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CLINICO- BIOCHEMICAL STUDIES ON BLOOD OF FIELD CASES OF HYPOPHOSPHATAEMIC BUFFALOES

BY

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SUMMARY

The aim of the present study is to investigate the effect of hypohposphataemia on buffaloes excessively feed on Trifolium Alexandrium (Barseem) during the green season in Egyptian village on the Electrophoretic pattern of serum proteins. Immunoglobulin G (lgG), some enzymes, minerals and Electrocardiocgraph (ECG) with trials for faster line of treatment.

Blood samples were collected from 10 clinically healthy buffaloes considered as control group and 24 buffaloes showing signs of pica, prefering dry feeds and loss of appetite, ten of them urinated reddish-coffi coloured urine (haemoglobinuria) which was the main clinical hypohosphatemic group (CHP); the others 14 voided normal coloured urine which considered as subclinical hypophosphatemic group (SCHP).

The biochemical serum analysis revealed that , the hypohosphataemia accompanied by a marked decrease in total proteins , Albumin, A/G ratio, Alkaline phosphatase (ALP); Ceruloplasmin (Cer); Copper; Iron, Total Iron Binding Capacity (TIBC), and inorganic phosphorous (Pi). levels. This decrease was followed by significant increases in total globulins, gamma globulin.

Immunoglobulin G (IgG), Acid phosphatase (ACP) and Lactate dehydrogenase (LDH). While the levels of Alpha globulin, Beta globulin, Zinc, Calcium and Magnesium showed no significant changes. The heamatological studies revealed a decrease in total Erythrocytes count (RBCs), Haemoglobin (Hb) concentration and Packed Cell Volume (PCV) , while the total leukocytes (WBCs) count was significantly increased. The ECG of SCHP showed normal trace while that of CHP demonestrated normal P wave, large amplitude of QRS complex and large T wave.

Both groups treated according to the severity of the case by different amounts and routs of phosphates supplementation . The complicated

cases were completely cured after blood transfusion.

INTRODUCTION

Haemoglobinuria indicates a disease accompanied by intravascular heamolysis, changes in the colour and / or constituents of urine, it is a clinical feature of many diseases of bovines as leptospirosis, babesiasis, and colistridium haemolyticum infection .The postparturient heamoglobinuria which was a type of haemolytic anaemia and excessive RBCs fragility associated with inadequate ATP formation developed in high producing muliparous cows reared on ration deficient in phosphates in early lactation, (Omran et al .,1987 and Yates, 1990).

The heamoglobinuria accompanied by hypophosphataemia, was firstly recorded in Egypt by (Awad and Abdel-Latif, 1963) who registered a syndrome simulating postparturient haemoglobinuria in buffaloes associated with prolonged feeding in Trifolium Alexandrium (Barseem).

Most reports of phosphorus deficiency have described cattle generally with clinical signs of pica, loss appetite, weakness, low milk production, poor reproductive performance and haemoglobinuria (Brintrup et al., 1993).

Ogawa et al (1989) demonestrated that, inadequate phosphorus in the plasma hindered ATP production by inhibiting the glucose -3-phosphate dehydrogenase step, thus leading to dysfunction of RBCs and ruptrue of its membrane.

Accordingly, this study was planned to investigate the effects of hypohosphataemia on the electrophoretic pattern of serum proteins and IgG concentration . Some enzymes minerals and trace elements which were suspected to be changed in this metabolic disease as ALP, ACP, LDH, Cer, Copper, Zinc, Iron, TIBC, Calcium, Magnesium and Pi. Also some heamatological studies were conducted to show the effect of the developed intravascular heamolysis on RBCs and WBCs count; Hb concentration and PCV. ECG tracing was applied with trials to attain a rapid and safe method for treatment specially with complicated cases.

MATERIAL AND METHODS

The present investigation was carried out in the clinic of the faculty of veterinary medicine Moshtohor, Zagazig University (Benha branch) and the villages surrounded it from December 1996 to May 1997, on 24 buffaloes 3 to 8 years old, pregnant at the mid - stage of pregnancy, with similar history of excessive prolonged feeding on

Barseem with signs of decreased appetite preferring the dry feeds, refuse barseem eating. Ten from them voided a coloured urine varied from reddish to coffe colour and foamy in characters, this group considered as CHP while

the other 14 buffaloes not void coloured urine and considered as SCHP group, other 10 clinically healthy ones used as control group.

Each animal subjected to clinical examinations, urine samples were collected by cathereization. ECG tracing was carried for each animal by Base-Apex lead system according to Broijamns,(1957).

In this sysem the right forlimb electrode was placed on the right side of the neck along the jugular groove one third of the wayup of the neck from the torso . The left forlimb electrode was placed on the ventral midline under the apex of the heart . Both the hind limb electrodes were remained attached to the stiffle. Blood smears were collected from ear vein of each animal.

