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Short Communication

THERAPEUTIC EFFICACY OF SODIUM ACID PHOSPHATE IN COMBINATION WITH DCP AND SSP IN HAEMOGLOBINURIC BUFFALOES

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ABSTRACT

This study was conducted to plan with the view to evaluate therapeutic efficacy of sodium acid phosphate in combination with Di-calcium phosphate (DCP) or single super phosphate (SSP). Total 28 buffaloes were subjected to this study from Faisalabad and Lyaah. Animals were splitted into Groups A and B (n=14). Group A; treated with sodium acid phosphate, (Inj.Alphos-40™), 200-250ml slow i/v b.i.d along with DCP @ 120g b.i.d. orally. Group B; treated with sodium acid phosphate (Inj.Alphos-40™)200-250 ml slow i/v b.i.d. and super juice @ 200mlb.i.d. orally. At the end of trial (7 days) blood samples were taken for serological tests. Phosphorous serum concentration was significantly ($P<0.01$) higher after the treatment in both groups than before the treatment. Similar trend was observed in total antioxidant system parameter but could not attain the optimal level as in healthy buffalo. Total cost on SSP given to one buffalo for 7 days was calculated to be low (PKR 4.00) as compared to DCP (PKR 90.78). The present study results indicate that super juice is effective as DCP, comparatively economical phosphorus supplement and acts as antioxidant in PHU affected buffaloes.

Key words: Di-calcium phosphate, super juice, total antioxidant status, phosphorus, buffaloes, parturient haemoglobinuria.

INTRODUCTION

Parturient hemoglobinuria (PHU) is one of the economically important metabolic disordersin dairy animals (Muhammad *et al*., 2000; Akhtar,2006b; Ghanem and El-Deeb, 2010). Parturient hemoglobinuriaoccurs as an acute sporadic disorderof high yielder dairy buffaloes and cows with intravascular hemolysis as the major pathogenic effect and clinically characterized by hemoglobinuria and anemia (Jubb *et al*., 1985; MacWilliams *et al.*, 1982; Radostits *et al*., 2007; Mahmood *et al*., 2013). Sequelae of this condition, low milk production, poor body condition, pica and reproductive loses (18.1%), add to the miseries of farmers already living with meager resources especially in impoverished countries (Muhammad *et al*., 2000).Several risk factors are associated with PHU. Dietary phosphorus deficiency and rations containing cruciferous plants (brassica, cabbage, turnips etc.) are the presumed causes of hypophosphatemia and associated hemolytic anemia in cows. Moreover, prolonged feeding of Berseem (*Trifoliumalexandrinum)* having low phosphorus contents is also associated with PHU. On the other hand, low levels of erythrocyte protective factors, antioxidants and indirect antioxidant enzymes (superoxide dismutase, glutathione peroxidase, reduced glutathione and decreased activity of glucose-6-phosphate dehydrogenase) have reportedly been associated with this condition, however, phosphorus deficiency has most consistent association with PHU (MacWilliams, 1982;Singari*et al*.,1991; Heuer and Bode,1998; Chhabra *et al*.,2015).

Standard treatment of PHU is based on parenteral and oral administration of sodium acid phosphate (Na_2HPO^4) together with oral dosing of Di-calcium phosphate (DCP) (Radostits *et al*., 2007). Super juice, prepared from single superphosphate fertilizer, has been used in dairy management practices in Australia mentioned by Radostitis *et al*. (2007). It's effect as nutritional supplementation on dry mater intake and weight gain in Sahiwal calves (Mohammad *et al*., 2005; Ishaq *et al*., 2006) has been reported while its use as a remedy of phosphorus deficiency related disorders like PHU has not been studied. This paper describes the therapeutic effects of sodium acid phosphate when combined with DCP or SSP against PHU in buffaloes. Additionally, cost effectiveness of DCP vis-à-vis SSP as phosphorus supplements and anti-oxidant status of PHU affected buffaloes (Pre- and post-treatment) are also studied.

