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BIOCHEMICAL AND HAEMATOLOGICAL STUDIES ON POST PARTURIENT HAEMOGLOBINUREA IN CATTLE REARED IN NEWLY RECLAIMED AREA

(With 5 Tables)

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دراسات بيوكيميائيه ودمويه علي مرض البول المدمم بعد الولاده بالماشيه المرباه في مناطق حديثه الاستصلاح

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أجريت هذه الدراسة بهدف معرفة تأثير مرض البول المد مم بعد الولاده فى الماشيه في المناطق المستصلحه حديثا علي صوره الدم وبعض القياسات البيوكيميائيه. وقد اجريت هذه الدراسة على عدد 40 رأسا من الماشية تتراوحت أعماره ا بين 6 الى 9 سنوات ظهرت عليها اعراض فقدان في الشهية ورفض أكل البرسيم وتغير لون البول الي الأحمر وأحيانا يميل الي لون القهوه وايضا نقص سريع في انتاج اللبن. وكذلك اشتملت هذه الدراسة على 15 رأسا من الماشيه السليمه ظاهريا كانت تنغذي علي برسيم وعليقه متوازنه في نفس مكان التجربه واستخدمت كضوابط. وقد تم دراسة الأعراض الأكلينيكية وأخذ عينات من دم ومصل وبول تلك الحيوانات قبل وبعد العلاج. وأسفرت نتائج التحليل انخفاض معنوي للعد الكلي لكرات الدم الحمراء وتركيز الهيموجلوبين وحجم الخلايا المضغوط وضغط الاوكسجين الوريدى وارتفاع معنوى للعد الكلى لكرات الدم البيضاء وضغط ثاني اكسيد الكربون وأيضا زياده غير معنوية في تركيز الاس الهيدروجينى والبيوكربونات فى الدم فى الحالات المريضه عند مقارنتها بالمجموعه الضابطه. وبالنسبه للتحليل البيوكيميائى لمصل الدم وجد انخفاض معنوي في مستوى البروتين الكلى والألبيومن والجلوبيولين والجلوكوز والكوليسترول والدهون الثلاثية والفوسفور والماغنسيوم والصوديوم والبوتاسيوم والزنك والحديد والنحاس. وكذلك وجد ارتفاع معنوى في مستوى نشاط انزيمات الكبد وانزيم الفوسفاتيز القاعدى ونسبة البولينا والمولبيدنيم فى المجموعة المريضة بمقارنتها بالمجموعة الضابطه. وعند تحليل البول للحيونا ت المريضة وجد ارتفاع معنوى للفوسفور والكالسيوم والماغنسيوم والبروتين واليوريا والكرياتينين بمقارنتها بالمجموعة الضابطه. وأظهرت النتائج بالنسبة لتحليل البرسيم ان تركيز النحاس والكالسيوم كان فى حدود معدله الطبيعى مع وجود نقص فى تركيز عنصر الفوسفور وزيادة نسبة عنصر المولبيدنيم بمقارنته بالبرسيم المنزرع فى القرى القديمة وقد ارجع ظهور

الاعراض الى النقص الاولى فى عنصر الفوسفور والنقص الثانوى فى عنصر النحاس. وتم علاج حالات الماشية المصابة بمرض البول المدمم بحقن مركب فوسفات الصوديوم الحامضى (H2PO4) (60 جرام فى 300 مل ماء معقم) فى الوريد وتحت الجلد كل 12 ساعة لمدة 3 الى 4 ايام. بالاضافة الى الحقن العضلى للتونوفوسفان 20% 20مل يوميا بالاضافة الى جلاسينات النحاس. وفى الحالات المريضة المتقدمة قمنا بنقل الدم لها. وقد عادت كل هذه القياسات فى الحيوانات التى تم علاجها الى مايقرب من مستوها الطبيعى خلال شهر كما تم التحسن من الناحية الصحية حيث أعراض البول المدمم. ويجب ان ننتبة الى الحرص فى التشخيص الاكلينيكى والمعملى للحيوانات المصابة وعلاجها مع توعية مالكى ومستصلحى هذه الاراضى الجديدة بضرورة اتباع الاسلوب السليم فى تغذية الحيوانات وعدم الاعتماد كلية على البرسيم والاعتماد على عليقة متزنة فى موسم البرسيم واضافة مخلوط الأملاح المعدنية والعناصر النادره والفيتامينات الى هذه العلائق اذا لزم الامر حتى نتلافى الخسائر الاقتصادية الناجمة عن ذلك.

