

Epidemiological studies of hypophosphatemia in buffaloes

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Abstract

The present study was conducted to record the occurrence of hypophosphatemia in buffaloes at Veterinary Clinical complex, NTR college of Veterinary science, Gannavaram during the period of one year. A total of 126 buffaloes presenting with clinical signs suggestive of phosphorus deficiency were screened. Serum phosphorus levels were estimated and the values below the physiological reference range were considered as hypophosphatemia. The overall occurrence of hypophosphatemia was found to be 22.22 per cent (28/126). Age-wise occurrence revealed a higher incidence in buffaloes aged 3 to 8 years (71.43%) followed by those above 8 years (17.86%) and the lowest in animals below 3 years of age (10.71%). Parity-wise occurrence was highest in the third lactation (39.29%) followed by fourth lactation (28.57%), second lactation (14.29%) and first and fifth lactation (3.57%) each. Stage of lactation wise occurrence showed the highest in the early lactation stage (0–3 months after calving) as 72.00 per cent followed by mid-lactation (3–6 months) with 20.00 per cent and the least in late lactation (above 6 months) with 8.00 per cent. Haematological and mineral profile studies in buffaloes with hypophosphatemia.

Key words: Andhra Pradesh, Buffaloes, Haematological Hypophosphatemia.

Introduction

India, with a population exceeding 1.44 billion as of 2024, relies heavily on agriculture and livestock for rural and peri-urban livelihoods. Cattle and buffaloes form the backbone of the dairy industry, with a combined population of 299.6 million (cattle: 190.9 million; buffaloes: 108.7 million) as per the 2019 census. These animals collectively contribute to an annual milk production of 230 million metric tons resulting in a per capita milk availability of 459 grams per day (NDDB, 2024). Despite this substantial output, the average milk yield per animal remains suboptimal necessitating targeted interventions to enhance productivity and ensure the sustainability of the dairy industry (Sharma *et al.*, 2025). Phosphorus primarily stored in bones and teeth and is essential for skeletal development, energy metabolism, cellular function and nucleic acid synthesis (Grunberg, 2014; Zhang *et al.*, 2017). It also supports acid-base balance and is a key component of adenosine triphosphate (ATP) (Manopriya *et al.*, 2022). Deficiency of phosphorus disrupts these functions causing ATP depletion, haemolysis, poor fertility and reduced milk yield (Zhang *et al.*, 2017). Buffaloes are particularly sensitive to phosphorus deficiency compared to cattle often showing decreased milk production, delayed maturity, impaired reproduction and increased disease susceptibility (Malafaia *et al.*, 2023). Although several studies have addressed hypophosphatemia in buffaloes from Andhra Pradesh, comprehensive epidemiological data remain scarce. Therefore, the present research was designed to assess the occurrence and epidemiological studies of hypophosphatemia in buffaloes in Andhra Pradesh.

Materials and Methods

The present study was conducted from May to December 2024 at the Veterinary Clinical Complex, NTR College of Veterinary Science, Gannavaram and on various dairy farms in and around Gannavaram, Andhra Pradesh. During this period a total of 1150 animals were screened for hypophosphatemia. A total of 126 buffaloes of different age groups and lactation stages presenting with clinical signs such as anorexia, reduced milk yield, anemia (Fig 1), decreased body weight (Fig 2), pica (Fig 3), haemoglobinuria (Fig 4), locomotor disturbances, leaky teats (Fig 5) and repeat breeding were selected based on anamnesis, clinical examination, and haemato-biochemical findings. Ten apparently healthy buffaloes with normal physiological parameters were taken as control group. Clinical history such as age, parity, stage of lactation, pregnancy status and management practices was collected using a standardized case sheet. Blood samples were aseptically collected from the jugular vein in EDTA and clot-activator tubes for haematological and serum biochemical analysis, respectively. Serum was separated and stored at -20°C until further use. Haematological parameters such as hemoglobin, packed cell volume, total erythrocyte count, total and differential leukocyte counts were estimated as per standard procedures. Serum biochemical parameters including phosphorus, calcium, magnesium, were analyzed using a semi-automatic analyzer (Indus BA-88A) with commercially available assay kits (ERBA Diagnostics, Mannheim, Germany).

