Email: sabaanwar68@gmail.com

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ABSTRACT

Introduction: Minerals play an essential role in the reproductive performance of dairy animals, especially in buffaloes. The present study aimed to evaluate the mineral concentrations in different types of samples, including soil used for fodder cultivation, canal water used for fodder irrigation, drinking water for buffaloes, fodder used for buffalo feeding, and serum of buffaloes and their effects on the reproductive efficiency of buffaloes.
Materials and methods: The current study was carried out for three months and samples were analyzed twice at the beginning and the end of the study. The concentrations of calcium, magnesium, and inorganic phosphorus in the soil were checked through the versinate method. The concentration of calcium, magnesium, and inorganic phosphorus in water was checked through the titration method and the concentration of calcium, magnesium, and inorganic phosphorus contents in fodder was checked through the wet digestion method. The minerals of blood serum (n = 80) were analyzed through commercial kits.
Results: In canal water, calcium concentration was higher for irrigation compared to buffalo drinking water. Similarly, on the upper surface of the soil compared to its lower surface, the level of calcium was high. Calcium values of serum were higher in cyclic buffaloes compared to buffalo heifers. In comparison to all groups, the levels of inorganic phosphorus were found to be lower in non-cyclic buffaloes. Similarly, the calcium-to-phosphorus ratio was found to be lower in non-cyclic buffaloes and heifers compared to repeat breeders and cyclic buffaloes. At the beginning of the study, cyclic buffaloes had higher magnesium levels than all other groups, except for non-cyclic buffaloes. By the end of the study, magnesium concentrations were higher in non-cyclic buffaloes and heifers compared to repeat breeders and cyclic buffaloes.
Conclusion: The mineral deficiencies in water and soil can affect the fodder content in the feed of buffaloes.

1. Introduction

The complex system of soil, water, and plants created by nature eventually transforms into fodder for animals when consumed by them. The mineral content present in the soil has a significant impact on both the quality of the forage and the animals that consume it[1]. The nutrient levels vary across different types of soil, impacting the growth of plants in each soil type. The roots of plants serve as a gateway for the entry of mineral content from water and soil. However, the mineral level in the soil is gradually diminishing as a result of the extensive cultivation of high-yielding crops[2].

The maturation of the plant can decrease the quality of fodder. Mature plants tend to accumulate nutrients primarily in their seeds. When it comes to enhancing the

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productivity of dairy animals, the primary emphasis is placed on supplementing protein and energy, while the significance of mineral availability is relatively diminished[3]. Generally, except salt bricks no specific mineral mixtures are supplemented to dairy animals[4]. Mineral supplementation is vital for dairy animals to gain economic benefits[5].

Buffalo is regarded as the second most important dairy animal globally, known for its exceptional ability to convert raw crop byproducts into milk[6]. Some minerals, such as calcium and phosphorus, are needed in larger quantities compared to other trace minerals in dairy animals[7]. During lactation, the calcium requirement in dairy animals doubles compared to normal levels[8]. Extensive research is essential to determine the mineral requirements of buffaloes for optimal production[9]. There is currently insufficient data on the mineral concentrations in water, fodder, and serum of buffaloes. This study aimed primarily to evaluate the concentrations of calcium, magnesium, and inorganic phosphorus in water, soil, fodder, and buffalo serum, as well as their impact on the reproductive efficiency of buffaloes.

2. Material and Methods

2.1. Ethical approval

Both studies were conducted following ethical regulations for animals and after proper approval from farm authorities, LES Bhunikey, Pattoki District Kasur Pakistan.

2.2. Location and season

The studies were conducted at Livestock Experiment Station (LES), Bhunikey, Pattoki District Kasur, Punjab, Pakistan located at (31°1′30.0324″ N and 73°50′52.3608″ E) during the breeding season of buffaloes from December to March 2023.

2.3. Selection of animal

A total of 60 Nili Ravi buffalo with an average body condition score of 3.0 ± 0.5 (1-5), and body weight (500-700 kg) with 3rd and 5th lactation at about 90 days postpartum were selected. The buffalo population was categorized into three distinct reproductive physiological groups based on their history, rectal palpation, age, reproductive status, and ovarian activity as determined by ultrasonography (Honda 3.5 MHz, Japan). Each group contained 20 buffaloes, including (a) Noncyclic buffaloes, (b) Repeat breeder buffaloes, and (c) Cycling buffaloes which the ultrasonography was repeated after ten days. Furthermore, 20 nonpregnant, anestrous buffalo heifers between 32 to 36 months with 300-350 Kg body weight were included in the study as group (d) Heifers. All the animals under study were on the green fodder (*Trifolium alxendrium)* and concentrate (Anmol wanda, Net Energy of Lactation = 1.24 mcal/kg of DM, Crude Protein, 17%, Pakistan) respectively. Access to water was free for all animals.

