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Clinico- haematobiochemical Profiling and Therapeutic Studies on Hypophosphatemic Dairy Buffaloes

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ABSTRACT

The present study was aimed to investigate the clinical, haemato-biochemical profile of hypophosphatemic dairy buffaloes, followed by therapeutic management. The investigation was conducted on 20 dairy buffaloes brought to Veterinary Clinical Complex, LUVAS, Hisar. The study revealed 4-7 years old buffaloes in their early lactation to be most commonly affected and the prominent clinical signs observed were anorexia, hypogalactia, straining during urination and difficulty in walking. Haematology showed significant ($P< 0.01$) increase in neutrophils count and mean values of ESR. Serum biochemistry revealed significant decrease in serum inorganic phosphorus with non-significant elevation in other biochemical parameters. Animals were randomly allocated into 2 groups. In group I, sodium acid phosphate @120 gm in 600 ml NSS (half dose IV and half SC), followed by same dose orally for 5 days was given along with supportive therapy while in group II, Injection Novizac^R (buffered phosphorous injection) along with supportive therapy was given for 5 days. Based on therapeutic trial, sodium acid phosphate therapy was found to be the most effective in management of hypophosphatemia in dairy buffaloes.

HIGHLIGHTS

- Increased Erythrocyte sedimentation rate, absolute neutrophilia and hypophosphatemia are characteristic.
- Standard therapy of hypophosphatemia is sodium acid phosphate with supportive therapy.

Keywords: Dairy buffaloes, ESR, Hypophosphatemia, Sodium acid phosphate

Phosphorous is the second most abundant mineral in animal body of which about 80% is found in bone and teeth. An early indication that phosphorous shortage seriously impairs livestock production came from pioneer work of Sir Arnold Theiler in 1912 (Suttle, 2010). Feeding of cruciferous plants, sugarcane tops, sugar beets exacerbate phosphorous deficiency either due to their low phosphorous content or an account of certain inhibitor factors that prevent proper absorption of dietary phosphorous (Purohit *et al.,* 2018). Hypophosphataemia results in decreased RBC glycolysis and ATP synthesis predisposing RBC to altered function and structure, loss of normal deformability and increase in fragility and hemolysis with resultant hemoglobinaemia and hemoglobinuria (Constable *et al.,* 2017). Metabolic profiling studies reported incidence of subnormal phosphorous on day of calving to be more than 50% while 10-15% in first two weeks of lactation (Grunberg, 2014). Singh *et al.* (2012) found prevalence of hypophosphatemia in buffaloes to be 78.57%, secondary to deficient phosphorous in fodder samples. Not any characteristic clinical signs have been observed by Kathiravan and Priya (2020). Bhikane *et al.* (2011) suggested that treatment with buffered phosphorous injection (Novizac^R) gave early recovery (2.5+0.22 days) in phosphorous deficiency hemoglobinuria affected buffaloes. By keeping above facts under consideration, the present study was aimed with the

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objective to study the clinical and haemato-biochemical profiling of hypophosphatemic dairy buffaloes, followed by comparison of therapeutic efficacy of sodium acid phosphate and buffered phosphorous injection in its management.

MATERIALS AND METHODS

The present study was conducted on 20 hypophosphatemic buffaloes brought to VCC, LUVAS, Hisar. The detailed anamnesis included age, parity, lactation status, pregnancy status, duration of illness, history of fever, previous treatment if any etc. were recorded. Different clinical parameters including rectal temperature, mucous membrane colours etc. were also noted. Blood samples collected in tubes coated with K_3EDTA were analysed in automated hematology cell counter (MS4s VET, Melet Schlosing Lab.) while serum samples were analysed using automated random access clinical chemistry analyzer (EM Destiny 180, Erba Diagnostics Mannheim GmbH). ESR was measured using Westergren tube method. The affected animals were randomly allocated into two therapeutic groups. In group I (n=5), sodium acid phosphate @ 120 gm in 600 ml half dose IV and half SC, followed by same dose orally for 5 days was given along with primary therapy as per the condition while in group II (n=3), Novizac^R (buffered phosphorous injection) was given intravenously along with primary therapy for 5 days. Supportive therapy included ascorbic acid, fluids and Vitamin B complex. Post therapeutic evaluation was done on day 5 of therapy on the basis of haemato-biochemical parameters. The obtained data was statistically analyzed using statistical software, SPSS version 23.