Results and Discussion

In the present study, 28 out of 126 buffaloes were confirmed with hypophosphatemia, based on serum biochemical findings thus representing an occurrence of 22.22 per cent. This finding was in close agreement with the earlier reports of Bhandari *et al.* (2015) who reported occurrence as 25.17 per cent. On the converse, some authors like Singh *et al.* (2012) reported a higher occurrence of hypophosphatemia as 78.57 per cent. The occurrence of hypophosphatemia was higher in buffaloes owned by individual farmers than in those at organized dairy farms. This lower occurrence in dairy farms could be attributed to better feeding and health management practices.

In the present study, the occurrence of hypophosphatemia was found to be higher in the age group of 3-8 years (71.43%) followed by buffaloes aged above 8 years (17.86%) and in the buffaloes less than 3 years of age (10.71%) (Table 2). These findings were similar to the observations of Jadhav *et al.* (2019) who opined that heavy drain of minerals like phosphorus in peak production period and decreased absorptive efficiency of phosphorus and bone metabolism with increasing age.

The present study revealed that the occurrence of hypophosphatemia was highest in buffaloes during the third lactation (39.29%), followed by the fourth (28.57%), second (14.29%) and both the first and fifth lactations (3.57% each). Hypophosphatemia was found higher during the early stage of lactation (0-3 months post-calving) at 72.00% followed by the mid-stage (3-6 months post-calving) at 20.00 per cent and the late stage (beyond 6 months) at 8.00 per cent. These findings align with the observations of Constable *et al.* (2017) and Kataria *et al.* (2022) who reported a higher occurrence of hypophosphatemia in lactating buffaloes than in pregnant ones. This was attributed to excessive phosphorus depletion through colostrum in early lactation and increased phosphorus demand by the developing fetus during late gestation. The present study suggests that continuous phosphorus loss through milk in lactating buffaloes fed low-phosphorus diets such as straw-based feed might lead to depletion of body reserves,

bone resorption and the manifestation of clinical signs during mid to late lactation (Constable *et al.*, 2017).



Fig 1. Pale Conjunctival mucous membrane



Fig 2. Extreme weight loss



Fig 3. Leaky teats



Fig 4. Pica



Fig 5. Haemoglobinuria

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Table 1: Haematological profile in buffaloes with hypophosphatemia

S. No	Parameter	Healthy control(n=10)	Buffaloes with hypophosphatemia(n=28)
1.	Haemoglobin(g/dL)	11.62±0.33 ^a	6.88±0.12 ^b
2.	Packed cell volume(%)	36.10±1.04 ^a	22.03±0.57 ^b
3.	Total erythrocyte count ×10 ⁶ /mm ³)	6.69±0.16 ^a	5.66±0.99 ^b
4.	Total leucocyte count(x10 ³ /mm ³)	10.72±0.07 ^a	11.78±0.18 ^b
5. Differential Leucocyte Count			
a.	Neutrophil (%)	31.40±1.30 ^a	45.67±0.74 ^b
b.	Lymphocytes (%)	62.30±1.25 ^a	50.07±0.76 ^b
c.	Eosinophils (%)	2.80±0.24 ^a	1.67±0.11 ^b
d.	Monocytes (%)	3.50±0.16 ^a	2.57±0.13 ^b

*Means with similar superscripts did not vary significantly in a row (P>0.05)

Table 2: Serum mineral profile in buffaloes with hypophosphatemia

S. N.	Parameter	Healthy control (n=10)	Buffaloes with hypophosphatemia (n=28)
1.	Phosphorus (mg/dL)	6.18±0.09 ^a	3.05±0.04 ^b
2.	Calcium(mg/dL)	10.94±0.18 ^a	10.42±0.07 ^b
3.	Ca:P ratio	1.77±0.26 ^a	3.43±0.56 ^b
4.	Magnesium(mg/dL)	1.98±0.05 ^a	0.76±0.02 ^b
5.	Iron (µg/dL)	138.90±3.98 ^a	200.71±1.86 ^b
6.	Copper (µg/dL)	118.20±1.10 ^a	72.60±0.96 ^b
7.	Zinc (µg/dL)	153.00±1.94 ^a	58.42±0.84 ^b
8.	Cobalt (µg/dL)	112.50±0.52 ^a	68.03±1.50 ^b

*Means with similar superscripts did not vary significantly in a row (P>0.05)