2.4. Sample collection and analysis

Water samples for buffalo drinking (n = 3) and soil irrigation (n = 3) were separately collected from LES Bhunikey, Pattoki District Kasur, Pakistan. Each sample was 500 ml. Six soil samples were collected from various locations at different depths ranging from 0 to 18 inches. Fodder samples, each weighing 2 kg, were taken from three different sites within an acre. Blood samples were drawn from the jugular vein of the animals to measure the concentrations of calcium, magnesium, and inorganic phosphorus, with each blood sample being 10 ml. All samples were collected twice, once at the beginning of the study in December and again at the end of the study in March, at six-month intervals. Calcium and magnesium levels in the samples were measured using the Versinate method, while inorganic phosphorus[10] levels were assessed through soil analysis. Minerals in water were determined using the titration method. Mineral content in fodder was evaluated via the wet digestion method[11]. The minerals in blood serum were analyzed using Randox commercial kits from the United Kingdom, as described by Teitz[12].

2.5. Statistical analysis

The relationship between the mineral profiles of soil, water, fodder, and buffalo serum was analyzed using correlation coefficients and multiple regression. The difference between the mineral profiles of 4 groups in buffalo's serum was analyzed through ANOVA[13]. Difference between means compared through DMR[14]. The level of significance was set as ($p < 0.05$). The data was analyzed using SPSS version 24.

3. Results and Discussion

3.1 Calcium

As can be seen in Table 1, calcium concentration was highly significant in canal water compared to buffaloes drinking water at Livestock Experiment Station, Bhunikey, Pattoki, District Kasur ($p < 0.05$). The obtained results are in line with the research of Sjors et al.[15] and Morr et al.[16], who reported 1-100 mg/L and 1.4 mg/L calcium levels in ground and tape water respectively. These results agree with Anonymous[17] and Van[18] who reported 2-141 mg/liter and 0.1-0.4 mg/liter levels of calcium in surface and canal water, respectively. Cotruvo and Bartram[19] reported 2-80 mg/L calcium levels in Asian region drinking water, which is slightly higher than these results. The findings are opposite to Kumar[20], who reported a higher concentration (8-343 mg / L) of calcium in drinking water. The disparity arises from the distinct geographic dispersion of minerals. The upper surface of the soil revealed a notably elevated calcium level in comparison to the lower surface ($p < 0.05$). Due to

Table 1. Calcium, inorganic phosphorus, and magnesium concentration (Mean ± SD mg/dl) in water and soil at Livestock Experimental Station, Bhunikey District Kasur, Punjab, Pakistan from different sources and surfaces

Variable	Water (mg / liter)		Soil (mg/kg)	
	Canal water	Drinking water	Upper surface	Lower surface
Number	6	6	6	6
Calcium	104 ± 0.02^{a}	100 ± 0.20^{b}	260 ± 1.95^{c}	147 ± 0.11^{d}
Inorganic phosphorus	0.09 ± 0.01^{a}	0.10 ± 0.03^{a}	9.76 ± 2.08^{c}	9.76 ± 2.08^{c}
Magnesium	7.2 ± 0.03^{a}	11.6 ± 0.21^{b}	17.33 ± 8.40^{c}	9.00 ± 6.24^{c}