Leishman's stain was used in staining blood smears. Two blood samples were taken by a vein punctruring from the jugular vein, the first sample was collected on EDTA as anticoagulent for heamatological study , the other sample collected for separation of serum . The sera were freshly used for electrophoretic study on serum proteins by Agarose Ge1 electrophoresis accoreding to Alper, (1974) ; serum IgG by (Fahey and Mckelvey, 1965); ALP (Belifield and Goldberg, 1971) ; ACP (Moss, 1984) ; LDH (Cabuud, 1958) ; Cer (Schosinsky et al., 1974) ; Iron, Zinc and copper were astimated by atomic absorption sepectrophotometer according to Bauer (1982); TIBC (Tietz, 1976); Calcium (Gindler and King, 1972); Magnesium (Tietz 1976) and Pi (Henry, 1974). The whole blood samples were subjected to total RBCs and WBCs counting and PCV determination according to (Schalm et al., 1975) ; haemoglobin was estimated according to (Wintrobe, 1965).

The SCHP cases were treated by daily oral adminestration of 60 gm sod. acid phosphates or daily I/m injection of 20 ml Catozal or Tonophosphan. While the CHP cases injected 20% sod. acid phospate I/V and then S/C 3 times with 12 hours interval in total amount of 300cc followed by similar dose orally. Second line of treament was the injection of Catozal® and Tonophosphan® till disappearance of the heamoglobinuria, the thrid line was by phosphorous supplementation with blood transfusion.Blood transfusion was conducted between animals of the same family . Blood was collected into an anticoagulant solution in an open - mouthed vessel.

Venepuncture into the jugular vein is the method of choice , using trocar and canula. Sodium citrate(10 ml of 3.85 per cent solution to each 100 ml of blood collected) is the standard anticoagulant used. About 4 to 7 ml of blood per lb of body weight can be drawn off at one time without danger . Antihistaminic and heart tonic used to overcome any side effect. (Abdel - Latif and Awad, 1964 blood et al , 1983 and Abdel-Ghani, 1989).

RESULTS

Microsopical examination of the stained blood films revealed no blood parasites. Laboratory chemical and microscopical examinations of the urine samples according to (Kelly, 1974)showed no sedements of intact RBCs or its stroma so it was haemoglobinuria (Postitive Benzidine test)

Table (1): Electrophoretic pattern of Total proteins , Albumin , Total globulin, A/G ration, Globulin fractions and Immunoglobuline G (IgG) levels of clinically healthy buffaloes (control) and those affected by SCHP and CHP

Parameter / Statements	Total Proteins (gm /d1)	Albumin (gm /d1)	Total globulins (gm /d1)	A/G Ratio	Globulin fractions (gm / dI) Alpha	Beta	Gamma	IgG (gm /d1)
Control ($\overline{X}$ ± S.E.)	7.954 ± 0.104	2.964 ± 0.061	5.008 ± 0.060	0.586 ± 0.012	1.377 ± 0.034	0.928 ± 0.018	2.709 ± 0.05	1.980 ± 0.09
SCHP ($\overline{X}$ ± S.E.)	7.322 ± 0.103	2.047* ± 0.032	5.275* ± 0.090	0.388* ± 0.011	1.292 ± 0.029	0.713 ± 0.017	3.270* ± 0.055	2.280 ± 0.039
CHP ($\overline{X}$ ± S.E.)	7.121* ± 0.176	2.007* ± 0.051	5.114* ± 0.129	0.386* ± 0.009	1.242 ± 0.033	0.712 ± 0.023	3.16* ± 0.084	371* ± 0.13

$\bar{x}$ ± S.E. = Mean ± standard error

* = Significant at (p < 0.05)

** = High significant at (P < 0.01)

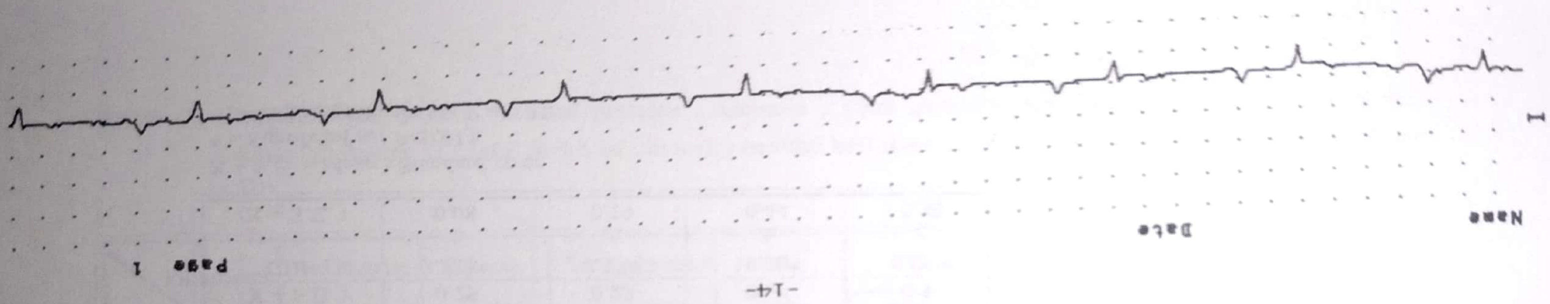
Table (2) The mean values of serum ALP (u/ml; ACP (u/ml); LDH (u/lm); Cer (u/ml); Copper (ug/dl); Iron (ug/dl); TIBC(ug/dl); Zinc (ug/dl); Calcium (mg/dl); Magnesium (Mg/dl); and Pi (mg/dl); in Clinically healthy buffaloes (control) and those affected by SCHP and CHP

