

## Evaluation of Stress and Metabolic Adjustments in Buffaloes with Post Parturient Haemoglobinuria

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**Abstract:** To evaluate stress and metabolic adjustments in buffaloes with Post Parturient Haemoglobinuria (PPH), certain serum enzymes viz. Gamma-Glutamyl Transferase (GGT), Glucose-6-Phosphatase (Glc-6-Pase), aldolase, glucose-6-phosphate dehydrogenase, glutamate dehydrogenase, malate dehydrogenase, arginase and 5'nucleotidase enzymes were determined. Simultaneously haemoglobin and serum calcium, phosphorus, glucose and proteins were also determined. Blood samples were collected from adult female healthy and buffaloes with post parturient haemoglobinuria. In affected group the mean values of all the parameters were compared with respective healthy mean values. Significantly ( $p \leq 0.05$ ) increased serum GGT activity in buffaloes with PPH indicated stress. Activities of the enzymes related to glycolysis and hexose monophosphate shunt were found to be decreased in affected animals. Activities of other enzymes like Glc-6-Pase, GDH, MDH, ARG and 5'NT were found increased significantly ( $p \leq 0.05$ ) in the affected cases. This pattern was suggestive of metabolic adjustments where rate of glycolytic pathway decreased and rate of gluconeogenesis was observed to be increased in the affected cases. Correspondingly, serum glucose concentration of affected animals was significantly ( $p \leq 0.05$ ) higher. Serum calcium showed non significant ( $p > 0.05$ ) difference whereas serum phosphorus, proteins and haemoglobin levels decreased significantly ( $p \leq 0.05$ ) in affected cases. Decreased levels of phosphorus and disturbed calcium to phosphorus ratio suggested that phosphorous deficiency plays a key role in causing haemoglobinuria in buffaloes. Pattern of variation in the activities of the enzymes related to various metabolic processes revealed the metabolic adjustments. The data obtained in the present investigation support the concept that sustained PPH stress may represent an integrating mechanism underlying metabolic adjustments for homeostasis. Better understanding between PPH stress and metabolic homeostasis can be instrumental in the design of novel therapeutic strategies.

**Key words:** Buffalo, glycolytic pathway, haemoglobin, post parturient haemoglobinuria, serum enzymes

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### INTRODUCTION

Phosphorus is essential for metabolic pathways like glycolysis and other important functions at cellular level. Its contribution in preventing the cell from oxidative damage is beneficial for the health of the animals. Imbalance of ions may bring about a series of events in the body culminating in a pathology. Stress is a frequent outcome irrespective of the presence of obvious disease. Timely measures to diagnose the diseases is becoming a mandatory object of the clinical laboratories in veterinary science. Many diseases, although may not be directly

related with stress but can disturb normal metabolic activity of the animals. In high yielders maintenance of normal metabolic rate is important as partitioning of energy is there to meet the demand for adequate production of milk. Though hypophosphataemia is documented consistently in the affected buffaloes (Chugh *et al.*, 1996), great stress on the metabolic machinery can not be ignored.

Post parturient haemoglobinuria is an acute disease in high yielding buffaloes and cows associated with hypophosphataemia and characterised by intravascular haemolysis (Kurundkar *et al.*, 1981). The transition

between late pregnancy and early lactation, from calving until a 3-4 weeks postpartum is a high-risk period for the occurrence of the disease. The risk is especially high around parturition (Whitaker *et al.*, 1999). A variety of etiological factors are responsible for the disease and dietary deficiency of phosphorus is one of them. Evaluation of degree of stress developed due to disease is required to regain the physiological status of the animals by providing supplements in addition to the proper line of treatment. Hypophosphataemia is known to decrease red blood cell glycolysis and ATP synthesis. Subnormal concentration of ATP predisposes red blood cells to altered structure and function and an increase in fragility and haemolysis with resultant haemoglobinaemia and haemoglobinuria. Decreased erythrocytic Glucose-6-Phosphate Dehydrogenase (G6PDH) activity in haemoglobinuric buffaloes may be partially responsible for a decrease in reduced glutathione thereby causing oxidative stress to erythrocytes which results in haemolytic syndrome (Singari *et al.*, 1991).

Looking towards the significance of PPH in economy of buffalo raisers, it is important to understand many underlying metabolic mechanisms so that affected animals can be managed in a scientific manner. Therefore, the present investigation was planned to evaluate stress and metabolic adjustments in the buffaloes affected with PPH.