SUMMARY

The aim of the present study is to investigate the effect of post parturient haemoglobinurea (PPHU) on haematological and biochemical parameters of cattle excessively fed on Trifolium Alexandrium (Barseem) during the green season in newly reclaimed area. Blood and urine samples were collected from 40 diseased cattle aged from 6-9 years showing signs of loss of appetite, refuse barseem eating, red to coffee coloured urine and milk production dropped rapidly. A number of 15 clinically healthy cattle were used as control group. The haematological studies showed significant decrease in TRBCs, Hb concentration, PCV and significant increase in TWBCs. The blood hydrogen ion concentration (pH) and bicarbonate were found non significant increased. Blood gas analysis showed significant increase in PCO2 and significant decrease in PO2. Serum biochemical analysis revealed that cattle with PPHU was accompanied by a marked decrease in total protein, albumin, globulins, glucose, total cholesterol, total triglycerides, inorganic phosphorus, magnesium, sodium, potassium, zinc, iron and copper levels. Moreover significant increase in AST, ALT, ALP activity levels, blood urea and molybdenum levels was noticed when compared with apparent clinically healthy ones. Moreover a marked increase in the excretion of phosphorus, calcium, molybdenum, protein, urea and creatinine through urine was observed only in PPHU affected cattle. Concerning the diet, analysis of barseem grows in newly reclaimed area, the obtained values of copper and calcium was adequate while phosphorus showed significant decrease levels. Molybdenum level has an opposite direction to copper level and

showed highly significant increases in its values when compared with barseem grow in old village. The affected PPHU cattle were treated according to the severity of the case by sodium acid phosphate and tonophosphan injection. Copper glycinate was also tried to halt haemolysis. Complicated cases were completed cured after blood transfusion. The symptoms disappeared, the appetite returned to normal and haematological as well as biochemical measured parameters in serum and urine returned nearly to normal. So attention must be paid for careful early clinical and laboratory diagnosis of diseased animals followed by therapeutic plan with good management. Further more excessive feeding on barseem must be avoided and feeding balanced ration mixed with minerals, trace elements and vitamins during the green season.

Key words: *Haemoglobinurea, blood analysis, urine analysis.*

INTRODUCTION

Post parturient haemoglobin urea (PPHU) is a major disease of dairy animals with detrimental economic consequences, Chugh *et al.* (1996). The post parturient haemoglobinurea is a type of haemolytic anaemia and excessive RBCs fragility associated with inadequate ATP formation developed in high producing multiparous cows reared on ration deficient in phosphates in early lactation, (Omran *et al.,* 1987). The exact aetiology and pathogenesis of PPHU are not known, as a variety of aetiological factors have been reported to be associated with the disease in different parts of the world, (Hussain *et al.,* 1991 and Chugh *et al.,* 1996). Dietary phosphorus deficiency and / or ration containing cruciferous plants are suspected causes of sever hypophosphataemia, (Pirzada and Hussain, 1998). Hypophosphataemia primarily affecting highly producing dairy cattle and buffaloes associated with prolonged feeding on barseem. Its prevalence during the period from March to May, (Nassif, 1995). It is hypothesised that hypophosphataemia results in decreased red blood cells glycolysis and ATP synthesis by inhibiting the glucose-6-phosphate dehydrogenase. Subnormal concentration of ATP predisposes red blood cells to altered structure and function, a loss of normal deformability and an increase in fragility and haemolysis, with resultant haemoglobinaemia and haemoglobinuria, (Ogawa *et al.,* 1989 and Radostits *et al.,* 2000). Copper deficiency is also an aetiological factor of Post PHU, as its deficiency reduces the activity of the copper-containing enzyme

superoxide dismutase, which is part of the erythrocyte protection mechanism against oxidative stress (Smith *et al.*, 1975). Phosphate deficiency also compromises oxygen release to the tissue due to the decreases in erythrocyte, (Jeffrey, *et al.*, 2005). Abdul-Samad (1997) recorded that at least 29%-42% of the cases of phosphorus deficiency, haemoglobinurea in buffaloes could be due to pregnancy and late stage of gestation. The author reported also that summer season is one of the putative causal factors in phosphorus deficiency haemoglobin urea. Bhikane *et al.* (1995) recorded that post parturient haemoglobinurea is characterized by coffee coloured urine, anaemia, loss of appetite and reduced milk yield with significant hypophosphataemia.