Statements \ Parameter	ALF (u /ml)	ACP (u /ml)	LDH (u /ml)	Cer (u /ml)	Copper (ug/dl)	Iron (ug/dl)	TIBC (ug/dl)	Zinc (ug/dl)	Calcium (mg/dl)	Magnisium (mg/gl)	Pi (mg /dl)
Control ($\bar{X} \pm$ S.E.)	23.01 ± 1.04	10.81 ± 0.99	454.46 ± 23.15	70.72 ± 3.65	76.81 ± 2.46	218.49 ± 6.99	549.32 ± 27.18	116.91 ± 3.89	9.60 ± 0.29	2.80 ± 0.09	4.99 ± 0.18
SCHP ($\bar{X} \pm$ S.E.)	19.98 ± 1.07	21.13* ± 1.76	5071.19* ± 37.18	58.77** ± 3.91	62.31* ± 1.76	159.00* ± 5.12	400.60* ± 31.81	114.35 ± 4.52	9.08 ± 1.03	2.56 ± 0.32	2.79* ± 0.81
CHP ($\bar{X} \pm$ S.E.)	9.13** ± 0.97	35.19** ± 2.61	686.81** ± 30.12	51.61** ± 4.18	69.18* ± 1.99	121.83** ± 9.08	304.83** ± 18.91	101.75 ±	8.75 ± 0.30	2.41 ± 0.81	2.01** ± 0.86

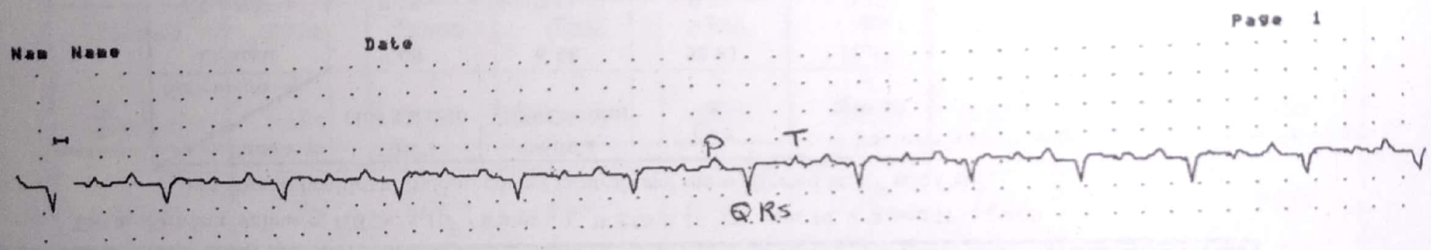
Table (3): Mean values of RBCs; x 10^6 / Cu.mm) , WBCs x 10^3 Cu. mm, PCV % and Hb (gm/dl) in normal healthy buffaloe - cows (control) and those affected SCHP and CHP .

Statements \ Parameter	RBCs (106 /Cu.mm)	WBCs (103/Cu.mm)	PCV %	hb (gm/dl)
Control ($\bar{X} \pm$ S.E.)	7.08 ± 0.18	6.98 ± 0.11	32.81 ± 0.31	11.53 ± 0.17
SCHP ($\bar{X} \pm$ S.E.)	6.48 ± 0.25	9.80* ± 0.23	30.79 ± 0.69	10.17 ± 0.19
CHP ($\bar{X} \pm$ S.E.)	3.82** ± 0.08	9.97* ± 0.19	19.30* ± 0.44	6.05 * ± 0.39

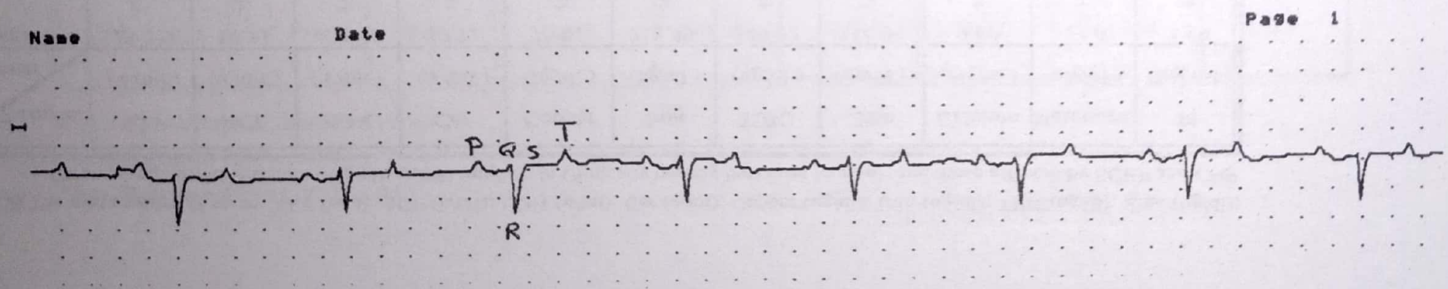
$\bar{X} \pm$ S.E. = Mean ±Standard error
* = Signifcant at (P<0.01).
** = High significant at (P < 0.01