## MATERIALS AND METHODS

**Animals:** Stress and metabolic adjustments in buffaloes with post parturient haemoglobinuria were assessed by determining some serum enzymes, phosphorus, calcium, glucose and proteins. Haemoglobin was also determined. Screening of buffaloes with PPH was based on clinical signs of haemoglobinuria, low haemoglobin and urine test. Urine samples of affected buffaloes were collected and centrifuged to differentiate haematuria and haemoglobinuria. Adult buffaloes selected (25 healthy and 25 with post parturient haemoglobinuria) for the study belonged to farmers' stock from Rajasthan State, India. From each animal, two types of blood samples were collected. To harvest sera, blood samples were collected without anticoagulant and for haemoglobin determination with anticoagulant.

**Analysis:** The serum enzymes included were Gamma-Glutamyl Transferase (GGT), Glucose-6-Phosphatase (Glc-6-Pase), Aldolase (ALD), Glucose-6-Phosphate Dehydrogenase (G6PDH), Glutamate Dehydrogenase (GDH), Malate Dehydrogenase (MDH), Arginase (ARG) and 5'Nucleotidase (5'NT). Methods as described by King were employed to determine Glc-6-Pase, ALD,

G6PDH, GDH, MDH and ARG by Wolf and Williams (1973) to determine GGT and by Varley to determine 5'NT. Serum enzyme activities were measured according to the specific reaction of each enzyme by using basic standard techniques. The required temperature maintenance was carried out according to the method for each enzyme and wherever required, necessary temperature corrections were done. All results of enzyme activities were expressed as per SI units in U/L. Haemoglobin and serum parameters like phosphorus, calcium, glucose and proteins were determined as described by Oser. Statistical significance for individual parameter between healthy and affected group was analysed as per Kaps and Lamberson (2004).

## RESULTS AND DISCUSSION

The mean values of serum parameters and haemoglobin are presented in Table 1. In affected buffaloes anorexia, depression, decreased milk production, pale mucous membranes and haemoglobinuria were detected.

**GGT:** In affected group the mean value of serum GGT increased significantly ( $p \leq 0.05$ ) as compared to healthy mean value. The mean value was 4.01 times higher in affected buffaloes. This clearly showed the stress to the affected animals. Kataria and Kataria (2012) also suggested serum GGT as a biomarker of stress and metabolic dysfunctions in cattle. Earlier scientists have reported higher activity of serum GGT in the animals affected with various disease conditions like gastrointestinal parasitism and pneumonia (Kataria *et al.*, 2011).

**Glc-6-Pase:** Increased Glc-6-Pase activity indicated higher rate of gluconeogenesis. Glucose-6-phosphate is

Table 1: Mean±SEM values of serum enzymes, phosphorus, calcium, glucose, proteins and haemoglobin in healthy and buffaloes with post parturient haemoglobinuria (n = 25)

Parameters	Healthy groups	Affected groups
Gamma glutamyl transferase (U L <sup>-1</sup> )	25.20±0.440	101.22±2.900*
Glucose-6-phosphatase (U L <sup>-1</sup> )	8.99±0.010	30.63±0.020*
Aldolase (U L <sup>-1</sup> )	9.88±0.040	4.21±0.010*
Glucose-6-phosphate dehydrogenase (U L <sup>-1</sup> )	8.34±0.020	4.22±0.010*
Glutamate dehydrogenase (U L <sup>-1</sup> )	22.33±0.110	112.00±1.670*
Malate dehydrogenase (U L <sup>-1</sup> )	35.22±1.000	120.21±3.610*
Arginase (U L <sup>-1</sup> )	10.52±0.030	65.30±2.230*
5'nucleotidase (U L <sup>-1</sup> )	32.22±0.100	73.22±1.440*
Phosphorus (mmol L <sup>-1</sup> )	1.73±0.001	0.60±0.001*
Calcium (mmol L <sup>-1</sup> )	2.63±0.030	2.61±0.040
Glucose (m mol L <sup>-1</sup> )	3.06±0.004	4.33±0.005*
Proteins (g L <sup>-1</sup> )	77.80±0.040	49.80±0.030*
Haemoglobin (g L <sup>-1</sup> )	119.50±1.140	59.23±0.310*

n = Number of buffaloes in each category; \*Significant difference ( $p \leq 0.05$ ) from respective healthy mean value

dephosphorylated by Glc-6-Pase to yield free glucose which is exported to replenish blood glucose. It can also increase markedly in diseases (Koide and Oda, 1959). Variations in the rate of metabolic pathways can be physiological or suggestive of metabolic syndrome. Endoplasmic reticulum stress increases expression and activity of glucose-6-phosphatase and the capacity for glucose release and glucose cycling. Experiments have demonstrated that several pathological stresses disrupt endoplasmic reticulum homeostasis and lead to stress (Kaufman, 1999). Probably stress developed in PPH is due to higher secretion of glucocorticoids. Latter doubtlessly stimulates gluconeogenesis.