The mean haemoglobin (g/dL), PCV (%) and total erythrocyte count (x 10⁶/mm³) (Table 1) was significantly low (P<0.05) in hypophosphatemic buffaloes which align with the findings of Purohit *et al.* (2018) and Jadhav *et al.* (2019) who documented that severe phosphorus depletion results in ATP depletion which predisposes red blood cells to alter its structure and function leading to haemolysis in hypophosphatemic buffaloes. Significant increase (P<0.05) in total leucocyte count was noticed in buffaloes with hypophosphatemia. This finding also gained support from the reports of Jadhav *et al.* (2019) and Kataria *et al.* (2022) who stated that it might be due to immune activation due to impaired cellular immunity or a stress leukogram triggered by chronic phosphorus deficiency.

The mean serum phosphorus (mg /dL) value in healthy control was 6.18±0.09 while it was 3.05±0.04 in buffaloes with hypophosphatemia. The lower values of phosphorus observed in hypophosphatemic buffaloes of the present investigation were similar to those observed by Nikvand *et al.* (2017), Wankahade *et al.* (2021) who attributed this to dietary high calcium and low phosphorus which impairs phosphorus absorption from the gut in buffaloes. Additionally, increased phosphorus demand for fetal development, its excretion through milk in lactating buffaloes and insufficient phosphorus levels in both fodder and soil contribute to this condition (Kataria *et al.* 2022).

In the present study, the mean calcium level in hypophosphatemic buffaloes was non significantly (p>0.05) decreased compared to healthy control which was in accordance El-Deen *et al.* (2021) and Abramowicz *et al.* (2022). However, Jadhav *et al.* (2019) and Kataria *et al.* (2022) reported significant decrease in calcium levels in buffaloes with hypophosphatemia. These variations might be attributed to differences in feeding practices, parity, pregnancy, and lactation status of buffaloes. Significant increase (P<0.05) in Ca:P ratio in phosphorus deficiency buffaloes was observed in the present study which was in accordance with Kumar *et al.* 2019, El-Deen *et al.* 2021 who stated that a high-calcium, low-phosphorus diet may widen the calcium-to-phosphorus ratio, hinder phosphorus absorption in the gut, and result in hypophosphatemia and also observed progressive increase in Ca:P ratio with the severity of the condition. Significant increase (P<0.05) in serum iron was noticed in buffaloes with hypophosphatemia when compared to healthy control. This finding also gained support from the reports of Zaghawa *et al.* 2019 and Abramowicz *et al.* 2022 could be due to intravascular haemolysis which increases serum iron and impaired absorption of iron in digestive tract..The mean serum copper (g/dl) was significantly less (P<0.05) in hypophosphatemic buffaloes compared to healthy buffaloes, which could be due to attributing it to nutritional deficiency and a three-way interaction between copper, molybdenum, and sulphur, where thiomolybdates formed in the rumen bind copper into an insoluble complex, rendering it unavailable for absorption and leading to hypocupraemia(Zaghawa *et al.* 2019) .Significantly low (P<0.05) zinc in anemic buffaloes could be attributed to the nutritional status due to anorexia(Zaghawa *et*

al. 2019). They also stated that hypophosphatemia results in weakness and anorexia, while zinc deficiency affects appetite regulation and protein metabolism, further compromising growth. The mean serum cobalt concentration ($\mu\text{g/dl}$) in hypophosphatemic buffaloes was significantly lower compared to healthy controls. These reduced cobalt levels in hypophosphatemic buffaloes align with the findings of El-Deen *et al.* 2021. Cobalt plays a crucial role in metabolism, primarily as a component of vitamin B12 (cobalamin), which was essential for energy production, erythropoiesis, and ruminal microbial activity. While hypophosphatemia is primarily linked to phosphorus deficiency, its relationship with cobalt is indirect but significant due to metabolic interdependence (Zaghawa *et al.*, 2019). Serum phosphorus positively correlated with magnesium, calcium, copper, zinc and cobalt and negatively correlated with Ca:P ratio and iron. Phosphorus deficiency leads to poor productivity and reproductive performance in buffaloes. Along with Phosphorus deficiency, in our study it was evident that there were deficiencies of copper, zinc, cobalt and increased iron profiles in buffaloes.

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