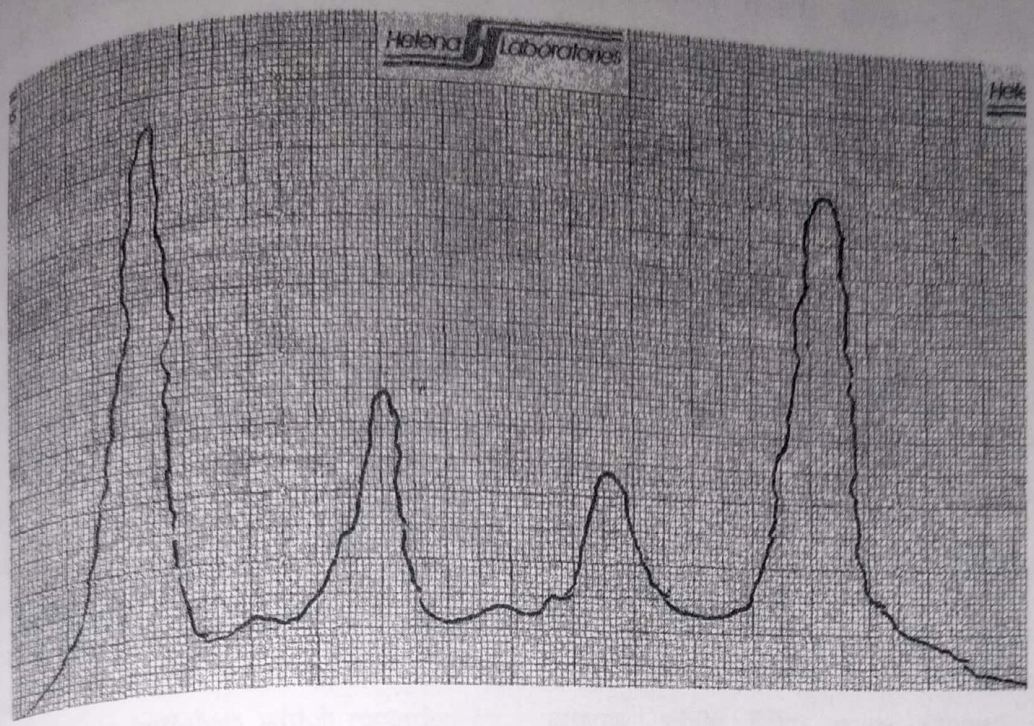
ECG trace of Clinically healthy buffaloes (Control)



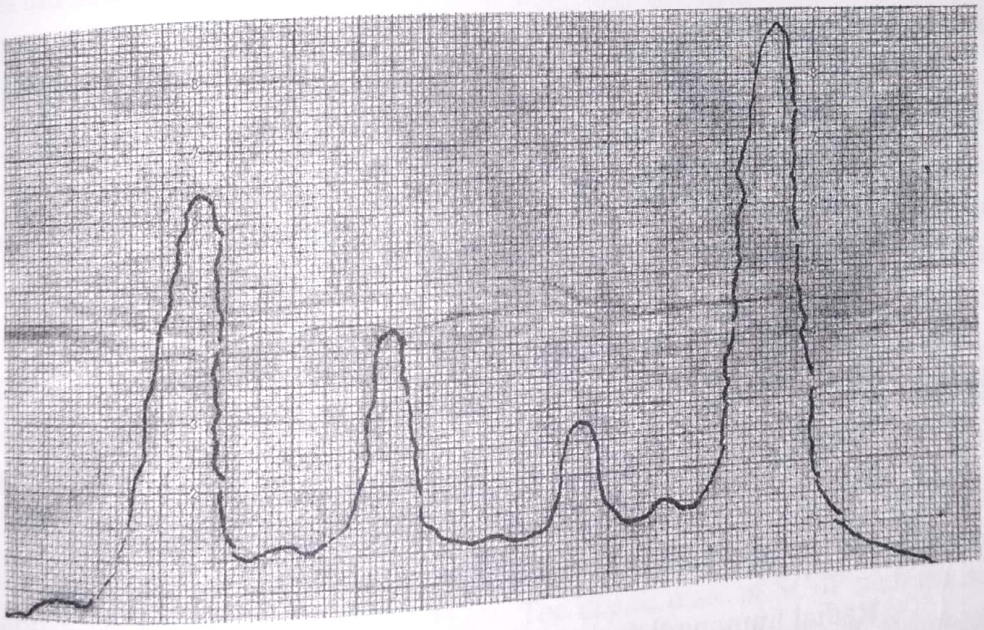
ECG trace of buffaloes with mild degree of hypophosphataemia