**ALD:** Mean value of serum ALD in affected animals was significantly ( $p \leq 0.05$ ) lower than healthy animals. Aldolase is a glycolytic enzyme and functions reversibly for gluconeogenesis, an important mechanism for generation of glucose in ruminants (Abdel-Fattah *et al.*, 2002). Serum aldolase activity is helpful in assessing rate of glycolytic cycle. Its decreased activity in affected animals indicated low ATP generation. Regulated glycolysis and gluconeogenesis prevent the futile cycling with loss of ATP. Since, phosphorus is necessary for glycolysis and the formation of adenosine 5'-triphosphate, probably low phosphorus in PPH is associated with decreased rate of glycolytic cycle.

**G6PDH:** Mean value of serum G6PDH in affected animals was significantly ( $p \leq 0.05$ ) lower than healthy animals. This enzyme is important for glucose oxidation through the Hexose Mono Phosphate (HMP) shunt. This pathway is essential for synthesis of fat and is the major source of NADPH to maintain the reductive environment for all biosynthetic processes. Glucose-6-phosphate dehydrogenase is considered to play a pivotal role in protection from oxidative stress. The expression of G-6-PDH is hypothesised to be modulated by free radicals during oxidative stress (Cramer *et al.*, 1995). The lower concentration of this enzyme in affected animals indicated its antioxidant type role.

**GDH:** Mean value of serum GDH in affected animals was significantly ( $p \leq 0.05$ ) higher than healthy animals. Glutamate dehydrogenase, a complex allosteric enzyme, plays a central role in amino group metabolism. Whenever a hepatocyte needs fuel for citric acid cycle, GDH activity increases making alpha-ketoglutarate available and releasing  $\text{NH}_4^+$  for excretion. The estimation of GDH as a liver function test is being emphasized in animals (Kataria *et al.*, 2010).

**MDH:** Mean value of serum MDH in affected animals was significantly ( $p \leq 0.05$ ) higher than healthy animals. Malate

dehydrogenase is an enzyme of immense significance in citric acid cycle. Malate dehydrogenase is also important in gluconeogenesis. Higher activity of MDH indicated the strategies of the animal to modulate the metabolic pathways for energy generation and glucose synthesis from noncarbohydrate precursors as in ruminants the major portion of carbohydrate available is supplied by gluconeogenesis (Abdel-Fattah *et al.*, 2002). Earlier researchers have shown the antioxidant role of MDH (Oh *et al.*, 2002) therefore higher activity of serum MDH during extreme ambiances indicated towards generation of reactive oxygen species (Vincent *et al.*, 2004).

**ARG:** Mean value of serum ARG in affected animals was significantly ( $p \leq 0.05$ ) higher than healthy animals. It is an important enzyme of urea cycle. All the enzymes of urea cycle are synthesized at a higher rate during starvation (Kour and Kataria, 2011). Increased activity of arginase indicated breakdown of proteins under the influence of glucocorticoids. Stress responses result in higher activity of glucocorticoids to increase blood glucose levels by stimulating gluconeogenesis and increased breakdown of proteins. This concept is supported by the evidence of low serum proteins and higher serum glucose in the affected animals.

**5'NT:** Mean value of serum 5'NT in affected animals was significantly ( $p \leq 0.05$ ) higher than healthy animals. The increased activity of 5'NT was probably an indicator of higher purine metabolism. Purine nucleotides are degraded by a pathway in which the phosphate group is lost by action of 5'NT. Serum 5'NT increases in diseases of liver and biliary tract in a roughly parallel manner. Serum levels of 5'NT rises in Inflammatory hepatic disease (Bardawill and Chang, 1963) and damage in the liver tissues (El Samani *et al.*, 1985). A strong link has been advocated between 5'NT and oxidative stress. Kirschner *et al.* (1996) demonstrated that 5'NT protected the cells against oxidative stress in animals. Jubb *et al.* (1990) reported that circulating oxidants may cause erythrocyte damage which may be predisposed to hypophosphataemia. Oxidative stress in the affected buffaloes could be the probable cause of increased 5'NT activity.