In view of the above speculations, the present investigation was undertaken to study the haematological and serum biochemical changes in cattle suffering from post parturient haemoglobinurea with history of excessive prolonged feeding on trifolium Alexandrium (barseem) in newly reclaimed areas of Egypt. This may help in the solution of this problem, as well as to reach ideal way for treatment and will contribute to the understanding of aetiopathology of post parturient haemoglobinurea. Suggestive measures for the prevention and control of disease in cattle was also aimed.

MATERIALS and METHODS

1- Animals:

The present investigation was carried out on 40 female multiparous dairy cattle suffering from post parturient haemoglobinurea (PPHU), aged 6-9 years, that were randomly selected from field cases that raised in newly reclaimed area in Salhia, Hosynia and the villages surrounded it districts in Sharkia Governorate, during the period between March till May 2008. All animals were heavily fed on Trifolium alexandrinum (Barseem) for at least 5 months. The disease was clinically diagnosed on the basis of specific signs during early lactation (2-4weeks) such as loss of appetite, refuse barseem eating, preferring dry feeds, red to coffee coloured urine, milk production dropped rapidly and straining while defecating. The control group included 15 clinically healthy cattle of similar description from the same location and received balanced ration (ration concentrate mixture consisted cotton seed cake 15%, soya bean meal 3%, wheat bran 55%, yellow corn 9%, rice polish 6%, molasses 7%, lime stone 3.5%, common salt 1.5%) during the green season. according to NRC (1985). History of affected examination

concerning animals' data, nutrition and management were recorded before the animals being clinically examined. A routine faecal examination was made for the detection of internal parasites. Blood film examination and standard laboratory methods were used to rule out other diseases that cause a reddish discoloration of urine such as babesiosis, leptospirosis or bacillary haemoglobinurea.

2-Sampling:

Two blood samples were collected from diseased group as well as from control animals before and after one month post treatment.

A- The first blood samples were collected in heparinized tube for the determination of total erythrocytes (TRBCs) and total leukocytes (TWBCs) counts, packed cell volume (PCV), haemoglobin concentration following the techniques described by Coles (1986) Red blood cells indices were also calculated. While blood gases and acid-base parameter (pH, HCO3, PO2, PCO2) estimated by using radiometer of Copenhagen ABLSIO Blood gases analyzer.

B- Another blood samples collected without anticoagulant for serum separation and preserved at -20^{o}C. Serum samples were used for determination of Aspartames aminotransferase (AST), Alanine aminotransferase (ALT) activity level according to Reitman and Frankel (1957), Alkaline phosphatase activity level (ALP) Bowers and Mc Comb (1975), total proteins level Doumos (1974), albumin level Drupt (1974) and serum globulins levels were calculated as difference between total protein and albumin. Serum glucose level was determined after Trinder (1969), while serum calcium level after Gindler and King (1972). Serum inorganic phosphorus level after Morinal and Prox (1973), magnesium level was determined according to Gindler and Heath (1971). Serum sodium and potassium levels were determined according to the method described by Hawk (1965) by using a flame-photometer (corning model AVL 988-3, made in U.S.A). Serum iron, zinc, copper and molybdenum levels were estimated by atomic absorption spectrophotometer according to Cowell (1973). Also serum samples were used for determination of blood urea level after Patton and Crouch (1977); creatinine level after Thomas (1992); total cholesterol level was estimated according to Stein (1986) and total triglyceride was analyzed after Mgtowan *et al.* (1983).

C- Urine samples:

Fresh urine samples were obtained from both diseased and apparently healthy cattle and used for biochemical analysis of phosphorus, calcium, magnesium, proteins, urea and creatinine levels according to the methods mentioned before in serum analysis.

D-Faecal samples:

Faecal samples were collected form each examined animal for detection of any internal parasite according to Soulsby (1986).

F-Diet samples:

Five samples of barseem from recently reclaimed area and from old villages were collect for phosphorus, calcium, copper and molybdenum analysis according to A.O.A.C. (1975).

3-Treatment trails:

The principal line of treatment based on correction of the feeding system of the affected animals and compensation of the occurred deficiency status

1- Correction of feeding system: avoiding the excessive feeding on barseem and prepared balanced ration during the green season.
2- Transfusion of large quantities of whole blood is the treatment for severely affected cows; blood transfusion was conducted between animals of the same family. Blood was collected into an anti coagulant solution in an open-mouthed vessel.
3- Treatment with sodium acid phosphate (60gm in 300ml of sterile water injected I.V. followed by subcutaneously injection every 12hours 3-4 days and daily I/M injection of 20 ml tonophosphan.^{R} 20% (Hookiest company)
4- Copper glycinate (120 mg available copper) one dose of intravenous injection will maintain adequate copper levels for about 60-90 days.