ECG trace of buffaloes with severe degree of hypophosphataemia

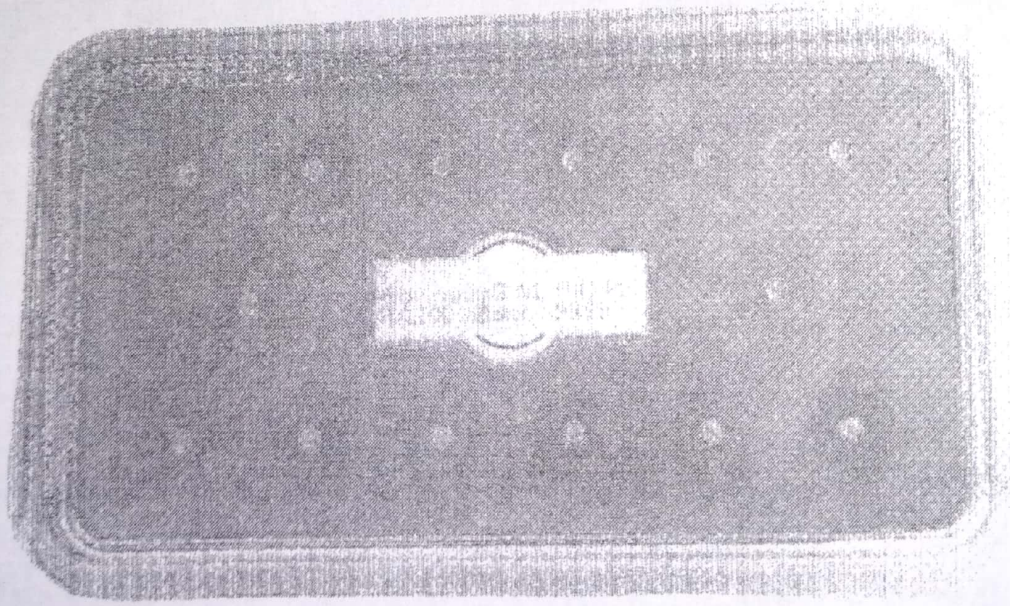


Scanning curve of serum protein fractions of clinical hypophoshatemic buffaloes.



Scanning curve of serum protein fractions of subclinical hypophoshatemic buffaloes.

Scanning curve of serum protein fractions of clinical hypophoshatemic buffaloes.



Radial immunodiffusion technique for determination of serum immunoglobulin IgG showed a preciptin rings of antigen antibody complexes.

with great albuminuria. On the other hand, there was no glucosuria, bile salts and bile pigments in the urine of all cases .

The clinical observation of the SCHP group showed decreased and depraved appetite , while exmination of those with CHP showed heamoglobinuria, decreased appetite, constipation, ruminal atony (one movement/2 minutes), normal body temperature (38 - 39.2°), accelerated pulse (81 - 101/min); accelerated respiration (27- 40/min) and the colour of mucous memebranes ranged from icteric to pale according to the severity of the case which were came in accordance to the signs of hypophosphataemic haemoglobinuria in buffaloes which recorded by (Awad and Abdel - Latif, 1963; Blood et al., 1983 and Omran et al., 1987).

The present data (Table,1) revealed a significant decrease ($P<0.05$) in serum total proteins level in CHP, while serum albumin level and A/G ratio were significantly decreased in both groups. Although there was significant increase in serum total globulins level in them , there was also significant increase in globulin levels in both SCHP and CHP and only in the serum IgG of the CHP buffaloes.

The recorded data (Table, 2) presented a high significant decrease ($P<0.01$) of serum ALP and Cer. levels and high significant increase in serum ACP and LDH levels in the CHP animals. The table also shows a significant increase in serum ACP and LDH levels and a significant decrease in Cer Level in the SCHP group.

The obtained results revealed a siginificant decrease in copper, iron and TIBC levels in both the CHP and SCHP animals, while the decrease in the level of iron and TIBC was highly significant in CHP group. The mean values of zinc, calcium and magnesium levels showed no significant changes in both groups.

The inorganic phosphorus levels showed a significant decrease in the serum of SCHP , while this decrcease was highly significant in CHP buffaloes .

The data in (Table, 3) showed a significant increase in WBCs count in the SCHP and CHP groups. while RBcs count showed a high significant decrease and a significant decrease only in both the PCV and HB concentration in the CHP.

ECG tracing of SCHP showed normal P wave (positive monophasic), normal QRS complex (negative monophasic R) and normal T wave (biphasic or small monophasic); while that of CHP was fluctuated according to the severity of the case, in mild CHP there were normal P wave, mild enlargement in QRS amplitude (0.5 mv) and biphasic T wave (negative then positive); while for the severe cases there were normal P wave, large amplitude of QRS complex (0.8 mv) and large T wave (positive monophasic).

The treated cases of SCHP with the second line were rapildy cured. While the third line of treatment in CHP cases was the rapid and safe method specially in severe cases.

DISCUSSION

The observed heamoglobinuria in the CHP can be considered due to the excessive RBCs fragility associated with the inadequate ATP formation (Yates et al., 1990). The increase in pulse rate might be attributed to the decreased blood volume following the intravascular haemolysis and anaemia, while the accelerated respiratory rate also due to the anaemic hypoxia (Blood et al., 1983).

