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Research Article

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Therapeutic Approach in Field Clinical Cases of Hypophosphatemia in Pregnant Murrah Buffaloes of Malwa Region

Shahzada Mudasir Rashid[1,2*], Muneeb U Rehman[1], Masrat Rashid[3], Rayeesa Ali[1], Shahzada Adil[4], Manzoor ur Rahman[1] , Sheikh Bilal[1] and Bilal ahmad Mir[1]

[1]Faculty of Veterinary Science and Animal Husbandry, Sher-e Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), Shuhama, Alustang, J&K-190006

[2]Collage of veterinary sciences Rasalpora Mhow Indore (MP)

[3]Department of Drug and food control organization, Govt of J&K

Jamia Millia Islamiya, Jamia Nagar, New Dehli

*Corresponding Author E-mail: mudasir@skuastkashmir.ac.in

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ABSTRACT

Present study was designed on hypophosphatemic pregnant Murrah buffaloes of different age groups belonging to Malwa region of MP. 30 field clinical cases of hypophosphatemia were diagnosed and treated. The diagnosis was made by symptoms like history of passing coffee colored urine, reduced appetite and milk production followed by interpretation of Hemato-biochemical parameters which revealed high mean concentration of iron, molybdenum potassium, blood glucose, creatinine, bilirubin, blood urea nitrogen, total bilirubin, serum alkaline phosphatase, serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), erythrocyte sedimentation rate (ESR), neutrophil and lymphocyte count . While significantly low concentration of glucose-6 phosphate dehydrogenase (G6PD), serum albumin, total protein, copper, red blood cells (RBC), hemoglobin (Hb) and packed cell volume (PCV) were found.

The study was conducted on 40 animals, out of which 10 normal pregnant Murrah buffaloes placed in group A were included in study as control. The remaining 30 diseased animals were placed in group B which was further subdivided into two sub groups: sub group B_1 comprising of 10 and sub group B_2 of 20 diseased animals. All buffaloes of group B were treated with sodium acid phosphate with difference of dose. The sodium acid phosphate was given @ 3mg/kg twice a day for 7 days by i.v route to sub group B_1 and @ 15 mg/kg twice a day for 7 days to sub group B_2 buffaloes. The supportive therapy was same in duo groups. No signs of recovery were seen in sub group B_1; however the recovery was seen in sub group B_2 in which phosphorus was given at higher doses. In conclusion the administration of inorganic phosphorus @ dose rate of 15 mg/kg b .wt facilitated the recovery in hypophosphatemic pregnant Murrah buffaloes.

Key words: *Hypophosphatemia, Hemoglobinurea, Murrah, Phosphorus*

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INTRODUCTION

Buffalo (*Bubalus bubalis*) plays an important role in the agricultural economy of India by providing essential proteins to human population in the form of milk and meat in addition to draught power. In spite of having good production potential the buffalo is vulnerable to various fatal diseases and thus the farmers bear heavy economic losses. Among the raging and fatal diseases parturient haemoglobinuria (Hypophosphatemia) is a major problem, which threatens a considerable number of buffaloes every year in India.

Hypophosphataemia is a well-known metabolic disorder of cattle and buffaloes resulted from deficiency of phosphorous or imbalance in Ca:P ratio. Phosphorus deficiency leading to hypophosphataemia may play a part in haemoglobinurea by decreasing red cell glycolysis and ATP synthesis. It is a sporadic disease affecting high ratio of buffaloes, and considered to be of dietary origin, as a result of prolonged feeding on barseem Nassif [1]; Selim *et. al*.[2] and Rizk *et. al*[3].

Hypophosphataemia affects recently calved and heavy lactating buffaloes. Buffaloes at the 3rd to 6th lactations are most commonly affected. The disease usually appears in the period from 2nd to 4th weeks after calving, it was considered that there was much more drainage of this element through milk Chugh *et. al*[4].