The mean values bearing different superscripts in a row for each parameter differ significantly ($p < 0.05$). The optimum value of calcium, inorganic phosphorus, and magnesium concentration to meet NRC is approximately 0.44-0.62 %, 0.32–0.40 %, and 0.18-0.20 % respectively for dairy animals in the water. The optimum value of calcium, inorganic phosphorus, and magnesium concentration in soil is > 750 mg/kg, > 50 mg/kg, and > 140 mg/kg respectively (Soil reference, Mehlich III SALAN; IPM Planning guide, Environmental Institute for Gulf)

different soil topography, the calcium values in soil reported by Ghafoor et al.[21], Akhtar et al.[22], and Khan et al.[23] were higher compared to the obtained values. Calcium concentration was fixed in fodder at the beginning and end of the study. The reported value of calcium in Berseem fodder by Ditta et al.[24] was higher (2.63 ± 0.09) in the DG Khan areas of Punjab Pakistan compared to the obtained calcium results. During December, mean calcium values of serum were high ($p < 0.05$) in cyclic buffaloes compared to anestrous heifers. However, the remaining differences between the groups at the Livestock Experiment Station, Bhunikey, Pattoki, District Kasur, were not significant ($p > 0.05$). In March, calcium levels were significantly lower ($p < 0.05$) in anestrous heifers (8.47 ± 0.22 mg/dl) compared to all other groups, while the differences among the other groups were non-significant ($p > 0.05$). Pasha et al.[5] found similar (9-11 mg/dl) calcium levels in the serum of buffaloes in Punjab. The obtained results are similar to Khan et al.[11], who reported (9.48 ± 0.43 mg/dl) calcium level in dry buffaloes of Kasur District of Punjab Pakistan. However, Husnain et al.[25] reported calcium levels in the serum of milking buffaloes were slightly lower (6.70- 8.00 mg/dl). These results agree with Ullah et al.[26], who indicated calcium deficiency affects adversely reproduction. Additionally, Hedaoo et al.[27] reported similar levels of calcium in cycling and anestrous buffaloes.

3.2. Inorganic phosphorus

The concentration of inorganic phosphorus exhibited no significant difference between the canal and drinking water samples collected at Livestock Experiment Station, Bhunikey, Pattoki, District Kasur ($p > 0.05$, Table 2). Similarly, the level of inorganic phosphorus was similar in the upper and lower surface of the soil ($p > 0.05$). Gowda et al.[28] reported a similar level of inorganic phosphorus in the soil compared to the present study. Above all, the critical level (45-130 mg/kg) described by Ramana et al.[29] and (20 mg / Kg) standardized by Mehlich[10] was higher than the level of inorganic phosphorus. There was no significant difference in the level of inorganic phosphorus between the beginning and end of the study in the fodder (Berseem, $p > 0.05$). The value of inorganic phosphorus in Berseem was close to values reported by Ditta et al.[24] in Punjab, Pakistan. Inorganic phosphorus mean values were significantly lower ($p < 0.05$) in non-cyclic buffaloes (5.12 ± 0.05 mg/dl) compared to all other groups at the beginning of the research period. At the end of the study, no significant alteration was observed in inorganic phosphorus levels across all groups ($p > 0.05$). The obtained results are in line with Hedaoo[27], who reported that inorganic phosphorus has no effect on cycling and anestrous cattle.

Table 2. Calcium, inorganic phosphorus, and magnesium concentration (Mean ± SD %) in fodder at Livestock Experimental Station, Bhunikey District Kasur, Punjab, Pakistan at different time intervals

Variable		Fodder (%)
Number	3	3
Interval	Dec	Mar
Calcium	1.46 ± 0.05^{a}	1.5 ± 0.04^{a}
Inorganic Phosphorus	0.20 ± 0.00^{a}	0.46 ± 0.18^{a}
Magnesium	1.65 ± 0.02^{a}	1.46 ± 0.1^{a}

The mean values bearing different superscripts in rows for each parameter differ significantly ($p < 0.05$). The optimum value of calcium, inorganic phosphorus, and magnesium concentration to meet NRC is approximately 0.44-0.62%, 0.32–0.40 %, and 0.18-0.20 % respectively for dairy animals in fodder; Dec: December; Mar: March

3.3. Calcium to inorganic phosphorus ratio

At the beginning of the study, anestrus heifers had a lower calcium-to-phosphorus ratio compared to other groups ($p < 0.05$). Similarly, both noncyclic and anestrus heifers exhibited a lower calcium-to-phosphorus ratio compared to repeat breeders and cyclic buffaloes ($p < 0.05$, Table 3). These findings agree with Kumar et al.[32], who reported that for better reproduction the Ca: P ratio should be 2: 1.