The significant decreases in both serum total proteins and albumin levels in CHP group, and in serum albumin level in SCHP were nearly came on contrary to the observed results of Abdel - All (1997).This decreased values could be attributed to the loss of protein from the distructed RBCs and its release in the urine as albuminuria was one of the positive results in the urine analysis of the investigated animals , the obtained result may be also due to the tubular degeneration in the kidney during heamoglobinuria or poor nutritional status and/or related to deprivation of the diet as reported by (Kurundikar et , al 1981, and Abdel - Salam et al., 1994) . The decreased total protein and albumin levels may also due to the mal-nutrition and high cortisol level as the common 17- hydroxy pregnenolone was shunted toward cortisol or reduced metabolic clearance and increased secretion of the hormone in posphorus deficiency (Henley and Judith, 1985).
The increase in serum total globulins levels in CHP and SCHP might be related to the depletion of albumin which simultneosly stimulated the formation of certain globulins (Latner, 1975) .The decrease in the A/g ratio in both groups is a consequence of the decrease of serum albumin level that associated with the increase total globulins level.

The non-significant decrease in the mean values of serum a and b globulins in both groups is similar to the oponion of Eidaroos et al. (1988) and Abdel - All (1997) as they found no significant decrease in the serum globulin fractions in buffaloes in the green season.

The significant increase in serum -globulin fraction level in both groups, might be increased to compensate the decrease of serum albumin with stimulation of the reticuloendothelial system by the toxins developed from the intravasular heamolysis (Eidaroos et al., 1988).

The increase in IgG level in CHP was confirmed by the results of Ahmed et al., (1993) who showed that , total WBCs sount, phagocytic activities and the total immunoglobulins were higher in buffaloes with gential disorders and the hypohosphataemia was a major cause of reproductive insufficiency, in addition increase in the IgG level could be attributed to the general reaction of the immune system to synthesize antibodies aganist any infections (Nagi, 1994) which in our investigation the intravascular heamolysis.

The decrease serum ALP level in the CHP was confirmed by the results of Omran et al. (1987) who found a positive correlation between the very high significant decrease of serum ALP and the decrease Pi in buffaloes with haemoglobinuria . This result is also similar to that obtained in

human by (Davidson and Henry, 1974); in cattle (El-Azab et al., 1988) and in sheep (Ragaa et al., 1992) . Such decrease could be related to the decreased phosphomonoesters and the pyrophosphates, where the enzyme released the Pi from them , this oponion was augmented by the recognition of Ogawa et al. (1989) who found depletion of ATP which released pyrophospates, Glutathion reductase and methemoglobin formation in all cases of hypohosphataemia.

The highly significant increase in the serum ACP in the CHP and the significant increase in the SCHP might be related to the intravascular hemolysis accompanied the hypohosphataemia, where the RBCs and platelets had abundant amount of ACP which increased in the serum after heamolysis (Wooton and Freeman , 1982) .

The increase in serum LDH in both groups was came on contrary to the results obtained by (Ragaa et al., 1992)in sheep . Such increase is due to the great amount of immature RBCs in the blood stream which had high ratios of the enzyme camming from the compensatory releasing of blood from its stores following the intravascular hemolysis accompanied hypohosphataemia (Abdel - Magid , 1996). Also it might be discussed due to the leukocytosis where the WBCs had high amounts of the enzme which considered as the main diagnostic parameter for leukaemia and myocardial infarction (Murray et al., 1996).

The decrease in serum Cer activities in both SCHP and CHP groups were similar to the results of Ragaa et al. (1992) in sheep . This decrease might be related to the decreased values of serum copper and iron followed the hypohosphataemia as the enzyme is a copper contaning ferroxidase, its activities depressed when serum copper level decreased (Mohamed , 1989).

The decreases in serum Iron , TIBC and copper levels in both groups were similar to the recorded results in phosphorous deficient cows (Ismaeil, 1984), buffaloe - cows (Ismaeil and Hussein , 1988) and sheep (Mandour, 1991).

The end result is the development of haemolytic anaemia and heamoglobinuria followed the hypohosphataemia where the haemoglobin is the major iron containing compound (Ogawa et al., 1987) . Also, the anemia might be due to the decreased serum copper level which is essential for iron absorption and metabolism through activation of ferroxidase enzyme (Abdel - Maksoud, 1991) .

The decreased serum copper level might be due to the poor-nutrition, which accompanied by decreased appetite and gradual starvation of the diseased animals through which the stimulation of gonadotrophins was inadequate, as the copper considered as gonadotrophic hormone index where the estrogen induced the synthesis of cerulopasmin " copper containing a2 globuline" initiate the absorption and metabolism of copper (Henley and Judith, 1985) .

The non signigicant changes in serum zinc level in both groups was came in agreement with the results noticed in phosphates deficient ewes (Mandour , 1991) and buffaloe - cows (Ismaeil

and Hussein, 1988).

The non -significant changes in serum calcium and Magnesium levels in both groups were came in agreement with the oponion of Brain and Eric (1996) who reported that, the serum calcium and magnesium levels were at the refrence limits in hypohosphataemia.

The decreases in serum Pi in CHP and SCHP could be related to the heavy feeding on trifolium Alexandrium (Barseem) which was deficient in phosphorus and had a lot of the decrease in serum Cer activities in both SCHP and CHP groups were similar to the results of Ragaa et al. (1992) in sheep . This decrease might be related to the decreased values of serum copper and iron followed the hypohosphataemia as the enzyme is a copper contaning ferroxidase, its activities depressed when serum copper level decreased (Mohamed , 1989).