Post-parturient haemoglobinuria/hypophosphataemia is characterized by intravascular haemolysis, anemia, and haemoglobinuria MacWilliams *et. al* [5].The high risk period for the occurrence of this disease is advanced pregnancy and early lactation Jubb *et. al*.[6]; Larsen *et. al*.[7] and Whitaker *et. al*.[8] The most prominent symptoms of hypophosphataemia in buffaloes include haemoglobinuria, inappetence, depression of milk yield, pale mucous membranes, weakness associated with osteomalacia, muscles weakness, lameness, locomotor disturbances, staggering gait, recumbency occurs later in severe hypophosphatemic animals El-Magawry *et. al*.[9]; Rizk *et. al*.[3] and Hoda[10].

The treatment protocol suggests parenteral or oral supplementation of phosphorus, which is given as inorganic phosphate. Sodium acid phosphate is the best studied PO_4 salt, with well documented action to treat hypophosphatemia in cattle and other animals after i.v infusion Charron *et. al.*[11] and Horner *et. al*.[12]. PO_4 salts show limited solubility with some minerals like Ca or Mg Sachs *et. al.*[13] To overcome this problem, more soluble phosphorus containing compounds like phosphite (Po_2), hypophosphite (Po_3), organic substances such as sodium glycerophosphate, butylamino-methylethyl-phosphoric acid, dimethylamino-methylphenyl-phosphinate or 4-dimethylamino-2-methylphenylphosphinic acid, and aminoethyl dihydrogen PO4 are in vogue to correct hypophosphatemia.

MATERIAL AND METHODS

30 advanced pregnant hypophosphatemic Murrah buffaloes of district Mhow (M.P, India) were selected with clinical signs of disease. Clinical examination revealed normal rectal temperature, increased respiration, pale mucous membrane, and anorexia. The color of the urine was red, dark red to coffee colored and its pH was strongly alkaline. For confirmation the animals were subjected to laboratory examination. Differential diagnosis was ruled out through laboratory tests.

About 15 ml of blood was collected from each animal by venipuncturing the jugular vein, out of which 5 ml of blood was stored in sterile anticoagulant (EDTA@ 1 mg/ml) containing test tube for carrying out hematological studies and remaining 10 ml was collected in sterile anticoagulant free test tube for separation of serum.

After keeping the serum containing test tubes in standing conditions for half an hour, they were centrifuged at 3000 rpm for 15 minutes for proper separation of serum from coagulated blood. Serum was separated and stored in aliquots at -20°C.

Blood glucose, blood urea, serum creatinine, serum total bilirubin, serum albumin, total protein, serum globulin, G6PD,

serum alkaline phosphatase, serum ALT and serum AST were determined by using autoanalyzer and commercially available kits. Serum samples were analyzed for the determination of phosphorus, calcium, copper, iron, potassium and molybdenum. These parameters were calculated by using atomic absorption spectrophotometer (Varian Spectr AA-5).

The animals of subgroup B_1 as encountered were treated with sodium acid phosphate @ 3mg/kg body twice a day by intra venous route (iv), however the latter encountered 20 animals placed in Sub group B_2 as selected during field practice were administered with inorganic phosphorus @ 15 mg/kg body weight twice a day; morning by IV route and evening by subcutaneous route (SC) for 5 days .The treatment protocol is summarized in the table 1 as hereunder:

RESULTS

Biochemical studies revealed high mean concentration of blood glucose, serum creatinine, blood urea nitrogen, total bilirubin, serum alkaline phosphatase, serum ALT, serum AST. While significantly low concentration of G6PD, serum albumin, total protein were found (Table-2). Mineral profile revealed high mean concentration of iron, molybdenum and potassium, while significantly low concentrations of phosphorous and copper were found (Table 3). These parameters are suggestive of confirmation of hypophosphatemia Akther *et. al*. [14]; Mehmood *et. al.*[15]. The animals of subgroup A_1 did not show any recovery signs as they continue to excrete red color urine even after 7 days of treatment. The animals belonging to subgroup B_2 recovered following 3 days of treatment.

