HEMATOBIOCHEMICAL ALTERATIONS IN CLINICAL AND SUBCLINICAL HYPOPHOSPHATEMIA IN EGYPTIAN BUFFALOES (*Bubalus bubalis*)

Nasr Nasr El-Deen[1], Ahmed Neamat-Allah[1,*], Laila Rizk[2] and Rania Samy Fareed[3]

ABSTRACT

This study aimed to verify the hematological and biochemical alterations in buffaloes with clinical (CH) and subclinical hypophosphatemia (SCH). For this intention blood and serum samples from 40 adult female water buffaloes were collected. These animals were divided into 3 groups according to the clinical signs and serum phosphorous level. The first group was ten apparently clinically healthy buffaloes and was assigned as a normal control group. The second and the third groups were fifteen buffaloes for each CH and SCH groups. Anorexia, weakness, emaciation, and pale mucous membrane were the most clinical findings in all affected cattle. Red urine was an additional sign in buffaloes with CH. Mean erythrocytic count, hemoglobin concentration and hematocrit were significantly decreased in CH and SCH, while total leukocytic counts were significantly increased in both groups when compared with the control group. The main biochemical changes were significant decrease in serum levels of phosphorous, total proteins, albumin in diseased groups. Serum urea, creatinine, glucose, bilirubin were significantly elevated in both affected groups. Meanwhile, serum calcium level was significantly elevated only in the CH when compared with the control group. It could be concluded that SCH and CH are accompanied with anemia, changes in total and differential leukocytic counts, and alterations in the hepatic and kidney functions.

Keywords: *Bubalus bubalis*, buffaloes, hematology, biochemistry, hypophosphatemia

INTRODUCTION

Phosphorus has many important biological functions which make it essential for animal health. Among these functions it is important in oxidative phosphorylation, oxygen delivery, glycolysis, and maintenance of cellular structure integrity (Grunberg, 2008). Phosphorus is an essential mineral necessary for normal cell functions. Bound to oxygen in all biological systems, phosphorus is found as phosphate (PO4) in the body. Approximately 85% of the body phosphorus is found in bones and teeth. It is the major structural component of bone in the form of calcium phosphate called hydroxyapatite. Phospholipids are the major structural components

[1]Department of Clinical Pathology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt, *E-mail: drnemovete@yahoo.com, anattia@vet.zu.edu.eg

[2]Department of Buffalo's Diseases, Animal Health Research Institute, Giza, Egypt

[3]Animal Health Research Institute, Sharkia, Egypt

of cell membranes. All energy production and storage are dependent on phosphorylated compounds such as (ATP) and creatine phosphate. Nucleic acids (DNA and RNA) are long chains of phosphate-containing molecules. A number of enzymes, hormones and cell signaling molecules depend on phosphorylation for their activation. As it also helps in maintaining normal acid base balance (pH) by acting as one of the body's most important buffers (Konchel, 2006).

Hypophosphatemia is a well-known metabolic disorder of cattle and buffaloes which resulted from insufficiency of phosphorus or Ca/P imbalance (Sarma *et al*., 2014). Also, decreasing intestinal phosphate absorption, transcellular shift of phosphorous from blood into cells and increasing renal excretion of phosphate are the most common cause of hypophosphatemia (Forrester and Moreland, 1989). Acute hypophosphatemia usually affect adult buffaloes immediately after parturition (1 to 60 days post parturient) (Durrani *et al*., 2010). Also, it is commonly seen during third to sixth lactation, and it can also occur during 6^{th} to 8^{th} month of pregnancy (Kumar *et al*., 2019). Severe phosphorous depletion has been documented to be the result of ATP depletion and also 2, 3 diphosphoglycerate synthesis in RBCs that predispose red blood cells to alter function and structure causing loss of normal formability and increase fragility leading to hemolysis (Purohit *et al*., 2018). Chronic phosphorous deficiency is commonly caused by inadequate intake for long period. This can be seen in grazing animals in region with low phosphorous content in the soil (Grunberg, 2017). Also, feeding animal on phosphorous deficient diet as cabbage, berseem, wheat, rice or straw will be a result in hypophosphatemia (Purohit *et al*., 2018).