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The decreases in serum Pi in CHP and SCHP could be related to the heavy feeding on trifolium Alexandrium (Barseem) which was deficient in phosphorus and had a lot of calcium , anti-vitamin D factor and high carotene level which henderanced the phsphorus absorption (Payne, 1977).

The decreased RBCs count, PCV and HB concentration in the CHP might be due to the excessive fragility and heamolysis of RBCs associated with inadequate ATP formation followed the low phosphorous intake and the

developed heamoglobinuria (Ogawa et al., 1987)

The increase in the total WBCs count in both groups could be related to the general reaction of the lymph nodes and reticulo-endothelial system against the heamolysed RBCs.

The normal ECG tracing of SCHP might be related to the mild decrease in Pi level which had no effect in cardiac muscles. While the increased QRS complex amplitude in CHP could be attributed to the massive destruction of RBCs and the hematogenic peripheral circulatory failure associated with cardiac dialation (Blood et al., 1983).

CONCLUSION

From this study it can be concluded, that the excessive feeding on barseem must be avoided, feeding balanced rations during the green season is essential and we suggested that the hypophospataemic buffaloes without clinical signs (SCHP) should be periodically examined and treated .

REFERENCES

Abdel-Aal, A.A. (1997) :" Seasonal Variation electrophoretic pattern of serum protein of fertile and infertile buffaloes" M. Vet. Sc. Fac. Vet. Med. " Moshtohor" Zagazig Univ.

Abdel-Ghani, A.A.(1989):Red urine in cattle , incidence, causes and treatment . M. Vet. Sc. Fac. Vet. Med. Zag Univ.

Abdel-Latif, K. and Awad, F.A. (1964): Haemoglobinuria of buffaloes associated with excessive feeding of trifolium alexanderium (Barseem) J. Vet. Sc. Un. A.K. (1) 69 - 74.

Abdel-Mageid, A.D. (1996) : " Biochemical concept of erythrocytes of normal sheep " M. Vet. Sc. Fac. Vet. Med. (Moshtohor) Zag. Univ.

Abdel-Maksoud, H.A. (1991) :" Biochemical altration of trace elements in relation to late pregnancy and peurperium in bovine" M. Vet. Sc. Fac. Vet. Med. (Moshtohor) Zag. Univ.

Abdel-Salam, N.M.; Ali, S.H; Sadiek, H.A.; Zaiton, M.A. and Sayed, S.A. (1994): "Haematological picture and serum protein analysis in fattening buffaloe calves naturally infested with theileria annulata" Assiut, Vet. Med. J. Vol. 30 No. 60.

Ahmed, W.M.; Nada, A.R. and Shalaby, A.I. (1993) :" Utrine hormonal and cellular immune response in some case of gential disorders in buffaloes". Reproduction in Domestic animlas, (28) : 6, Pp. 298 - 301.

Alper, C.A. (1974):" Plasma proteins " Measurements as a diagnostic aid" Neng.J. Med., (29) , pp. 287 - 291.

Awad, F.A. and Abdel-Latif, F.A. (1963) : " The first recorded of disease condition in Egyptian buffaloes stimulating post - parturient haemoglobinuria " Vet. Res. 75) : 298 - 300.

Bauer, J.D. (1982) : " Clinical laboratory methods" 9th (ed.), the c.v Mosby Co. II, 830 westline industrial missouri, 63146 U.S.A.

Belifield, D.A. and Goldberg, S.R. (1971): Enzyme, (12) : PP. 561. Cited in biochemical Kits.

Blood, D.C.; Henderson, J.A. and Radostits, O.M. (1983): "In veterinary medicine" 6th (ed), Williams and Wikins Co. Baltimor, U.S.A.

Brain, J.G. and Eric, P.S. (1996) "Acute recumbernсy and marginal phosphorus deficiency in dairy cattle" J.A. V.M.A.; Vol. 208, No. 5, 716 - 719.

Brintrup, R.; Mooren, T. and Meyer, (1993): "Effects of two levels of phosphorus intakes on performance and fecal phosphorus excretion of dairy cows: J. Anim. Physiol.

Anim. Nut. (69) : 29 - 36.

Brooijamns , A.W.M. (1957): Electrocardiography in horses and cattle . Laboratory of Vet . Physiology , State University , Utrecht.

Cabuud, P.C. (1958): "Colourimeter measurement of lactate dehydrogenase activity of body fluids" J. Clinic. Path. (30): 234 - 236.

Davidson, I. and Henry, J.B. (1974): In "clinical diagnosis by laboratory methods" 15th ed. W.B. SaundersCo. Philadelaphia, U.S.A. : 857 - 860.

Eidaroos, A.; Abdel - Rehiem. A. and Mansour, H. (1988) : "A study of the electrophoretic pattern of the serum total protein in buffaloe - cows with normal and banormal reproduction Zagazig Vet. J. (16), 4 : 81 - 91.

