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CLINICAL AND BIOCHEMICAL STUDIES ON HYPOPHOSPHATEMIA (POST-PARTURIENT HAEMOGLOBINURIA) IN CATTLE

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ABSTRACT

This study designed to verify the haematological and biochemical changes that occur in cattle with post-parturient haemoglobinuria (PHU). For this intention, blood and serum samples from 30 PHU-affected and 20 apparently and clinically healthy cattle were collected and analyzed. Mean erythrocyte count, haemoglobin concentration, and haematocrit of the PHU-affected cattle were lower ($P < 0.05$), while their erythrocyte sedimentation rate was higher ($P < 0.05$) in comparison to the healthy cattle. Neutrophils, urea and creatinine concentrations were significantly higher in the PHU-affected cattle, while lymphocytes, erythrocytic glucose-6-phosphate dehydrogenase (G6PD) and glucose were lower than in the healthy cattle. There were significant increase in the levels of GGT and AST with significant decrease in total protein, albumin and globulin in PHU affected cattle in comparison with control group. Serum phosphorus was lower, while calcium was higher in the PHU-affected cattle as compared to those values in the healthy cattle. It was concluded that PHU affected cattle usually suffer from severe anemia and hypophosphataemia, and erythrocytes with significantly reduced G6PD are prone to haemolysis, leading to haemoglobinuria in cattle.

KEY WORDS: Parturient haemoglobinuria, cattle, haematology, biochemistry

INTRODUCTION

The transition from gestation to lactation is a period of great metabolic stress for dairy cows. Homeorhetic mechanisms in early lactation partition nutrients toward the mammary gland to support lactation even at

the expense of other body tissues. At the same time, voluntary DMI is markedly decreased **(Rollin et al., 2010)**

Parturient haemoglobinuria (PHU) is a major disease of dairy animals with detrimental economic consequences **(MacWilliams et al., 1982 ; Chugh et al., 1996).** It is an acute disease of high yielding dairy animals characterised by hypophosphataemia, intravascular haemolysis, haemoglobinuria, and anaemia **(Radostits et al., 2007).** The exact aetiology and pathogenesis of PHU are not known, as a variety of aetiological factors have been reported to be associated with the disease in different parts of the world. Nonetheless, hypophosphataemia is documented consistently in affected animals (**Hussain et al., 1991 ; Chugh et al., 1996).** Dietary phosphorus deficiency and/or rations containing cruciferous plants are suspected causes of severe hypophosphataemia and have been associated with haemolytic anaemia in cows (**MacWilliams et al., 1982**). 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).**

MATERIALS AND METHODS

Animals

The study included 30 cattle (376 ± 22Kg body weight) in AL-Ahsa, Saudi Arabia, suffering from PHU that were randomly selected from field cases arrived to the veterinary teaching hospital, faculty of veterinary medicine and animal resources, king Faisal university and all of them having a normal labor. The controls were 20 clinically healthy cattle of similar description from the same localities. The study animals were fed on seasonal green fodders, including Trifolium alexandrinum (Berseem) and hay.

Clinical examination

All buffaloes were clinically examined every day until 4 weeks after parturition, according to **Radostits et al. (2007).** The disease was clinically diagnosed on the basis of specific signs, such as haemoglobinuria, anorexia, normal rectal temperature and characteristic straining while defecating during early lactation or advanced pregnancy **(Akamatsu et al., 2007).** Other diseases that cause a reddish

discolouration of urine, like babesiosis, leptospirosis, and bacillary haemoglobinuria, were ruled out through laboratory tests.

Collection and analysis of blood and serum samples

Haematological and biochemical studies Blood samples from were collected from each cow, with and without anticoagulant. Blood samples with anticoagulant were used for the determination of erythrocyte and leukocyte counts (haemocytometer method), haemoglobin concentration (cyanmethaemoglobin method), haematocrit (microhaematocrit method), and erythrocyte sedimentation rate (Westergren tube method), following the techniques described by **Benjamin (1978).**

Differential leukocyte counts were determined by staining the blood smears with Giemsa stain **(Benjamin, 1978).** Serum was separated from blood samples collected without anticoagulant and preserved at -20 C° for further biochemical analysis. Serum urea (Crescent Diagnostics, Jeddah, Saudi Arabia), creatinine (Biocon Diagnostik, Germany), and erythrocytic G6PD (Randox Laboratories Ltd., UK) were estimated spectrophotometrically using the diagnostic kits according to the manufacturer's instructions. Spectrophotometric assays was conducted for colorimetric determination of AST **(Reitman and Frankel, 1957),** GGT **(Yang et al., 1998)** glucose **(Lott, 1975),** phosphorus **(Morinal and Prox, 1973)** and serum calcium **(Glinder and King, 1972).** All steps were performed using a selective chemistry analyzer (Abbott Alcyon 3001, USA).