4- Statistical analysis:

The obtained data was statistically analyzed according to Snedecor and Cochran (1982) using the "SAS" computer program.

RESULTS

A-Clinical signs:

The main clinical signs observed included inappetence and depraved appetite with refuse barseem eating, preferring dry feeds. Pale to icteric visible mucous membranes, weakness, accelerated heart rate, rapid and shallow respiration was also detected. Red to coffee coloured urine, straining while defecating or during early lactation appeared. Body temperature ranged from 38-39.1 C and milk production dropped rapidly.

B- Haematological results

Post parturient haemoglobinurea (PPHU) induced very highly significant $P<0.001$ decrease in total erthrocytic counts, haemoglobin

concentration and packed cell volume reading Table (1). Mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) showed non significant increase. But mean corpuscular haemoglobin concentration (MCHC) showed non significant decrease. Concerning the total leucocytic count in the present study showed very highly significant increase. Mean while, cattle affected by (PPHU) showed highly significant increase in Pco_2 while Po_2 showed very highly significant decrease. The results referred to a type of anaemic anoxia. The blood hydrogen ion concentration (pH) and bicarbonate (Acid-base parameters) were found non significant increased. These parameters were returned to the normal levels nearly 30 days post treatment.

C- Biochemical results:

The present data (Table2) revealed significant $P<0.001$ decreases in serum total proteins, albumin, globulins, glucose, total cholesterol, and total triglycerides levels. On contrary significant increases $P<0.01$ in blood serum activities of AST, ALT, ALP and blood urea levels were noticed. Meanwhile creatinine level showed non significant increase. The obtained results in blood serum macro and micro elements in cattle affected with PPHU (Table 3) revealed a very highly significant $P<0.001$ decrease in phosphorus, magnesium, sodium, potassium, iron and copper levels. But the mean values of calcium and zinc levels showed non significant decrease. While the molybdenum level recorded very highly significant $P<0.001$ increase.

Concerning urine analysis, post parturient haemoglobinurea (PPHU) induced very highly significant increase $P<0.001$ in phosphorus, calcium, magnesium, protein, urea and creatinine in urine of the diseased cattle when compared with healthy one Table (4). This biochemical parameters returned to the nearly normal levels 30 days after treatment. Analysis of samples of barseem from recently reclaimed area and from old agricultural area (old villages) revealed that the concentrations of phosphorus, calcium, copper and molybdenum content were suggestive of primary phosphorus and secondary copper deficiency in recently reclaimed area, while the level of molybdenum showed very highly significant increase than the old villages Table (5).

Table 1: Haematological variables (mean ± SE) in blood of healthy cattle and those affected with post parturient haemoglobinurea (PPHU) before and one month after treatment.

Items / Parameters	Control healthy cattle	PPHU affected cattle	Post treatment
TRBCs ($x10^6$cum)	7.60 ± 0.41	3.92 ± 1.60***	6.7 ± 0.35
Hb (gm/dI)	11.79 ± 0.28	6.48 ± 0.48***	9.97 ± 0.59
PCV (%)	34.50 ± 1.04	17.92 ± 0.66***	30.2 ± 1.75
MCV (cuu)	46.05 ± 1.87	49.66 ± 6.60N	45.46 ± 1.57
MCH (ug)	15.62 ± 0.75	19.24 ± 1.87N	14.75 ± 0.52
MCHC (gm/dl)	33.96 ± 0.92	30.47 ± 1.42N	32.36 ± 1.37
TWBCs ($x10^3$ cum)	7.90 ± 0.29	10.06 ± 0.46***	7.29 ± 0.37
Hydrogen ion (pH)	7.41 ± 0.008	7.67 ± 0.02N	7.36 ± 0.009
Bicarbonate (mmol/L)	26.53 ± 2.30	31.39 ± 2.50N	27.40 ± 1.41
Pco2 (mm Hg)	46.95 ± 2.02	55.45 ± 2.70**	48.44 ± 2.0
Po2 (mm Hg)	42.90 ± 1.14	36.95 ± 0.66***	43.10 ± 1.53

N: non significant
**: highly significant at ($p<0.01$)
***: very highly significant at ($p<0.001$)

Table 2: Biochemical variables (mean ± SE) in blood of healthy cattle and those affected with post parturient haemoglobinurea (PPHU) before and one month after treatment.