El-Azab, A.I.; El-Zayat, M.A. and Nasr, M.T. (1988): "Estimation of some serum chemical constituents in fertile and infertile buffaloe - cows" Prac. 3th Sci Cong. Fac Vet. Med. Assiut Univ. (5). PP 20-22.

Fahey, J.I. and Mckelvey, E.M. (1965): Quantitative determination of serum immunoglobulins in antibody - agar Plantes" J. Immunol. (94): 48-90.

Gindler, E.M. and King, J.D. (1972): "Rapid colorimetric determination of clacium in biologic fluids with methylethmol blue" Am. J. Clin. Path. (58) 376-382)

Henley, M.K. and Judith, L.V. (1985):"Hormonal changes associated with changes in body weight" Clin Obest. and Gynec. (28), 3 : 615-620.

Henry, R. (1974): In "clinical chemistry principles and techniques" 2nd ed Harper and Row: pp. 543.

Ismaeil, M. (1984): " A study of some mineral and enzymes in buffaloes and cattle in healthy and disease" Ph. D. Thesis, Fac Vet. Med Alex. Univ.

Ismaeil, M. and Hussein, F.M. (1988) : "Studies on the relationship between trace elements and some blood components and fertility in Egyptian buffaloe" Alex. J. vet. Sci. 4 (1) : 555 - 559.

Kelley, W.R. (1974) : In "Veterinary clinical diagnosis" 2 nd Ed. Baillier Tindall- London : 964.

Katner, A.L. (1975) : In " Clinical biochemistry" 7th ed. Sanders. Co. Philadlaphia, Toronto: 186.

Kurundkar, V.D.; Deshpande, B.D.; Singh, B. and Anantwar, L.G (1981): Biochemical and pathological changes in Clinical cases of haemoglobinuria in buffaloes . Ind .J. Anim . Sci , 51 ,35 -38.

Latner , A.L (1975): In " Clinical Biochemistry " 7 th ed Baillier Tindall, London :964

Mondour, A.A. (1991) : " Biochemical profile of some nutritional disorders in sheep. Ph. D. Fac. Vet. Med. Alex. Univ.

Mohamed, L.M. (1989) : " Biochemical analysis of some constituents in blood of buffaloes during pregnancy" Ph. D. Thesis, Fac. Vet. Med. Zag. Univ.

Moss, D.W. (1984) : " Methods of enzymatic analysis" Am. J, Clin Path. (54) : 92 - 106.

Murray, R.; Granner, D.; Mayes, P. and Rodwell, V. (1996): In " Harper's Biochemistry" 24th ed. Long. Medical Pub. Murazin Co. LTD. Sanfrancisco, U.S.A.

Nagi, N. (1994) : Biochemical profile of immunoglobulin (IgG) and electrophoretic pattern of serum proteins in relation tomycoplasma gallisepticum and new castle" Ph. D. Fac. Vet. Sc. (Moshtohor), Zagazig Univ.

Ogawa, E.; Kobayashi, K. and Yoshiura, N. (1987): "Bovine post-parturient hemoglobinaemia : hypophosphataemia and metabloic disorder in red blood cells" Am. J. Vet. Res. (48) : 1300 - 1303.

Ogawa, E.; Kobayashi, K. and Yoshiura, N. (1989): "Hemolytic anemia and red blood cell metabolic disorder attributable to low phosphorus intake in cows" Am. J. Vet. Res. (5) , 3: 388 - 392.

Omran, H.; Abdel-Razik, W. and El-Nagar, E. (1987): " Some clinical and Biochemical studies on blood and urine of buffaloes in some diseased conditions

accompanied with heamoglobinuria". Alex. J. Vet. Sci. (3) : 345 - 356.

Payne , J.M. (1977) : " Metabolic diseases in farm animals " 1 st ed. Willinam. Heiniman Medical Books, LTD London 228.

Ragaa, M.; Taha, N. Korshom, M. and Mandour, A. (1992): "Relationship between steriod hormones, certain trace elements and some enzymatic activities in ewes suffering from phosphorus deficiency" Benha, Vet. Med. J. (3) : 51 - 60.

Schalm, O.W.; Jain, N.C. and Carrol, J. (1975) : In "Veterinary Haematology".3rd ed. Lea and Gebiger, Pholadephia U.S.A. PP. 105.

Schosinsky, K.H.; Lekman , H.P. and Beeler, M. (1974) : " Measurement of ceruloplasmin from its oxidase activity in serum by use of O-diansidine dihydrochloride" Clin. Chem. (20), 125 - 1556.

Tietz; N.W. (1976): In " Fundamentals of clinical chemistry" 2nd ed. NW Tietz, Editor, Philadephia: 924 - 929.

Wintrobe, M.M. (1965) : In " clinical Haematology".4th ed. Lea & Febiger, Philadelphia. PP. 98.

Wooton , I.D. and Freeonan, H. (1982) : In " Microanalysis in medical biochemistry" 6th ed Churchill living stone, Edinburgh, London and New York Pp. 67.

Yates, D.J. (1990): " Chronic phosphorus deficiency / hypophosphataemia in : Smith B.P.ed. " Large animal internal medicine" St. Louis . CV, Mosby Co. : 1322 - 1324.