RESULTS AND DISCUSSION

Etiological characterization of hypophosphatemic buffaloes

In the present study, hypophosphatemia is mainly recorded as a secondary disorder. Based on clinical examination, hemato-biochemical alterations and primary diagnostic tools i.e. radiography interpretation, hypophosphatemic condition was characterized as per diagnosed primary etiology as depicted in Table 1. Pneumonia and post-parturient hemoglobinuria (PPH) were the major disease conditions in which hypophosphatemia was the predominant finding. Purohit *et al.* (2018) also reported PPH as a major hazard to buffalo dairy industry that mainly attributed to hyphophosphatemia. While pneumonia affected animals did not receive any mineral supplementation since one year, so hypophosphatemia might be a dietary origin, not related to disease condition.

Table 1: Etiological characterization of hypophosphatemia in buffaloes

Disease category	Number of cases (n=20)
Pneumonia	5
Mastitis	3
Traumatic pericarditis	1
Foreign body syndrome	1
Impaction	1
Pica	1
Post-parturient hemoglobinuria	4
Renal affection	2
Hepatic affection	1
Cystitis	1

Clinical profile of hypophosphatemic buffaloes

In 20 clinical cases, anorexia and hypogalactia were the predominant clinical findings observed as depicted in table 2 and figure 1. Kathiravan and Priya (2020) also reported hypogalactia and inappetence as the major findings in a hypophosphatemic buffaloes. Buffaloes in 4-7 years age group and in their early lactation were found to be most affected. Heavy drain of minerals including phosphorous might be observed during early lactation while the requirement of phosphorous to developing foetus goes to peak at late pregnancy may lead to hypophosphatemia (Dhonde *et al.*, 2007; Constable *et al.*, 2017).

Table 2: Clinical profile of hypophosphatemic dairy buffaloes

History	No of cases (n=20)	Percentage (%)
Anorexia	19	95
1 month post-parturient	10	50
Advanced pregnancy	2	10
Nasal discharge	6	30
Tympany	2	10
Hypogalactia	11	55
Pica	1	5
Coffee colored urine	3	15
Straining during urination	4	20
Ventral edema	1	5
Difficulty in walking	3	15

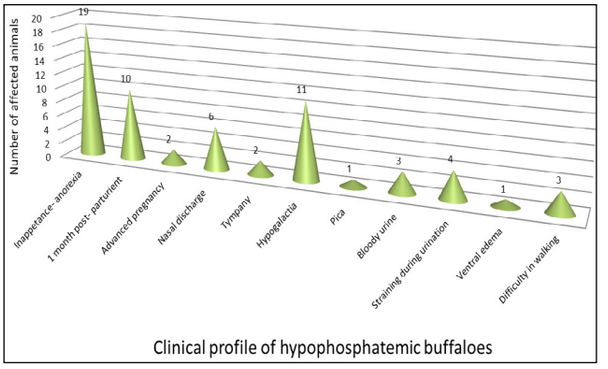

Fig. 1: Graphical presentation of clinical profile of hypophosphatemic buffaloes

Haemato- biochemical alteration in hypophosphatemic buffaloes

Table 3, 4 and 5 depict alteration in haemato-biochemical parameters. Out of 20, 9 animals have haemoglobin (11.99±0.55 g/dl) and PCV (36.51±3.37%) within reference range while 10 animals have increased ESR (94.5±22.3%) and absolute neutrophilia (76.6±7.42%). Haematological analysis in our study concluded that there is significant increase in neutrophils percentage and mean values of ESR as compared to normal reference values while non-significant alteration in other haematological parameters recorded. Similar variation in leucogram i.e. absolute neutrophilia in post-parturient hyphophosphatemic buffaloes was observed by Sarma *et al.* (2014). In contrast to our findings, Wankhede *et al.* (2021) and Sharma *et al.* (2020) reported significant decrease in RBC indices in hypophosphatemic buffaloes. Significant increase in mean value of ESR observed in our study is similar to that reported by Rahmati *et al.* (2021) in hypophosphatemic buffaloes.