MATERIALS AND METHODS

A total of 28 buffaloes suffering from PHU either in early lactation(n=22) or late gestation(n=08) were included in this study. These buffaloes were randomly divided into two groups of 14 viz. A and B. Matching co-hort, healthy buffaloes (n=10) were kept as control. The PHU was diagnosed clinically on basis of specific signs such as haemoglobinuria and characteristic straining while defecation (Radostits *et al*., 2007;

Muhammad *et al*., 2000). Possibility of hemoparasites was ruled out in study animals by microscopic examination of Giemsa stained blood smear. Information regarding management, clinical signs and treatment of the affected animals was entered in a "data capture form".

Treatment: Group A: buffaloes pertaining to this group were treated with sodium acid phosphate, (Inj.Alphos-40™[1], 200-250ml slow i/v b.i.d along with DCP @ 120g b.i.d. orally for seven days consecutively (Radostitis *et al*., 2007). Group B was treated with treated with sodium acid phosphate (Inj.Alphos-40™ 200-250 ml slow i/v) b.i.d . and super juice @ 200ml per animal b.i.d. orally up to seven days. Super juice was prepared according to the method used by Radostits *et al*. (2007) described as one Kg of the single superphosphate fertilizer was dissolved in 16 liters of water and stirred vigorously. After 12 hrs. the supernatant of the settled solution was used for oral administration in affected animals.

Blood sampling: A 15ml blood sample was collected from healthy and affected animal before and after treatment (7 days) to determine serum inorganic phosphorus level and total antioxidant status (TAS). Evaluation criteria for recovery were clinical examination and the urine color of the affected animals.

Statistical analysis: Comparison of serum phosphorus levels and total antioxidant status before and after treatment in PHU affected buffaloes were determined by using One-Way ANOVA (Steel *et al.,* 1997) at P<0.01 significant level

RESULTS

All the study animals were stall fed. Berseem (*Trifoliumalexandrinum*) was the major fodder for all (100%) the animals. In the present study,32% of buffaloes were in their 4th lactation. At the time of treatment, buffaloes suffering from PHU in both groups exhibited clinical signs as dullness (100%), anemia (100%),dehydration (100%), complete anorexia(21%), reduced milk production (70%), straining with constipation (17%) and straining without constipation (46%). The color of urine in hemoglobinuric buffaloes ranged from dark red oxide (46%), to red oxide (54%) depending upon the severity of the disease.

On the basis of disappearance of clinical signs (hemoglobinuria) and net survival rates, both treatments had same efficacy (85.71%) and mortality rate was 14.28% in each group. Mean serum phosphorus concentration was 2.057 ± 0.102 mg/dl in group A and 1.979 ± 0.140 mg/dl in group B before treatment. Mean phosphorus levels in healthy buffaloes was 5.64±0.309 mg/dl. Statistical analysis revealed significantly lower (P≤0.01) phosphorus level in diseased buffaloes compared to healthy ones prior to treatment. (Table1). Mean serum phosphorus concentration was 6.136±0.250 mg/dl in group A and 5.879 ± 0.131 mg/dl in group B after treatment. The comparison of these mean values with normal buffaloes having serum phosphorus level 5.64±0.309 mg/dl showed that the diseased buffaloes had a more serum phosphorus levels than healthy buffaloes after treatment. Group A had a slightly higher serum phosphorus level than group B after treatment but statistically non-significant (Table 1). The local price of one kg of DCP and SSP was PKR 54 (0.51 USD) and 20 (0.18 USD), respectively. DCP and SSP were given orally up to seven days.

Total antioxidant status (TAS) was 0.305 ± 0.024mM in group A and 0.284 ± 0.035mM in group B before treatment (Table 2). The comparison of aforementioned values with normal value determined in healthy control buffaloes (1.59 Mm) revealed that the deficiency of TAS was highly significant (P≤0.01) in the hemogobinuric buffaloes. (Table 2). After seven days treatment, TAS values showed improvement (1.309 ± 0.067 and 1.290 ± 0.056 Mm in group A and B, respectively) than that of before but still a significant deficiency of TAS was observed. (Table 2).

Table 1. Comparison of serum phosphorus levels (mean±SD) of buffaloes in different groups.

Group	Serum phosphorus levels (mg/dl)	
	Pre-treatment	Post-treatment
A	2.057± 0.102[b]	6.136± 0.250 [a]
B	1.979±0.140[b]	5.879±0.131 [a]
Control	5.649±0.309[a]	5.649±0.309 [a]

Means sharing different superscripts ([a, b]) in a columnare statistiscally different at P<0.01 significant level

Table 2. Comparison of total antioxidant status (mean±SD) of buffaloes in different groups.