The aim of this research is to evaluate the impact of subclinical and clinical hypophosphatemia on the hematological and biochemical indices in Egyptian buffaloes.

MATERIAL AND METHODS

Animals

Forty female water buffaloes (2 to 5 years old) were selected from different areas in Sharkia governorate, Egypt through the period from January 2020 to April 2021, fed on the seasonal green fodder and were used in this study. They divided into 3 groups; the first group was 10 apparent healthy animals kept as normal control group. The second group was 15 animals suffering from moderate hypophosphatemia (sub clinical cases). The third group was 15 animals suffering from severe hypophosphatemia (clinical cases) ten of these animals were 3^{rd} to 5^{th} weeks post-partum and five were in late gestation period 8^{th} month.

Sampling

Two blood samples were collected from each animal via jugular vein puncture. The first sample was collected on EDTA vacutainer tubes for hematological studies according to standard techniques (Feldman *et al*., 2000). The second portion was collected in centrifuge tubes for separation of serum for biochemical studies.

Hematological studies

Red blood cells (RBCs), hemoglobin (Hb) concentration, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and total and differential leukocytic count were determined using automatic cell counter (Sysmex KX-21N).

Biochemical studies

Serum phosphorus and calcium were estimated by spectrophotometer using special kits according to Tietz (1990); Tietz (1995). Serum total proteins, albumin, glucose, urea and creatinine were estimated using special kits (Doumas *et al*., 1981; Doumas *et al*., 1971; Trinder, 1969; Vassault *et al*., 1986; Rock *et al*., 1987). Serum alkaline phosphatase (ALP), total, direct, and indirect bilirubin were estimated spectrophotometrically using special kits (Belfield and Goldberg, 1971; Tietz, 1995).

Statistical Analysis

All values were presented as mean (±) standard error (SE). The significant difference between the mean of the groups was statistically analyzed by one way ANOVA. The significance was set as $P<0.05$ (Tamhane and Dunlop, 2000).

RESULTS

The clinical examination of the hypophosphatemia-affected animals revealed, anorexia, pale mucous membranes, labored breathing, jugular pulsations in SCH affected buffaloes, in addition to red urine and some animals were recumbent in CH affected buffaloes (Figure 1 and 2).

DISCUSSION

Hypophosphatemia is a sporadic, seasonal metabolic disorder that affects cattle and buffaloes. It is mainly occurring due to prolonged grazing on green fodder (berseem) that resulted in inadequate phosphorous content (Zaghawa *et al*., 2019).

Subclinical hypophosphatemia can be seen in grazing animals in region with low phosphorous content in the soil and also feeding the animals on inadequate ration as feeding on cabbage, berseem, wheat, or straw (Purohit *et al*., 2018) and the disease characterized by poor growth, loss of weight, anorexia (Grunberg, 2017).

In the present study the observed clinical symptoms in SCH were low body weight, anorexia, stiffness in gait, pale mucous membrane of vulva and conjunctiva, and serum phosphorous level of 2.6 mg/dl. The same clinical signs were reported by Forrester and Moreland (1989); Grunberg (2008); Durrani *et al*., (2010); Grunberg, (2017). While animals suffering from CH had red urine and sometimes recumbency with serum phosphorous level of 1.40 mg/dl. These clinical signs agreed with those recorded by Al-Majalli (2010); Durrani *et al*. (2010); Navjot *et al*. (2017); Ziwei *et al*. (2017); Purohit *et al*. (2018).

Regarding to the hematological results, SCH revealed a significant decrease in RBCs, hemoglobin concentration, and PCV with the development of macrocytic normochromic anemia. This might be attributed to inadequate dietary intake of phosphorous, decrease absorption by the intestine and disturbed metabolism of liver. Previously the same results were obtained by Forrester and Moreland (1989); Grunberg (2008); Durrani *et al*. (2010); Kurek *et al*. (2010); Grunberg (2017).