Statistical analysis

The obtained data of the examined acute phase proteins were compared between groups within different concentrations by using computer package of the statistical analysis system **(SAS 1997).** All data are presented as means ± standard error (S.E.) of the means.

RESULTS

Table 1. The blood picture of control and diseased animals

Variable	Control group	Diseased group
Erythrocyte count (× 10^6 /µl)	8.72 ± 1.32	4.8 ± o.42*
Haemoglobin concentration (g/dl)	12.46 ± 1.32	6.87 ± 0.56*
PCV (%)	37. 23 ± 1.22	18.23 ± 1.11*
Erythrocyte sedimentation rate (mm/1 h)	71.65 ± 12.3	111.45 ± 15.23*
Total leukocyte counts (/µl)	7254 ± 1163.3	10132 ± 1460.3*
Neutrophils (%)	36.24 ± 2.35	45.35 ± 3.45*
Lymphocytes (%)	54. 26 ± 3.56	44.36 ± 4.23*
Monocytes (%)	5.62 ± 0.34	5.53 ± 0.32
Eosinophils (%)	3.2 ± 0.71	3.1 ± 0.62

*Means are significantly different at the level ($p \leq 0.05$).

Table 2. The elemental and biochemical parameters in diseased and control animals

Variable	Control group	Diseased group
Phosphorus (mg/dl)	5.56 ± 0.56	1.8 ± 0.57**
Calcium (mg/dl)	9.92 ± 1.32	10.12 ± 1.22
Erythrocytic G6PD (mU/10^7 TECs)	116.34 ± 14.33	88.43 ± 12.67*
Urea (mg/dl)	25.34 ± 3.54	43.22 ± 8.31*
Creatinine (mg/dl)	1.3 ± 0.22	2.44 ± 0.21*
AST (U/L)	67.65 ± 4.61	99.26 ± 6.67*
GGT (U/L)	7.2 ± 1.54	13.67 ± 1.45**
Glucose (mmol/L)	3.69 ± 0.23	1.66 ± 0.24**

*Means are significantly different at the level ($p \leq 0.05$).
**Means are highly significantly different at the level ($p \leq 0.01$).

DISCUSSION

Hypophosphataemia in PHU-affected animals is consistently documented **(Chugh et al 1996 and Radostits et al., 2007).** In the present study, significantly decreased serum phosphorus in PHU affected cattle was recorded (Table 2) as has been reported previously in PHU-affected cattle **(Stockdale et al., 2005).** Heavy drainage of phosphorus through milk, particularly in high milk yielding animals, leads to hypophosphataemia **(Bhikane et al., 1995).** In advanced gestation, more phosphorus and calcium are required for the developing foetus if supplementary phosphorus is not provided, thereby leading to hypophosphataemia. Moreover, a high calcium to phosphorus ratio results in decreased phosphorus absorption from the intestinal tract and ultimately leads to hypophosphataemia **(Benjamin, 1978).** It is well-known that Berseem as a green fodder is a rich source of calcium. It was concluded that PHU is strongly associated with Berseem feeding in winter season, probably because Berseem has high calcium to phosphorus ratio (>2:1) **(Macwilliams et al., 1982).**

Moreover a significant decrease in erythrocyte count, haemoglobin concentration, and haematocrit in PHU affected cattle (Table 1) indicates severe anaemia. This could be attributed to intravascular haemolysis (**Benjamin, 1978** and **Smith, 1990**) due to an impaired glycolytic pathway and depletion of ATP in erythrocytes, which results from phosphorus deficiency. Subnormal concentration of ATP predisposes red blood cells to alter functions and structure, a loss of normal formability, and an increase in fragility, ultimately leading to haemolysis **(Wang et al., 1985 and Suttle, 1991).**

In the present study, total erythrocyte count was optimistically correlated with hemoglobin concentration and hematocrit in PHU-affected cattle, which were also anaemic. In anaemic cases, total erythrocyte count, haemoglobin concentration, and haematocrit were reported to decrease simultaneously **(Benjamin, 1978).**, indicating a possible positive correlation between total erythrocyte count and both haemoglobin concentration and haematocrit.