3.4. Magnesium

In this study, drinking water magnesium concentration was higher compared to canal water ($p < 0.05$). Similarly, Khan et al.[33] reported a minimum level of magnesium (8.48 mg/L) in drinking water. Magnesium levels were similar in the upper and lower surface of the soil. These results were slightly lower than Fardous et al.[34], who reported a range of magnesium between 21.1-37.7 mg/kg in the soil. Likewise, the magnesium concentration in the fodder (Berseem) was similar at the beginning of the study and the end of the study. Khan et al.[11] reported 0.20% magnesium in winter forages, which was higher than Berseem. Magnesium values reported by Pasha et al.[5] were 2.68 mg/dl in Punjab in buffalo's blood and were slightly higher. Similar to the obtained results, Iqbal[35] and Hussain[36]

Table 3. Serum calcium, inorganic phosphorus, magnesium concentration (Mean ± SD mg/dl), and calcium to inorganic phosphorus ratio in buffaloes at Livestock Experimental Station, Bhunikey District Kasur, Punjab Pakistan at different time intervals

Variable	Interval	Calcium (mg / dl)	Inorganic Phosphorus (mg/dl)	Magnesium (mg/dl)	Ca: IP
No.	20	20	20	20	20
Non-cyclic buffaloes	December	10.27 ± 0.16^{ab}	5.12 ± 0.05^{c}	2.54 ± 0.08^{f}	2.00 ± 0.02^{g}
	March	9.5 ± 0.23^{b}	4.73 ± 0.08^{c}	2.51 ± 0.09^{f}	2.02 ± 0.04^{g}
Repeat breeder buffaloes	December	10.18 ± 0.30^{ab}	5.56 ± 0.08^{d}	2.17 ± 0.05^{e}	1.82 ± 0.05^{g}
	March	9.89 ± 0.23^{b}	4.68 ± 0.08^{c}	2.34 ± 0.03^{e}	2.10 ± 0.04^{g}
Cyclic buffaloes	December	10.74 ± 0.38^{ab}	5.67 ± 0.13^{d}	2.61 ± 0.10^{f}	1.91 ± 0.07^{g}
	March	9.95 ± 0.18^{b}	4.74 ± 0.09^{c}	2.31 ± 0.03^{e}	2.11 ± 0.03^{g}
Anestrus heifers	December	9.54 ± 0.25^{b}	5.48 ± 0.14^{d}	2.17 ± 0.03^{e}	1.77 ± 0.07^{h}
	March	8.47 ± 0.22^{a}	4.90 ± 0.08^{c}	2.51 ± 0.05^{f}	1.78 ± 0.06^{h}

The mean values bearing different superscripts (small letters) in the column for each parameter differ significantly ($p < 0.05$). The optimum value of calcium in the blood serum of dairy animals is 10-12 mg/dl.[30] The optimum value of inorganic phosphorus, Ca: IP ratio, and magnesium in the blood serum of dairy animals is 4-5 mg/dl, 1.5-2: 1, and 1.7-3 mg/dl respectively[31]; no: Number

reported 2.57-2.58 mg/dl and 1.75-280 mg/dl magnesium in the blood serum of cattle, contrary to Oba and Ramos[37], who described 3.84 ± 1 mg/dl magnesium in the serum of cattle. The diversity of minerals found in water and soil had a notable impact on the mineral composition of the fodder in this research. Khan et al.[23] and Akhter et al.[38] reported similar results, in which deficiency of inorganic phosphorus in soil exerts its effect on fodder mineral profile.

4. Conclusion

The mineral deficiency in soil and water in District Kasur, Punjab, Pakistan, affects fodder quality, indicating a need for phosphorus supplementation in the soil. In the canal water, the level of calcium was high and the level of magnesium was low compared to drinking water for buffaloes. The low concentration of calcium and imbalance of calcium to inorganic phosphorus ratio can be a contributing factor to delayed puberty in buffalo heifers. Further studies on a large scale are required to evaluate the mineral supplementation on the reproductive performance of buffaloes.

Declarations

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Muhammad Binyameen and Saba Anwar conducted the experiment and write-up. Nasim Ahmad, Khalid Javed, and Abdul Sattar revised the manuscript and did the statistical analysis. All the authors read and approved the final version of the manuscript.

Authors' relationships and activities

Authors are responsible for disclosing all relationships and activities that might bias or be seen to bias their work.

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Availability of data and materials

The data used in this study can be made available from the corresponding author upon reasonable request.

Ethical considerations

The authors have checked plagiarism, publication consent, misconduct, data fabrication or falsification, double publication or submission, and redundancy.

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