The clinical cases of CH showed a significant decrease in RBCs count, hemoglobin concentration, and PCV with the development of macrocytic hypochromic anemia. The intravascular hemolysis is due to hypophosphatemia as mammalian red blood cells depend on phosphorous. So, phosphorous depletion resulted in reduced availability in adenosine triphosphate (ATP) and 2,3

diphosphoglycerate (2,3 DPG) synthesis in RBCs (Wang *et al*., 1985). RBCs require ATP to control cell volume and deformability, without sufficient ATP the intracellular sodium concentration rises, and the cells become more rigged and rupture (Goff, 2000). Also, the reduced ATP resulted in decrease of the membrane phospholipids which help in maintaining the shape and integrity of red cells and leads to sphero-echinocytosis (Rana and Bhardwaj, 1988). The same results were obtained by Durrani *et al*. (2010); Al-Majalli (2010); Navjot *et al*. (2017); Ziwei *et al*. (2017); Purohit *et al*. (2018).

Regarding to leukogram, the present study revealed a significant increase in total leukocytic count among the affected groups. The increase in the total leukocytic count is due to neutrophilia in both groups. The increase in neutrophilic count could be attributed to the endogenous release of stressful condition as hypophosphatemia (metabolic disorder) is the source of the release of corticosteroids (Stockdale *et al*., 2005). Also, the present study showed a significant decrease in lymphocytes in both affected groups. Such decrease is due to the release of corticosteroids that suppress lymphoid tissue and bone marrow resulting in lymphopenia (Bremmer *et al*., 2000). The same results were previously recorded by previous authors (Al-Majalli, 2010; Durrani *et al*., 2010; Navjot *et al*., 2017; Ziwei *et al*., 2017; Purohit *et al*., 2018). The present study revealed a significant decrease in serum phosphorous level in both affected groups. This decrease could be attributed to the prolonged feeding on phosphorous deficient diet as berseem, cereals, concentrates leading to a decrease in the phosphorous absorption from intestine (Akhtar *et al*., 2007). Similar results were obtained by Muhammad *et al*. (2001); Mahmut *et al*. (2009); Navjot *et al*. (2017); Ziwei *et al*. (2017); Kumar *et al*. (2019); Rahmati *et al*. (2021).

The serum calcium level was not significantly changed in the SCH group. Similar results were obtained by Al-Majalli (2010); Sarma *et al*. (2014). While in the CH group had a significant decrease in serum calcium level when compared with normal control group. The decrease could be attributed to decreased feed intake, decreased calcium absorption from the intestine and or hypoalbuminemia. Nearly similar results were obtained by Grunberg (2008); Kurek *et al*. (2010); Kumar *et al*. (2019).

The proteingram of both groups revealed significant decline in serum levels of total serum proteins, albumin and globulin, albumin globulin ratio in comparison with normal control group. The decrease in albumin globulin ratio was due to the decrease in serum albumin. The hypoalbuminemia and hypoglobulinemia might be due to the decrease in feed intake and decrease production by damaged liver. Our results come in agreement with Al-Majalli (2010); Kurek *et al*. (2010); Fayed *et al*. (2018).

Serum glucose level showed a significant rise in both groups in comparison with the normal control group. The hyperglycemia might be due to the increase in the metabolic stress in the diseased condition which leads to an elevation in the cortisol level (Marik and Bellomo, 2013). Also, hyperglycemia might occur as a result of hypocalcemia in the CH group as calcium ions are required for insulin secretion from pancreas (Kaneko *et al*., 1997). Our results come in agreement with Muhammad *et al*. (2001); Purohit *et al*. (2018); Kumar *et al*. (2019). In contrary, Mahmut *et al*. (2009); Al-Majalli (2010) reported a significant decrease in serum glucose level.

Serum urea and creatinine levels showed a significant increase in subclinical and clinical cases.

Table 1. Effect of subclinical and clinical hypophosphatemia on hematological parameters of buffaloes.