Items / Parameters	Control healthy cattle	PPHU affected cattle	Post treatment
T.Proteins (gm/dl)	7.12 ± 0.19	4.82 ± 0.17***	6.27 ± 0.26
Albumin (gm/dl)	3.40 ± 0.16	2.22 ± 0.15**	2.86 ± 0.35
Globulins (gm/dl)	3.70 ± 0.17	2.60 ± 0.13***	3.08 ± 0.14
Glucose (gm/dl)	80.82 ± 1.62	46.96 ± 1.75***	71.04 ± 2.17
AST (u/L)	59.91 ± 3.17	73.62 ± 2.96**	58.36 ± 2.42
ALT (u/L)	17.45 ± 1.11	30.65 ± 1.88***	18.57 ± 0.78
ALP (u/L)	15.90 ± 1.69	22.62 ± 1.61**	15.40 ± 1.87
B. Urea (gm/dl)	30.87 ± 2.26	47.89 ± 4.69**	33.17 ± 2.47
Creatinine (gm/dl)	1.13 ± 0.07	1.84 ±0 .10N	1.06 ± 0.08
T.Cholesterol (gm/dl)	126.31 ± 3.56	83.27 ± 2.49***	118.40 ± 4.91
T.Triglycerides (gm/dl)	96.2 ± 2.30	81.67 ± 2.20***	95.53 ± 1.50

N: non significant
**: highly significant at ($p<0.01$)
***: very highly significant at ($p<0.001$)

Table 3: Blood serum macro and micro elements levels (mean ± SE) in healthy cattle and those affected with post parturient haemoglobinurea (PPHU) before and one month after treatment.

Items / Parameters	Control healthy cattle	PPHU affected cattle	Post treatment
Calcium (gm/dl)	10.0 ± 0.29	9.40 ± 0.39^{N}	9.92 ± 0.40
Phosphorus (gm/dl)	5.49 ± 0.14	2.15 ± 0.29***	4.34 ± 0.45
Magnesium (gm/dl)	2.76 ± 0.10	2.42 ± 0.09***	2.56 ± 0.09
Sodium (mmol/L)	142.96 ± 1.18	134.3 ± 1.60***	140.72 ± 1.46
Potassium (mmol/L)	5.28 ± 0.14	3.18 ± 0.21***	4.64 ± 0.17
Zinc (ug/L)	161.13 ± 5.69	143.19 ± 7.15^{N}	165.15 ± 4.14
Iron (ug/L)	156.24 ± 1.26	142.20 ± 2.66***	152.59 ± 1.11
Copper (ug/dl)	72.14 ± 1.35	54.54 ± 3.45***	62.88 ± 2.27
Molybdenum (ug/dl)	55.54 ± 0.89	72.11 ± 1.47***	57.29 ± 0.87

N: non significant ***: very highly significant at (p<0.00

Table 4: Mean ± SE of minerals, total proteins and protein non nitrogenous compounds in the urine in healthy cattle and those affected with post parturient haemoglobinurea (PPHU) before and one month after treatment.

Items / Parameters	Control healthy cattle	PPHU affected cattle	Post treatment
Phosphorus (gm/dl)	14.91 ± 0.43	18.60 ± 0.51***	16.38 ± 0.46
Calcium (gm/dl)	11.85 ± 0.21	15.01 ± 0.37***	10.12 ± 0.38
Magnesium (gm/dl)	28.56 ± 0.43	31.42 ± 0.32***	30.32 ± 0.19
Protein (gm/dl)	31.54 ± 0.16	295.53 ± 0.39***	37.48 ± 0.50
Urea (u/L)	0.398 ± 0.052	1.11 ± 0.107***	0.424 ± 0.008
Creatinine (u/L)	29.15 ± 0.26	37.32 ± 0.52***	27.42 ± 0.30

***: very highly significant at (p<0.001)

Table 5: Mean ± SE of phosphorus, calcuim, copper and molybdenum of barseem from newly reclaimed area and old agricultural area (old villages) on dry matter basis.