Biochemical analysis showed significant decrease in mean value of phosphorous (2.31 mg/dl) as compared to reference range. Similarly decrease in serum phosphorous value was also observed by Iqbal (2014) in post-parturient hemoglobinuria affected buffaloes while non-significant variation in mean values of other biochemical parameters. Paul *et al*. (2018) also showed no significant variation in ALP mean values of hypophosphatemic buffaloes similar to our study.

Table 3: Haematological parameters in hypophosphatemia affected buffaloes

Parameters	Reference value	Mean value (Mean ±SE) (n= 20)
Hemoglobin (g/dl)	10.8- 13.3	11.18 ± 2.89
PCV (%)	30-41	33.5 ± 9.41
TEC ($\times 10^6$ µl)	5.8-6.2	5.38 ± 1.48
TLC ($\times 10^6$ µl)	8.61- 12.61	14.57 ± 5.81
N (%)	43-45	**69.94 ± 13.41***
L (%)	45- 48	34.91± 13.06
ESR	55-60 mm/hr	**72 ± 28.13***

*Imply significant increase in mean values.

Table 4: Haematological parameters in hypophosphatemia affected buffaloes (contd..)

Parameters	Mean value (Mean ±SE) (n= 20)	Reference value
Hemoglobin (g/dl)	**11.18 ±2.89**	
(a) Below lower limit (n= 8)	8.39±1.64	10.8- 13.3
(b) Within normal limit (n= 9)	11.99 ± 0.55	
(c) Above upper limit (n= 3)	16.17 ±0.31	
PCV (%)	**33.5 ±9.41**	
(a) Below reference (n= 8)	23.14± 4.7	33.5 ± 9.41
(b) Within normal range (n= 9)	36.51±3.37	
(c) Above reference (n= 3)	48± 3.26	
ESR (mm/hr)	**72 ±28.13**	
(a) Below reference (n= 6)	42 ±7.72	55-60 mm/hr
(b) Within normal (n= 4)	56.25 ±4.14	
(c) Above normal (n= 10)	94.5 ± 22.3	
Neutrophils (%)	69.94 ± 13.41	
(a) Relative neutrophilia (n= 7)	70.71 ± 8.27	43-45
(b) Absolute neutrophilia (n= 10)	76.6 ± 7.42	

Table 5: Biochemical alteration in hypophosphatemia affected buffaloes

Parameters	Reference value	Mean value (Mean ±SE) (n= 20)
SGOT (IU/L)	78- 132	131.45 ± 108.42
Albumin	2.1- 3.6	2.43 ± 1.07
Total protein (mg/dl)	5.7- 8.1	5.19 ± 1.46
Calcium (mg/dl)	9.7- 12.4	10.0 ± 0.97

Phosphorous (mg/dl)	**5.6- 6.5**	**2.31 ± 0.74***
BUN (mg/dl)	6- 27	24.9 ± 21.2
Creatinine (mg/dl)	1-2	1.89 ± 2.71
ALP (IU/l)	0-200	126.7 ± 28.3
Triglyceride (mg/dl)	0-14	13.18 ± 4.05
Glucose (mg/dl)	45-75	50.85 ± 14.9
Cholestrol (mg/dl)	65- 220	75.7 ± 14.68
GGT (IU/l)	6.1- 17.4	25.84 ± 7.6

* depict significant increase in mean value.