Parameters	Control	SCH	CH
RBCs (10^6/µl)	8.02 ± 0.23[a]	6.07±0.12[b]	4.06±0.16[c]
Hb (g%)	11.00±0.31[a]	9.35±0.05[b]	6.90±0.11c
PCV (%)	33.00±0.45[a]	28.05±0.42[b]	22.60±0.33[c]
MCV (fl)	41.14±2.09[c]	46.21±1.01[b]	5.66±2.40[a]
MCH (pg)	13.70±0.35[c]	15.40±0.56[b]	16.99±0.42[a]
MCHC (%)	33.33 ±1.11[a]	33.33±0.62[a]	30.01±1.06[b]
WBCs (10^3/µl)	9.23±0.45[b]	10.17±0.60[b]	11.18±0.95[a]
Neutrophils (10^3/µl)	3.44±0.34[c]	6.10±0.05[b]	7.60±0.05[a]
Lymphocytes (10^3/µl)	5.30±0.20[a]	3.50±0.10[b]	3.00±0.16[b]
Eosinophils (10^3/µl)	0.09±0.02[a]	0.07±0.01[a]	0.08±0.01[a]
Monocytes (10^3/µl)	0.40±0.08[a]	0.50±0.15[a]	0.50±0.03[a]

Values (mean±SE) within the same raw carrying different superscript letter are statistically different at P<0.05.

Table 2. Effect of subclinical and clinical hypophosphatemia on serum minerals and proteinogram of buffaloes.

Parameters	Control	SCH	CH
P (mg/dl)	6.94±0.31[a]	2.65±0.14[b]	1.42±0.43[c]
Ca (mg/dl)	10.53±0.13[a]	10.40±0.10a	8.11±0.46[b]
Ca: P ratio	1:1.52	1:3.92	1:5.71
Total proteins (g/dl)	7.13±015[a]	6.16±0.14[b]	5.88±0.13[b]
Albumin (g/dl)	3.76±0.11[a]	3.04±0.11[b]	2.72±0.12[b]
Globulin (g/dl)	3.38±0.13[a]	3.12±0.10b	3.16±0.08[b]
A/G ratio	1.05±0.50a	0.95±0.03[b]	0.86±0.08[b]

Values (Mean ± SE) within the same raw carrying different superscript letter are statistically different at P<0.05.

Table 3. Effect of subclinical and clinical hypophosphatemia on some biochemical parameters of buffaloes (Mean values ± SE).

Parameters	Control	SCH	CH
Glucose (mg/dl)	52.15±0.24^{c}	69.40±1.10^{b}	101.45±2.42^{a}
Urea (mg/dl)	32.86±0.15^{c}	39.00±1.21^{b}	48.76±1.42^{a}
Creatinine (mg/dl)	1.21±0.07^{c}	1.61±0.07^{b}	2.30±0.23^{a}
Total bilirubin (mg/dl)	0.30±0.01^{c}	1.15±0.05^{b}	2.35±0.06^{a}
Direct bilirubin (mg/dl)	0.16±0.02^{c}	0.24±0.02^{b}	0.95±0.05^{a}
Indirect bilirubin (mg/dl)	0.14±0.03^{c}	0.91±0.01^{b}	1.40±0.02^{a}
ALP (U/L)	57.00±2.12^{c}	69.27±5.00^{b}	131.00±6.50^{a}

Values (Mean ± SE) within the same raw carrying different superscript letter are statistically different at $P<0.05$.

Figure 1. Buffalo with Subclinical hypophosphatemia (SCH) showing rough coat and loss body weight.