Table 1: Treatment protocols envisaged in Group B_1 and Group B_2

S.No.	Treatment Protocol	Subgroup B_1 (10 cases)	Subgroup B_2 (20 cases)
1.	Inj. Sodium acid phosphate	Given @ 3mg/kg body .wt every day (twice) for 7 days by intra venous (iv) route	Given @ 15mg/kg body wt twice a day-Morning by i.v and evening by subcutaneous route every day for 5 days
2.	Inj Ferritas (Fe, Follic acid)	5 ml daily for 3 days by i.m route	5 ml daily for 3 days by i.m route
3.	Inj Rintose (Dextrose, Sodium choloride, potassium chloride, calcium chloride)	2 liters every day for 5 days by i.v route	2 liters every day for 5 days by i.v route

Table 2: Biochemical parameters (Mean ±SE) in hypophosphatemic buffaloes

S.No.	Parameters	Group A (N=10)	Group B (N=30)			
			Sub Group B1 N=10		Sub Group B2 N=30	
			Before Treatment	After Treatment	Before Treatment	After Treatment
01	Blood Glucose(mg/dl)	58.75 ± 1.878	83.5± 3.338**	78.32 ± 0.3211	85.5± 2.198**	60.58 ± 0.6756
02	Blood urea(mg/dl)	36.88±1.505	52.13± 1.747**	51.33±0.212	49.98± 1.342**	39.81±1.458
03	Serum creatinine(mg/dl)	1,353±0.08235	2.179 ±0.1044**	2.532±0.0345	2.214 ±0.1074**	1.934±0.2892
04	Serum total bilirubin (mg/dl)	0.7 ± 0.0319	4.,613± 0.6226**	3.117 ± 0.2092	4.212± 0.5189**	1.645 ± 0.1563
05	Serum albumin(g/dl)	3.04±0.10	2.58±0.20*	3.01±0.50	2.54±0.13*	2.98±0.20
06	Total protein(g/dl)	6.94±0.12	5.44±0.27**	6.48±0.10	5.81±0.54**	6.45±0.22
07	Serum globulin(g/dl)	3.90±0.18	3.30±0.36 NS	3.47±0.41	3.27±0.41 NS	3.47±0.22
08	G-6PD mU/10^9	110.78± 17.76	90.67± 12.63**	95.31± 9.34	91.22± 13.40**	97.80± 13.68
09	Serum alkaline phosphatase (U/I)	79.5± 6.059	170.7± 5.53**	128.2± 4.256	173.1± 4.86**	85.4± 5.329
10	Serum ALT(U/I)	14.42± 0.6882	39.47± 1.119**	37.33± 1.6588	38.66± 1.0746**	16.94± 0.9753
11	Serum AST(U/I)	97.35 ± 1.765	121.8 ± 2.557**	119.32 ± 1.472	119.3 ± 3.652**	96.96 ± 0.964

** Highly significant * Significant NS Non Significant

Table 3: Serum mineral profile (Mean ± SE) in Hypophosphatemic buffaloes

S.No.	Parameters	Group A (N=10)	Group B (N=30)			
			Sub Group B1 N=10		Sub Group B2 N=20	
			Before Treatment	After Treatment	Before Treatment	After Treatment
01	Phosphorus(mg/dl)	4.95 ± 0.254	1.88± 0.1133**	4.95 ± 0.254	1.88± 0.1133**	4.67 ± 0.765
02	Calcium mg/dl)	9.53± 0.2495	8.405 ± 0.7935NS	8.53± 0.2495	8.405 ± 0.7935NS	7.87± 0.611
03	Copper (ug/dl)	117.7 ± 1.476	68 ± 1.513**	117.7 ± 1.476	68 ± 1.513**	111.8 ± 2.341
04	Iron(ug/dl)	161.4 ± 3.545	221.96 ± 3.438**	161.4 ± 3.545	221.96 ± 3.438**	164.8 ± 4.222
05	Molybdenum(mmol/l)	61 ± 3.576	179.1 ± 12.9**	61 ± 3.576	179.1 ± 12.9**	75.88 ± 4.151
06	Potassium (mmol/l)	4.45± 0.27	12.68 ± 0.44**	4.45± 0.27	12.68 ± 0.44**	5.77± 0.88