Group	Total antioxidant status (mM)	
	Pre-treatment	Post-treatment
A	0.305±0.024 [b]	1.309±0.067 [a]
B	0.284±0.035 [b]	1.290±0.056 [a]
Control	1.590±0.052 [a]	1.590±0.052 [a]

Means sharing different superscripts ([a, b]) in a column are statistiscally different at P<0.01 significant level

DISCUSSION

Therapeutic effects of sodium acid phosphate when combined with Dicalcium phosphate or single super phosphate, and total antioxidant status in bubaline parturient hemoglobinuria were investigated in this study. Phosphorus deficient, Berseem (*Trifoliumalexandrinum*), is considered to be responsible for this metabolic condition (Heuer and Bode, 1998; Muhammad *et al*.,

2000). Use of rich sources of phosphorus e.g. concentrates, grains and cotton seed meal were not observed by resource-poor dairy farmers of study area. Maximal milk production is yielded in fourth lactation when drainage of phosphorus from body through milk causes imbalance of phosphorus along with other minerals (Dhillon *et al*., 1972). These findings are generally in line with previous reports (Akhtar *et al*., 2006a; Jain *et al*., 2009). Arif, 1997 had reported range of urine colors due to intravascular hemolysis i.e. coffee color, reddish brown, brown and red oxide color of urine in PHU affected animals.

Mortality in PHU might be due to anemic anoxia caused by excessive hemolysis (Radostitis *et al*., 2007). Low serum phosphorus level in PHU affected animals has been recorded in various studies (Joshi *et al*., 1991; Jain *et al*., 2009; Heuer and Bode, 1998; Durrani *et al*., 2010). Use of sodium acid phosphate and DCP in PHU has been evaluated for their therapeutic effects (Radostitis *et al*., 2007; Gahlawat *et al*., 2007). Use of DCP may cause detrimental effects that attributed to the high amount of iron i.e 1% (10,000 ppm), above than recommended level by National Research Council (NRC), which potentiate the oxidant or pro-oxidant biochemical processes in the body. Moreover, iron interacts with zinc, copper and manganese in the intestine leading to their reduced absorption (Chhabra *et al.,* 2007). Although effects of single superphosphate fertilizer as a phosphorus supplement on weight gain and dry matter intake in Sahiwal calves has been reported in different studies (Muhammad *et al*., 2005; Ishaq*et al*., 2006), but its use as a remedy in PHU is reported for the first time in the present study. In present study super juice feeding affirmed to be comparatively cheaper than DCP for the treatment of PHU in buffaloes.

Oxidative stress has been reported (Bhardwaj *et al*.,1988; Singar*et al*.,1989; Gahlawat*et al*.,2007; Sordillo and Aitkena,2009; Ahmed *et al*.,2010) for reproductive disorders and PHU in buffaloes. Researchers (Stern, 1985; Gahlawat*et al*., 2007) opined that oxidative stress in PHU affected buffaloes was due to excessive lipid peroxidation of red blood cell membranes. Gahlawat*et al*. (2007) established for the first time that use of sodium acid phosphate elevate the glutathione level in PHU and results of present study followed the same pattern. Antioxidant action of sodium acid phosphate might be attributed to the phosphate ions which is necessary mineral for the the synthesis and action of many antioxidant enzymes (Valdimirov*et al*., 1980).

Since TAS increased in both groups of present study, it can be assumed that SSP also worked much the same and increased TAS of the animals. Mean values of TAS increased after treatment but remained lower than the healthy animals.It could be rationalized due to decreased activity of glucose-6-phosphate dehydrogenase essential for regeneration of reduced glutathione) in PHU affected buffaloes (Singari *et al*., 1991). Besides, rate of regeneration of antioxidants in red cells was reported to be slower in buffaloes as compared to other ruminants (Suzuki, 1985).

Conclusion: It is conceivable from these findings that single super phosphate fertilizer potentially compensates the phosphorus requirements and enhances the total antioxidant status in PHU affected buffaloes.

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