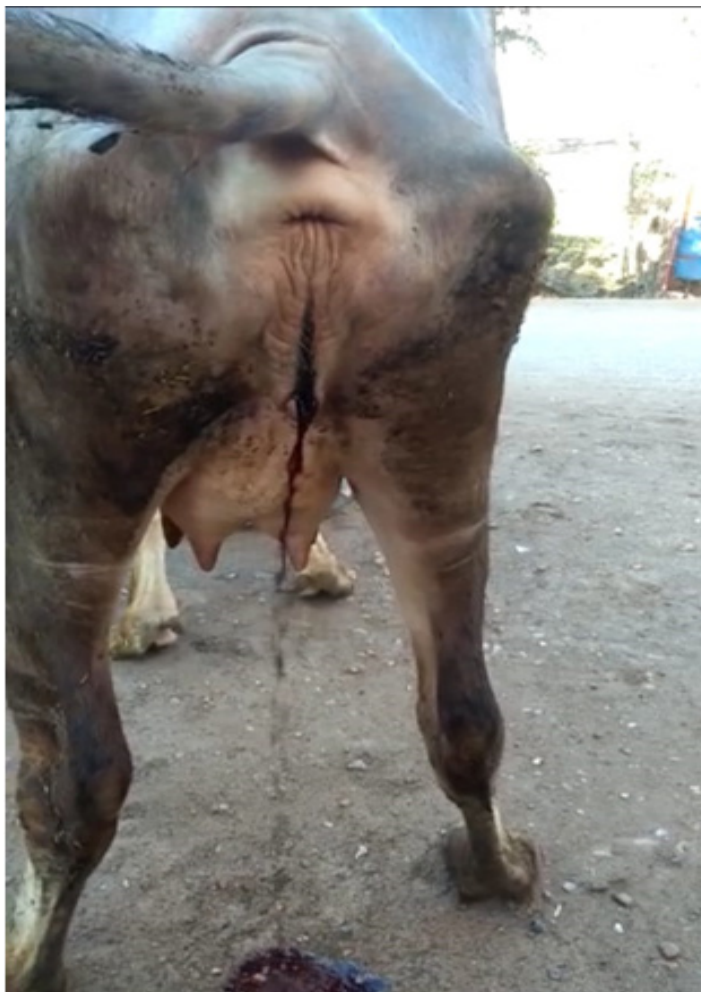

Figure 2. Figure 2. buffalo with Clinical hypophosphatemia (CH) showing red urination.

This could be attributed to the pathological kidney function (Latimer *et al*., 2003). This improper function resulted from the endogenous release of corticosteroids and tubular epithelial necrosis which leads to a decrease in renal perfusion and leads to a decrease in glomerular filtration leading to increase in the blood urea and creatinine levels (Benjamin, 1978; Stockdale *et al*., 2005). The same results go hand in hand with Muhammad *et al*. (2001); Navjot *et al*. (2017).

Serum bilirubin content is a specific indicator for biliary duct disease, hepatocellular damage, and disorders of red blood cell system (Kurek *et al*., 2010). Our study reported a significant rise in the serum total bilirubin in the second group. This might be attributed to hepatocellular failure; while in the third group there was a highly significant elevation in serum total bilirubin which could be attributed to the increase in unconjugated bilirubin that was induced by intravascular hemolytic anemia (Kaneko *et al*., 1997). The same results were agreed with those reported by Kurek *et al*. (2010); Purohit *et al*. (2018); Zaghawa *et al*. (2019).Concerning the activities of serum alkaline phosphatase (ALP), both 2nd and 3rd groups showed a significant increase when compared with the normal Control group. This could be ascribed to anemia that creates generalized hypoxia which, causes hepatocellular damage resulting in leakage of ALP into the circulation (Latimer *et al*., 2003). The same result was obtained by previous authors (Mahmut *et al*., 2009; Kurek *et al*., 2010; Purohit *et al*., 2018; Zaghawa *et al*., 2019).

CONCLUSION

It could be concluded that hypophosphatemia is a metabolic disorder characterized by low serum phosphorous level. Prevention of such cases should be via maintaining high serum phosphorous level. Thus phosphorous supplementation is very important especially in late gestation and early lactation. Supplementation of other minerals could be helpful in the prevention of hypophosphatemia.

ACKNOWLEDGEMENT

The authors express thanks. Dr. Mohamed Abdo at Animal Health Research Institute, Sharkia province, Egypt for his aid in sample collection.

REFERENCES

Akhtar, M.Z., A. Khan and G. Muhammad. 2007. Haemato-biochemical aspects of parturient haemoglobinuria in buffalo. *Turk. J. Vet. Anim. Sci*., **31**(2): 119-123.

Al-Majalli. 2010. Clinical and biochemical. *The 4th Congress of Egypt*, Egypt.

Belfield, A. and D.M. Goldberg. 1971. Revised assay for serum phenyl phosphate activity using 4-amino-antipyrine. *Enzyme*, **12**(15): 561-573. DOI: 10.1159/000459586

Benjamin, M.M. 1978. *Outline of Veterinary Clinical Pathology*, 3rd ed. Iowa State University Press, Ames, Iowa, USA.

Bhikane, A.U. and A.M. Syed. 2014. Recent trends in management of metabolic disorders of transition cows and buffaloes. *Intas Polivet*, **15**(2): 485-496.