** Highly significant * Significant NS Non Significant

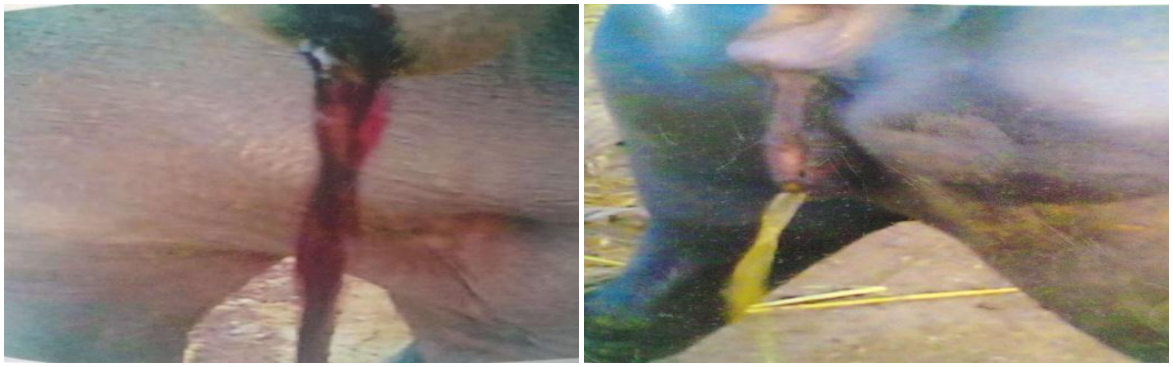

Fig. A **Fig. B**

Fig. 1: A. Urine of Hypophosphatemic buffalo showing haemoglobinuria. **B**. Recovered buffalo after treatment.