Parameter / Items	DM basis			
	Copper mg/kg	Molybdenum mg/kg	Phosphorus %	Calcium %
Barseem from newly reclaimed area	5.78 ± 0.14^{N}	4.9 ± 0.27***	0.17 ± 0.01**	3.43 ± 0.15
Barseem from old villages	7.1 ± 0.02	0.86 ± 0.01	0.31 ± 0.02	3.51 ± 0.02

N: non significant m **: highly significant at (p<0.01)
***: very highly significant at (p<0.001)

DISCUSSION

The clinical observation of cattle suffering from post parturient haemoglobinurea (PPHU) during early lactation (2-4 weeks) showed

decreased and depraved appetite, refuse barseem eating, preferring dry feeds, pale to icteric visible mucous membranes, weakness, accelerated heart rate, increase in pulse rate, rapid and shallow respiration, red to coffee coloured urine, straining while defecating. Body temperature ranged from 38 – 39.1 °C and milk production drop rapidly. The clinical signs were similar to those reported by Abdel – Maksoud and Abdle – Raoef (1998), Radostits *et al.* (2000), Mobark and El-Bealawy (2002) and Emam *et al.* (2005). The above mentioned observations were also supported by previous studies by Wang *et al.* (1985) who mentioned that the phosphorus deficiency leading to hypophosphataemia may play a part in haemoglobinurea by decreasing erythrocyte glycolysis**.** The increase in pulse rate might be attributed to the decrease blood volume following the intra vascular haemolysis and anaemia, while the accelerated respiratory rate also due to the anaemic hypoxia (Radostits *et al.*, 2000). Analysis of samples of barseem from newly reclaimed area and from old agricultural area, revealed that the concentrations of phosphorus, copper, molybdenum and calcium content were suggestive of primary phosphorus and secondary copper deficiency.

There would appear significant difference at ($P<0.01$) in phosphorus content between both samples. This low levels of phosphorus in barseem from newly reclaimed area may be attributed to the low phosphorus content in the parent rock from which the soil are derived (Radostits *et al.*, 2000). Meanwhile no significant difference was detected (Table 5) in copper content between both diet samples.

Obtained values of copper content of barseem from newly reclaimed area, where a deficiency occurred, are generally considered adequate for cattle unless complicating factors cause secondary deficiency (Under wood and Suttle, 1999). In this study it appeared that copper deficiency is caused by the relatively high concentration of molybdenum in barseem of recently reclaimed area, which effectively reduce the availability of dietary copper in cattle. In this regard, molybdenum and sulphur react to form tetrathiomolybdates that then react with copper resulting in the formation of highly stable compounds that can not be digested and absorbed. Such correlation go in parallel with the results previously recorded by Ward (1978) and Allen and Gawthoren (1987). The fodders in particular barseem grown on newly reclaimed area have high molybdenum content, the excess of this element reduces phosphorus content of the body by interfering with its absorption from the gastro - Intestinal tract and increasing phosphorus elimination through urine (Dhillon *et al.*, 1972). Analysis of blood in this

study showed very highly significant decreases (P<0.001) in TRBCs count, Hb concentration and PCV% values in cattle affected by PPHU indicating severe anaemia. This could be attributed to intra vascular haemolysis (Smith, 1990; Digraskar *et al.,* 1991 and Akhtar *et al.,* 2007) due to an impaired glycolytic pathway and depletion of ATP in erythrocytes which results from phosphorus deficiency. This ATP is essential for maintenance of shape and deformability of erythrocyte (Kaneko *et al.,* 1997). Subnormal concentration of ATP predisposes red blood cells to alter function and structure, a loss of normal formability, increased osmotic fragility and shortened life span ultimately leading to haemolysis (Wang *et al.,* 1985 and Ogawa *et al.,* 1989). El-Amrousi *et al.* (1977) attributed these change to destruction of erythrocyte with release of haemoglobin content causing decrease in haemoglobin concentration accompanied by oligcythemia. The higher MCV with lowered MCHC indicate that the anaemia is of macrocytic hypochromic type which may be due to malnutrition and malabsorption (Abdel-Maksoud and Abdel-raoef, 1998). The explanation of the anaemic condition in hypocupremic cattle based on the role of copper in the production of haemoglobin through the reutilization of iron liberated from normal breakdown of haemoglobin by the activation of ferroxidase enzyme, (Radostits *et al.,* 2000 and Shalaby, 2003). Concerning, the total leucocytic count in the present study showed very highly significant increases in PPHU cattle in comparison to control group. The increased TWBCs might be due to the endogenous release of corticosteroids. Increase stress due to PPHU (a metabolic disorder) is the source of the release of corticosteroids. These observation are in agreement with those mentioned by (Singari *et al.,* 1991; Abdel-Maksoud and Abdel-Roaef, 1998 and Akhtar *et al.,* 2007).