Bremmer, D.R., S.L. Trower and S.J. Bertics. 2000. Etiology of fatty liver in dairy cattle: Effects of nutritional and hormonal status on hepatic microsomal triglyceride transfer

protein. *J. Dairy Sci*., **83**(10): 2239-2251. DOI: 10.3168/jds.S0022-0302(00)75108-3

Digraskar, S., B. Singh and B.B. Deshpande. 1991. Epidemiology and clinico-pathology of hemoglobinuria in buffalo (*Bubalus bubalis*). *Livestock Advisor*, **16**: 32-38.

Doumas, B.T., D.D. Bayse, R.J. Carter, T. Peters Jr and R. Schaffer. 1981. A candidate reference method for determination for total protein in serum. I. Development and validation. *Clin. Chem*., **27**(10): 1642-1650.

Doumas, B.T., W.A. Watson and H.G. Biggs. 1971. Albumin standards and the measurement of serum albumin with bromocryzol green. *Clin. Chem. Acta*., **31**(1): 87-96. DOI: 10.1016/0009-8981(71)90365-2

Durrani, A.Z., N. Kamal, A. Shakoori and R.M. Younus. 2010. Prevalence of post parturient haemoglobinuria in buffalo and therapeutic trials with toldimfos sodium and tea leaves in Pakistan. *Turk. J. Vet. Anim. Sci*., **34**(1): 45-51.

Fayed, H., M. Ghanem, Y. Abdel-Raof and H.M. El-Attar. 2018. Hematobiochemical and urological alterations in buffaloes with post parturient haemoglobinuria. *Benha Veterinary Medical Journal*, **34**(3): 287-294. DOI: 10.21608/BVMJ.2018.47846

Feldman, G.S., N.R. Perkins and N.C. Jain. 2000. *Schalm's Veterinary Heamatology*, 4th ed. Lea and Fibiger, Philadelphia, USA.

Forrester, S.D. and K.J. Moreland. 1989. Hypophosphatemia. Causes and clinical consequences. *J. Vet. Intern. Med*., **3**(3): 149-159. DOI: 10.1111/j.1939-1676.1989.tb03091.x

Goff, J.P. 2000. Pathophysiology of calcium and phosphorus disorders. *Vet. Clin. N. Am*., **16**(2): 319-337. DOI: 10.1016/s0749-0720(15)30108-0

Grunberg, W. 2008. Phosphorous homostasis in dairy cattle: Some answers, more questions. *In Proceedings of The 17th Annual Tri-State Dairy Nutrition Conference, Fort Wayne*, Indiana, USA.

Grunberg, W. 2017. Metabolic disorders of phosphorous metabolism, hypophosphatemia. *Veterinary Manual*. Available on: www.msd.vet.manual.com

Kaneko, J.J., J.W. and M.L. 1997. *Clinical Biochemistry of Domestic Animals*, 5th ed. Academic Press San Diego, California, USA.

Konchel, J.P. 2006. *Modern nutritional in Health and Disease*, 10th ed. Baltimore, Lippincott Williams and Wikins, Philadelphia , USA.

Kumar, A., V. Thakur, P. Sandeep, S. Harpreet, R. Swati, G. Anita, R. Biswa and R.S. Bisla. 2019. Study on incidence, haemato biochemical changes and therapeutic management of post parturient haemoglobinuria in Murrah buffaloes. *The Pharma Innovation Journal*, **8**(1): 147-150.

Kurek, L., K. Lutnicki and A. Barch. 2010. Various types of hypophosphatemia in dairy cows and the clinical implications depending on the intensity of the deficiency. *B. Vet. I. Pulawy*, **54**(1): 35-41.

Latimer, K.S., E.A. Mahaffey, K.W. Prasse and S. Ducan. 2003. *Veterinary Laboratory Medicine: Clinical Pathology*, 4th ed. Iowa State Press, Iowa, USA.

Mahmut, O.K., H. Guzelbektes, I. Sen, A. Coskun and A.S. Ozturk. 2009. Post-parturient haemoglobinuria in three dairy cows. A case report. *B. Vet. I. Pulawy*, **53**(3): 421-423.

Malik, H.U. and A. Samad. 1996. Pathogenesis of

phosphorous deficiency haemoglobinuria in buffaloes. Influence of extracellular inorganic phosphorous on glucose metabolism in buffalo erythrocytes. *Buffalo Journal*, **12**: 73-84.