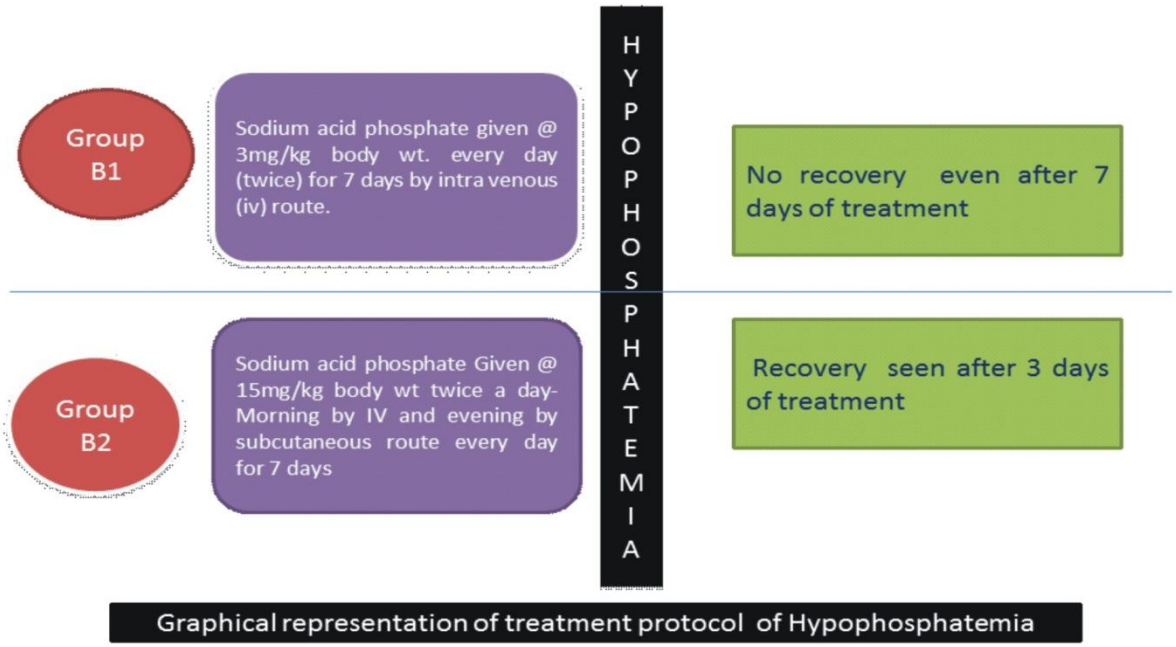

Fig 2: Graphical Abstract

DISCUSSION

The exact aetiology and pathogenesis of hypophosphataemia is not known as yet, variety of aetiological factors had been reported to be associated with disease in different parts of world. The pregnant buffaloes are mostly affected during summer season possible due to consumption of cruciferous fodder or unavailability of phosphorous rich fodder Akther *et. al*.[16] Cellular integrity depends on phosphorus as it forms an integral part of the phospholipids that form cell membrane. The phosphorus deficiency can reduce the adenosine triphosphate content in red blood cells, influencing the structure and function of cell, thereby increasing fragility and haemolysis, which may lead to acute haemoglobinuria Mahmutet *et. al*.[17] and Ogawa *et. al.*[18]. The normal reference value of 4-8mg/dl in dairy animals is suggested by literature Satter [19]. Walter [20] reported the low plasma phosphorus level of 1.8mg/dl as the phosphorus requirements are high during late gestation and onset of copious milk production. The buffaloes could not meet out the requirement of phosphorus due to less dietary supplementation and it served as a predisposing factor for the onset of disease.

The treatment protocol as followed was to delimit any use of solutions containing phosphite or hypophosphite salts because the animal is unable to convert biologically inactive phosphite into phosphate, instead sodium acid phosphate was used. The first 10 clinically ill animals of subgroup B_1 which were given inorganic phosphorus @ 3mg/kg body weight twice by intra venous (i.v) route along with other supportive therapy showed no signs of recovery even after 7 days of treatment signifying under dosage of phosphorus. While as the latter encountered 20 animals of subgroup B_2 administered with inorganic phosphorus @ 15 mg/kg body weight twice a day morning by i.v route and during evening by subcutaneous route for 7 days showed signs of recovery with red color of urine changing to normal in 3 days, improvement in appetite was seen after 4 days of treatment. The different dosage recommendations in cattle as suggested by the literature are from 13 to 20/Kg body weight Goff [21]; McGaughan [22] and Staufenbiel [23]. The Ca: P does not show marked derangement in pregnant Murrah buffaloes due to the reason the buffaloes in advanced pregnancy were dry, limiting the drainage of Ca through milk. This finding is in contrast with the findings of Walter [24] in cattle, who have reported the decline of serum Ca after i.v administration of Pi in lactating hypophosphatemic cows Walter [24]. The subcutaneous administration of sodium acid phosphate in buffaloes as done during evening does not cause any local tissue irritation; the tissue irritation has been seen by subcutaneous administration of sodium acid phosphate in cows by McGaughan[23].

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REFERENCES

1. Nassif, N.M., Nutritional problems affecting calcium, phosphorus and agnisium metabolism in Egyptian cattle and buffaloes. Ph.D. thesis. *Fac. Vet. Med. Za.* University of Egypt. (19995).
2. Selim, H.M., Abd-Allal, Attia., Field investigations on hypophosphataemia in Egyptian buffaloes: Risk factors, clinical, haematological and biochemical studies with trial of treatment. 8th sci. con. *Fac. Vet. Med. Assiut.* Univ. Egypt. 543-557 (1998).
3. Rizk, H.I., Kamel, A.A. and Razek, M.S.A., Biochemical changes in subclinical cases associated with phosphorus deficiency in buffaloes at sharkia Governorate. *J. Vet. Med. Res.* **1:** 165-174 (1999).
4. Chugh, S.K., Maa, M.M. and Malik, K.S., Lowered antioxidant status of red blood cells in post parturient haemoglobinuria of buffaloes. *Vet. Rec. Commun*. **22(6):** 385-388 (1996).