The blood hydrogen ion concentrations (pH) value showed non significant increase in PPHU cattle. These results similar to that obtained by Barzanji and Daniel (1987) who attributed the increase level of pH to the reduced oxygen tension as a sequel to the alteration in the acid base balance. The obtained results in this study revealed highly significant increase in Pco2 and non significant increase in bicarbonate. While Po2 showed very highly significant decrease in PPHU cattle. There is a type of anaemic anoxia which lead to decrease in oxygen level in the blood. These results were supported by previous studies Barzanji and Daniel (1987) who mentioned that the up take of O2 by the peripheral tissues was reduced during metabolic disorders. It was found also that the

decreases in blood flow to tissues have been shown to cause increases in blood PCO2 levels.

The highly significant decreases in serum total proteins, albumin and globulins levels in PPHU cattle in present study were nearly in accordance to the observed results of Abd-Allah and El-Fadali (1996); Abdel-Aal (1997) and Abdel-Maksoud and Abdel-Roaef (1998), who stated that the decreased values could be attributed to the loss of protein from the destructed RBCs. The case may be also due to the tubular degeneration in the kidney during haemoglobinurea or/poor nutritional status as represented by Kurundikney *et al.* (1981), and /or related to deprivation of the diet as reported by Abdel-Maksoud and Abdel-Roaef (1998) and Tawfik *et al.* (2004). Meanwhile Scott (1988) stated that the decreased levels of total proteins in PPHU might be attributed to the diet which was poor in proteins or due to the inhibition of the liver to synthesis more proteins. Mohga (2000) reported that the reduction of albumin concentration might be attributed to either a decreased food intake or albumin loss as a result of increased capillary permeability in copper deficient animals due to abnormalities in the blood vessel wall elastin and collagen structures. The highly significant decrease in the mean values of serum globulin level is similar to the opinion of Abdel-Aal (1997) and Tawfik *et al.* (2004) as they found no significant decrease in the serum globulins fractions in cattle in the green season.

The present investigation revealed that AST, ALT and ALP enzymes activity levels were highly significantly increased in cattle suffering from PPHU. The increases, in these enzymes, were previously reported by Wikes *et al.* (1992) and Tawfik *et al.* (2004), who recorded that the changes in the activities of the liver specific enzymes in combined phosphorus and copper deficiency in cattle may be due to alteration in the metabolic rate which accompanied that deficiency. Another explanation, for such increase in enzymes activities, was reported previously by Mohga (2000) and Shalaby (2003), who attributed to the hepatic dysfunction and skin lesions. Hypoglycaemia was noticed in this study in PPHU may be due to loss appetite or loss of blood through the urine and / or depraved metabolic processes which consequently reflected upon glucose metabolism and on its levels. Same results were previously reported by Mobark and El-Bealawy (2002); Tawfik *et al.* (2004) and Emam *et al.* (2005).

A high significant increase in blood urea was noticed in PPHU cattle when compared with control healthy ones. Such increases can be explained by the state of negative protein balance in the affected animals

which may leads to catabolism of body protein, producing excess of urea (Coles, 1986). Or could be attributed to the endogenous release of corticosteroids, starvation and tubular epithelial necrosis (kurundikney *et al.,* 1981 and Digraskar *et al.,* 1991). Additionally, dehydration usually occurs with PPHU which is a source of decreased renal perfusion, resulting in a reduced glomerular filtration rate and increased blood urea level Stogdale, (1981 and Latimar *et al.,* 2003). Alternatively, increased blood urea could be due to the failure of the urea recycling process through salivary glands and its non-utilisation by microbes in the rumen during digestive disorders. Most of the urea formed by the liver circulation in the circulatory system and remain unutilised (Singh *et al.,* 1989). In the present study, creatinine was increased in PPHU cattle when compared with control healthy once. This increased level may be attributed to the deficiency of inorganic phosphate which plays an important role in the intermediary metabolism of creatinine in the chemical reaction occurring in the muscle contraction (Radostits *et al.,* 2000). Both urea and creatinine levels were elevated and positively correlated to each other in PPHU affected cattle. Urea and creatinine are waste products that the kidneys normally filter from the blood and these are interrelated if the kidneys are not working properly (Kurundikney *et al.,* 1981; Digraskar *et al.,* 1991 and Mousa, 1998).

A high significant decrease in serum total cholesterol and total triglycerides levels was observed in PPHU when compared with control ones. The obtained results were similar to those previously reported by Tawfik *et al.* (2004) who found reduction in serum cholesterol level in copper deficient animals. These reductions of cholesterol and triglycerides may reject either reduced food intake or early hepatic damage (Uivund, 1990).