Marik, P.E. and R. Bellomo. 2013. Stress hyperglycemia: An essential survival response. *Crit. Care*, **17**(2): 305. DOI: 10.1186/cc12514

Muhammad, G., M. Saqib and M. Athar. 2001. A rational approach to diagnosis, treatment and control of parturient hemoglobinuria (red water) in buffaloes and cattle. *Pak. Vet. J.*, **21**(4): 214-219.

Navjot, S., K. Palneez, S. Harbir and Sh. Neelesh. 2017. Postpartum hemoglobinuria (PPH) in bovine. *Theriogenology Insight*, **7**(1): 51-59. DOI: 10.5958/2277-3371.2017.00016.X

Purohit, G.N., G. Trilok, K. Amit, Sh. Atul, G. Mitesh and Sh. Chandra. 2018. Perspectives of parturient hemoglobinuria (pph) in buffaloes. *International Journal of Development Research*, **2**: 23513-23520.

Rahmati, S., A. Aziz, M. Tawfeeq, J. Zabuli and S. Nazhat. 2021. Clinical features of post-parturient hemoglobinuria in dairy cattle and buffaloes. *Open Journal of Veterinary Medicine*, **11**(4): 143-155. DOI: 10.4236/ojvm.2021.114010

Rana, J.P. and R.M. Bhardwaj. 1988. Relationship of intraerythrocytic ATP and membrane phospholipids to red cell shape in hemoglobinuric buffaloes. *In The Proceeding 2nd World Buffalo Congress*, New Delhi, India. p. 178-184.

Rock, R.C., W.G. Walker and C.D. Jennings. 1987. Nitrogen metabolites and renal function. *In Fundamentals of Clinical Chemistry 3rd ed.* WB Saunders, Philadelphia, USA.

Sarma, K., M. Saravanan, K. Pankaj, M. Kumar, R.K. Jadav and D.B. Monda. 2014. Influence on haemato-biochemical and oxidative indices of post parturient haemoglobinuric (PHU) buffalo. *Buffalo Bull.*, **33**(4): 343-348. Available on: https://ibic.lib.ku.ac.th/e-bulletin/IBBU201404013.pdf

Stockdale, C.R., T.E. Moyes and R. Dyson. 2005. Acute post-parturient haemoglobinuria in dairy cows and phosphorus status. *Aust. Vet. J.*, **83**(6): 362-266. DOI: 10.1111/j.1751-0813.2005.tb15635.x

Tamhane, A.C. and D.D. Dunlop. 2000. *Statistics and Data Analysis from Elementary to Intermediate*. Upper Saddle River, USA.

Tietz, N.W. 1990. *Clinical Guide to Laboratory Tests*, 2nd ed. WB Saunders Company, Philadelphia, USA. p. 444-447.

Tietz, N.W. 1995. *Clinical Guide to laboratory Tests*, 3rd ed. WB Saunders Company, Philadelphia, USA. p. 102.

Trinder, P. 1969. Enzymatic determination of glucose. *Ann. Clin. Biochem.*, **6**: 24-26.

Vassault, A., D. Grafmeyer, J. de Graeve, R. Cohen, A. Beaudonnet and J. Bienvenu. 1986. Protocol for the validation of methods. *Ann. Biol. Clin.-Paris*, **44**: 686-745.

Wang, X.L., C.H. Gallager, T.J. Mcclure, V.E. Reeve and P.J. Canfield. 1985. Bovine post-parturient haemoglobinuria: Effect of inorganic phosphate on red cell metabolism. *Res. Vet. Sci.*, **39**(3): 333-339.

Zaghawa, A. Yousef and H, Nayel M. 2019. Clinical, epidemiological and clinic-pathological aspects of hypophosphatemia in buffaloes in Egypt. *Bioscience Research*, **16**: 31-41.

Ziwei, Z., B.I. Mingyu and X.U. Shiwen. 2017. Effect of phosphorus deficiency on erythrocytic morphology and function

in cows. *Vet. Sci*., **18**(3): 333-340. DOI: 10.4142/jvs.2017.18.3.333