5. MacWilliams, P.S., Searcy, G.P., Bellamy, J.E.C., Bovine post-parturient haemoglobinuria. *Can. Vet. J.* **23:** 309-312 (1982).
6. Jubb, T.F., Jerrett, I.V., Browning, J.W. and Thomas K.W., Haemoglobinuria and hypophosphatemia in post parturient dairy cows without dietary deficiency of phosphorus. *Aust. Vet. J.* **67:** 86-89 (1990).
7. Larsen, T., Moller, G. and Bellio, R., Evaluation of clinical and clinical chemical parameters in periparturient cows. *J. Dairy Sci*. **84:** 1749-1758 (2001).
8. Whitaker, D.D., Goodger, W.J., Garcia, M., Perera, B.M.A.Q. and Wittver, F., Use of metabolic profiles in dairy cattle in tropical and subtropical countries on small holder dairy farms. *Prev. Vet. Med.* **38:** 119-131 (1999).
9. El-Magawery, S., Nasr, M.H., Naser M.Y. and Abo El-Eneen G.E., Clinicobiochemical and epididemiological aspects of Egyptain buffaloes. *Ind. J. Sci*. **66 (11):** 1123-1125 (1995).
10. Hoda, I.M., Clinico-Biochemical studies on hypophosphataemia in baffaloes with some therapeutic traits. Ph.D. *Vet. Med. Aaaiut. Univ*. Egypt (2006).
11. Charron, T., Bernard, F., Skrobik, Y., Simoneau, N., Gagnon, N. and Leblanc , M., Intravenous phosphate in the intensive care unit: more aggressive repletion regimens for moderate and severe hypophosphatemia. *Int Care Med*. **29(8):** 1273-8 (2003).
12. Horner, S. and Staufenbiel, R., The influence of different therapeutic substances applieable for phosphat substitution on the concentraion of phosphore in the blood. *Int. Care Med*. 29: 1273–8 (2004).
13. Sachs, M. and Hurwitz, S., The efficacy of calcium and hypophosphite in raising plasma calcium and inorganic phosphate levels in the blood of normal dairy cows. *Refu Vet.* **29:** 153–8 (1972).
14. Akhtar, M.Z., Khan, A., Khan, M.Z. and Muhammad, G., Haemato-Biochemical phosphates of Parturienthaemoglobinurea in buffalo. *Turk. J. Vet. Anim. Sci*. **31(2):** 119-123 (2007).
15. Mahmood, A., Khan, M.A., Younis, M.A., Khan, M.A., Ahad, A., Ahmad, M., Iqbal, H.J., Fatima, Z. and Anees, M., Haematological and Biochemical risk factors of Parturient haemoglobinuria in buffaloes. *J. of Ani. and Plant Sci*. **23(2):** 364-368 (2013).
16. Akhtar, M.Z., Khan, A., Zaman, T., Ahmad, N., Some clinic-epidemiological and biochemical observations of parturient haemoglobinuria in Nili Ravi buffaloes. *Pak. Vet. J.* **26(4):** 151-156 (2006).
17. Mahmutet, O.K., Guzelbektes, H., Ismail, S., Alparslan, C., Aliye, S. O., Post-Parturient haemoglobinuria in three dairy cows. *Bull Vet. Inst. Pulawy*. **53:** 421-423 (2009).
18. Ogawa, E., Kobayashi, K., Yoshiura, N. and Mukai, J., Hemolytic anaemia and red blood cell metabolic disorder attributable to low phosphorus intake in cows. *Am. J. Vet. Res*. **50:** 388-392 (1997).
19. Satter, L., What goes in must come out-phosphorus balance on dairy farms. *The Amer. Assoc. Bov. Pract. Proceedings*. **35:** 125-130 (2002).
20. Walter, G., Treatment of phosphorus balance disorders. Clinic for cattle, Uni. Of Vet Med Hannover foundation Bischofsholer Damm 15,30173 hand over, Germany. (2011).
21. Goff, J.P., Treatment of calcium, phosphorus, and magnesium balance disorders. *Vet. Clin. North Am. Food Anim. Pract*. **15:** 619–39 (1999).
22. McGaughan, C.J., Treatment of mineral disorders in cattle. *Vet. Clin. North Am. Food Anim. Pract.* **8:** 107–45 (1992).
23. Staufenbiel, R., Dallmeyer, M. and Horner, S., Hinweise, Z., Therapie des atypischen Festlegens Proc. 2. Leipziger Tiera¨rztekongress. Leipzig, Germany, **17:** 288–91 (2002).
24. Walter, G., Treatment of Phosphorus Balance Disorders. *Vet Clin Food Anim*; 383-408 (2014).