Post- therapeutic evaluation/ follow-up

Follow up of 5 animals from group I and 3 animals from group II was available after 5 days of therapy. Animals belonging to Group I show improvement following 5 days of treatment evident clinically as well as hemato-biochemically while animals of Group II not show any recovery signs even after 5 days of treatment as shown in table 6 and 7 respectively. Haemato-biochemical findings of group I buffaloes show significant increase in phosphorous (5.2+0.75 vs 2.66+0.78 mg/dl) and non-significant decrease in neutrophil (57.2+5.27 vs 72.8+3.3) and ESR (68.2+9.58 vs 100+35.78) mean values after 5 day of therapy. Similar response to sodium acid phosphate therapy was observed by Purohit *et al.* (2014) in post-parturient hemoglobinuria affected buffaloes. Similar to our findings in group I, Cohrs and Grunberg, (2018) suggested that oral supplementation of sodium acid phosphate salt for the correction of hypophosphatemia found suitable to replace or at least complement parenteral P-supplementation and have a rapid onset of action and sustained effect lasting for at least 24 hours. While in group- II buffaloes, no variation in mean values of different parameters observed. In contrast to our findings, Bhikane*et al.* (2011) found significant improvement in phosphorous deficiency hemoglobinuria affected buffaloes following buffered phosphorous injectable (Novizac^{R}). Rashid *et al.* (2018) based on his trial suggested that administration of inorganic phosphorus @ dose rate of 15 mg/kg b.wt facilitated recovery in hypophosphatemic pregnant Murrah buffaloes in comparison to 3 mg/kg bwt. And in our study, in group I sufficient dose of phosphate salt (120 gm) given while in group II, Novizac contain insufficient phosphorous concentration. So sodium acid phosphate is the recommended therapy for hypophosphatemic buffalo.

Table 6: Group-I Post therapeutic hemato-biochemical evaluation

Parameters	Mean values (0 day of therapy) (n=5)	Mean values (5 day of therapy) (n=5)
Hemoglobin (g/dl)	8.94 ± 1.51	10.03 ± 2.07
PCV (%)	27.2 ± 13.1	32.16 ± 4.97
TLC	12.37 ± 4.7	11.54 ± 0.79
N (%)	72.8 ± 3.3	57.2 ± 5.27
L(%)	30.6 ± 7.86	34.8 ± 3.86
ESR (mm/hr)	100 ± 35.78	68.2 ± 9.58
TEC	4.38 ± 2.19	5.07 ± 0.79
SGOT (IU/l)	96.8 ± 15.18	100.06 ± 14.03
Ca (mg/dl)	9.8 ± 0.94	9.6 ± 0.48
Ph (mg/dl)	2.66 ± 0.78	5.2 ± 0.75*

*Depict significant variation in value.

Table 7: Group- II Post therapeutic hemato-biochemical evaluation

Parameters	Mean values (0 day of therapy) (n=3)	Mean values (5 day of therapy) (n=3)
Hemoglobin (g/dl)	10.43 ± 0.65	10.7± 0.51
PCV (%)	32 ± 2.94	33.3± 4.64
TEC	5.04 ± 0.34	5.11± 0.19
TLC	24.83 ±1.75	21.17± 1.86
N (%)	86.33 ± 3.77	82 ± 2.45
L(%)	28.67 ± 14.83	33 ± 4.09
ESR (mm/hr)	75 ± 10.8	75.6 ± 11.14
SGOT (IU/l)	98.87 ± 16.01	82.07 ± 21.32
Ca (mg/dl)	10.65 ± 0.89	9.57 ± 0.65
Ph (mg/dl)	2.40 ± 0.31	2.25 ± 0.19

CONCLUSION

Present study of 20 hypophosphatemic dairy buffaloes inferred that inappetance- anorexia (95%) and hypogalactia (55%) are the predominant findings in affected animals. Post- parturient buffaloes in 4-7 year age group found to be most affected. Haemato-biochemical analysis revealed ESR and phosphorous level as prognostic indicators following therapy. There is significant increase in phosphorous level (5.2+0.75 mg/dl vs 2.66+0.78 mg/dl) following sodium acid phosphate administration in

comparison to buffered phosphorous injection 2.55+0.19 vs 2.4+0.31 mg/dl). Based on present clinical trial, it is concluded that line of treatment for hypophosphatemic buffaloes should be sodium acid phosphate @120 gm in 600 ml half dose i/v and half subcutaneously followed by same dose orally for 5 days along with supportive therapy.

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