The inorganic phosphorus level in the serum showed very highly significant decrease in the PPHU cattle in this study. This result is in agreement with that reported by (Abdel-Maksoud and Abdel-Roaef, 1998; Mobark and El-Bealawy, 2002; Emam *et al.,* 2005 and Akhtar *et al.,* 2007), who mentioned that the decreased phosphorus level may be due to long feeding on barseem with low level in phosphorus content. Also due to heavy drainage of phosphorus through milk particularly in high milk yielding animals (Bhikane *et al.,* 1995 and Ivanov *et al.,* 1995). Moreover, soils in the newly reclaimed area have high molybdenum content. The fodders in particular barseem grown on such soils have high molybdenum content. The excess of this element reduces phosphorus content of the body by interfering with its absorption from

the gastro-intestinal tract and increasing phosphorus elimination through urine (Dhillon *et al.,* 1972).

The non-significant changes in serum calcium levels in PPHU cattle in this study were in agreement with the opinion of Brain and Eric (1996) who reported that, the serum calcium levels were at the reference limits in hypophosphataemia. The obtained results revealed that the magnesium level was highly significantly decreased in cattle affected by PPHU. Present results were in agreement with those obtained by Mobark and El-Bealawy (2002) and Emam *et al.* (2005).

Serum electrolytes levels including sodium and potassium were highly significant decrease in cattle affected by PPHU. The obtained results were similar to those previously reported by El-Amrousi et al. (1977), who reported that the decreases may be due to loss of these electrolytes as a result of diarrhoea which was observed in same individuals of this group.

The non-significant change in serum zinc level in PPHU cattle was in agreement with the results, noticed on phosphate deficient buffaloes-cows (Ismaeil and Hussein, 1988).

Concerning the blood serum iron and copper levels the obtained results in PPHU cattle, suffering from primary phosphorus and secondary copper deficiencies behaved very highly significant decrease when compared with control. Nearly similar data were recorded by Georgievskij (1982) and proved that there are direct interactions between copper and iron in the formation of haemoglobin, copper is essential for iron metabolism. Therefor the serum iron level is decreased in copper deficient animals (Nabila, 1983). Also the decreased serum copper level might be due to the poor-nutrition, which accompanied by decreased appetite and gradual starvation of the diseased animals (Abdel-Maksoud and Abdel-Roaef, 1998).

The serum molybdenum levels were highly significantly increase in PPHU affected cattle suffering from combined phosphorus and copper deficiency if compared with clinically healthy ones. Similar results were previously obtained by Tawfik *et al.* (2004). Such elevation can be explained by the antagonist relationship between copper and molybdenum which was previously discussed, (Allen and Gawthoren, 1987 and Shalaby, 2003).

The result of urine analysis showed very highly significant increase in the concentrations of urine phosphorus and calcium in PPHU affected cattle. Similar observation was recorded by Singh *et al.* (1974) and Mousa (1998). The increased excretion of these substances through

urine indicated renal damage (Teotia *et al.*, 1973). Depressed tubular reabsorption of phosphorus in case of renal damage was reported by Shtacher (1969).

The elevated protein concentration in urine of diseased cattle supports the involvement of kidney glomeruli. Proteinurea occurs as a result of injury of glomeruli (Kaneko, 1997). The high levels of urea and creatinine in the urine might be the result of kidney dysfunction. Estimation of creatinine is a valuable diagnostic test for haematouria (Singh *et al.*, 1974).

After treatment:

An observable advance in both clinical and laboratory results of cattle suffered from PPHU after correction of the diet system was achieved by avoiding the excessive feeding on barseem and providing the animals with balanced ration. Transfusion of large quantities of whole blood is the treatment for severely affected cows. The diseased cases were treated by sodium acid phosphate injection, plus to daily injection with 20 ml tonophosphan 20%. Also one dose of copper glycinate injection (120 mg available copper) halt haemolysis. This result agreed with the obtained by Mousa (1998); Radostits *et al.* (2000); Mobark and El-Bealawy (2002) and Emam *et al.* (2005). Who observed disappearance of clinical signs, normalization of macro and micro elements and improvement in haematological values after treatment with sodium acid phosphate and copper glycinate.

It could be concluded that PPHU noticed in cattle fed excessively on barseem from recently reclaimed area could be attributed to phosphorus deficiency which plays a key role in causing PPHU in cattle. Post parturient haemoglobinurea affected cattle showed hypocopraemia and reduce erythrocytic counts and this in turn reflects the disturbance in general healthy condition of the affected animals. So attention must be paid for careful early clinical and laboratory diagnosis of diseased animals followed by therapeutic plan with good management. Furthermore excessive feeding on barseem must be avoided and feeding balanced ration during the green season is also utmost.

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