**Glucose:** Serum glucose in affected animals were significantly ( $p \leq 0.05$ ) higher than healthy animals. Increased blood glucose levels in affected buffaloes observed in the present study were nearly the same as reported by Pandey and Misra (1987) and Akhtar *et al.* (2008). Anorexia develops non-availability of volatile fatty acids in sufficient quantity. This stimulates glycogenolysis and gluconeogenesis. In present study the activity of Glc-6-Pase was found higher. Higher serum

activity of GGT clearly reflected stress in the affected animals. Most likely stress related higher glucocorticoids resulted in higher serum glucose concentration in affected animals.

**Proteins:** Serum proteins in affected animals were significantly ( $p \leq 0.05$ ) lower than healthy animals. Probably anorexia and increased gluconeogenesis resulted in lowered proteins in the present study. Lowered values clearly indicated mobilization of proteins to meet the energy demand of the body. Earlier scientists (Iqbal *et al.*, 2011) also observed significant decrease in serum protein concentration in affected buffaloes.

**Calcium and phosphorus:** A non significant ( $p > 0.05$ ) difference was observed in the mean values of serum calcium between healthy and affected cases. In affected buffaloes serum phosphorus was significantly ( $p \leq 0.05$ ) lower. Earlier researchers also reported lower serum phosphorus in PPH affected buffaloes (Kurundkar *et al.*, 1981; Stockdale *et al.*, 2005). The inorganic phosphate level in the blood provided an indication of the low dietary phosphate intake (Grunwaldt *et al.*, 2005). The phosphorus requirements of buffalo vary depending on the stage of growth, lactation and pregnancy. Early lactation is often associated with a decrease up to 35% in mean herd serum phosphorus concentrations from parturition to peak lactation (Betteridge, 1986). Small intestine is the site for absorption of phosphorus and faeces and urine are main excretory ways. Anorexia reduces flow of saliva and cause increased losses of phosphorus via urine (Reinhardt *et al.*, 1988). Low serum inorganic phosphorus is a frequent finding in postparturient haemoglobinuria (McCaughan, 1993). Probably heavy drainage of phosphorus through milk in high milk yielding animals leads to hypophosphataemia (Bhikane *et al.*, 1995). Developing foetus during advanced pregnancy requires more calcium and phosphorus (Digraskar *et al.*, 1991). Supplementation of minerals is essential to prevent hypophosphataemia. Maintenance of proper calcium phosphorus ratio is important for adequate absorption of phosphorus. Increased calcium to phosphorus ratio results in decreased phosphorus absorption from the intestinal tract and ultimately leads to hypophosphataemia. In present study, a higher ratio was observed in the affected animals.

**Haemoglobin:** In present study, haemoglobin concentration of affected animals reduced to a greater degree. Earlier scientists (Pandey and Misra, 1987; Iqbal *et al.*, 2011) also observed significant decrease in haemoglobin concentration in affected buffaloes which indicated severe anaemia. Probably intravascular haemolysis due to impaired glycolytic pathway and

depletion of ATP in erythrocytes results from phosphorus deficiency. Subnormal concentration of ATP predisposes red blood cells to alter functions and structure causing a loss of normal formability and an increase in fragility, ultimately leading to haemolysis.

Activities of the enzymes related to glycolysis and pentose phosphate pathway were found to be decreased in affected animals. Activities of other enzymes like Glc-6-Pase, GDH, MDH, ARG and 5'NT were increased in the affected cases. This was suggestive of metabolic adjustments in affected cases where rate of glycolytic pathway decreased and rate of gluconeogenesis increased. The data obtained in the present investigation support the concept that sustained PPH stress may represent an integrating mechanism underlying metabolic adjustments for homeostasis. Better understanding between PPH stress and metabolic homeostasis can be instrumental in the design of novel therapeutic strategies.

## CONCLUSION

It can be stated that the present investigation may be the first to provide values of certain serum enzymes viz. GGT, Glc-6-Pase, ALD, G6PDH, GDH, MDH, ARG and 5'NT in PPH affected buffaloes from arid tracts at one place. The variations observed in the present study could help in realistic evaluation of the management practice and nutrition of the affected animals. Enhanced activity of serum GGT level was suggestive of stress to buffaloes. Decreased levels of phosphorus and disturbed calcium to phosphorus ratio proposed that phosphorous deficiency plays a key role in causing haemoglobinuria in buffaloes. Pattern of variation in the activities of the enzymes related to various metabolic processes revealed the metabolic adjustments. The present study can contribute to understand the stress responses and modulations in the physiological mechanisms of PPH affected buffaloes.

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