The erythrocytic G6PD activity and glucose levels in PHU-affected cattle was significantly lower than that in healthy cattle (Table 2). **Singari et al.**

(1991) suggested that decreased erythrocytic G6PD activity in haemoglobinuric buffaloes may be partially responsible for the decrease in reduced glutathione, thereby causing oxidative stress to erythrocytes, which leads to haemolytic syndrome. Among the 2 major pathways of glucose metabolism in red blood cells, the pentose phosphate pathway (PPP) is of critical significance for normal red cell survival. The first reaction in PPP is the catalytic action of the enzyme G6PD in oxidising glucose-6-phosphate. NADPH generated by the cells PPP has a reducing potential on glutathione, and glutathione maintained in a reduced state protects red cells from oxidative stress; thus, a deficiency of G6DP will result in haemolytic anaemia **(Agar and Board, 1983).**

Deficiency of G6PD, owing to mutation, is the most common enzymatic abnormality in humans and has a high incidence rate, and over 300 genetic variants of the enzyme have been identified; at least 100 million people are deficient in this enzyme owing to these variants **(Agar and Board, 1983).**. G6PD may exist in haemoglobinuric buffaloes, but this needs further exploration. The increasing and decreasing trend in neutrophil and lymphocyte counts, respectively, in PHU-affected cattle could be attributed to the endogenous release of corticosteroids. Increased stress due to PHU (a metabolic disorder) is the source of the release of corticosteroids (**Singari et al., 1991 and Stockdale et al., 2005)** that results in increased neutrophils and depressed lymphocytes. Neutrophils are short-lived and normally the entire neutrophilic population in circulation is replaced 2.5 times daily **(Benjamin, 1978),** therefore, these have to leave circulation rapidly (about 9-10 h), but under disease conditions these are retained in circulation.

Moreover, marginal neutrophils are pooled in the main circulation and increased release of neutrophils from the maturation pool (**(Benjamin, 1978** and **Latimer et al 2003)**.) seems to be the main source of neutrophilia in PHU-affected cattle. According to **Latimer et al. (2003),** recirculating lymphocytes under the influence of corticosteroids remain transiently sequestered in the lymphoid tissues or bone marrow rather than entering efferent lymph and blood, resulting in lymphopenia. Furthermore, lysis of lymphocytes in all tissues and a decline of lymphoid mitosis in lymph nodes, due to corticosteroids, can also lead to lymphopenia. The reduction in glucose level may occur in response to energy restriction in the diet **(Bremmer et al., 2000)** specially at the early

stage of lactation when high rate of glucose utilization in the mammary gland is required **(Nazifi et al., 2008).**

The Erythrocyte sedimentation rate is governed by the balance between pro-sedimentation factors, mainly fibrinogen, and those factors resisting sedimentation, namely the negative charge of the erythrocytes (zeta potential). When an inflammatory process is present, the high proportion of fibrinogen in the blood causes red blood cells to stick to each other. The red cells form stacks called 'rouleaux' which settle faster. In the present study the increased Erythrocyte sedimentation rate is attributed to intravascular haemolysis and the anemic state of the examined cattle (**Benjamin, 1978 and Smith, 1990**).

The presence of AST in many organs of animals makes serum level a good marker of soft tissue damage but preclude its use as an organ-specific enzyme **(Kramer, 1989).** However, determining AST activities in dairy cows is most often connected with fatty liver syndrome **(Cebra et al., 1997).** Moreover, **Steen et al. (1997)** found that AST activity was greater in cows with ketosis (115 U/L) and hepatic lipidosis (252 U/L) than in healthy cows (70 U/L). The infiltration of hepatic cells with fat increases cell membrane permeability with subsequent release of AST enzyme that serves as a good tool for metabolic liver diseases **(Karasai and Schefar, 1984).** Therefore, the increased level of AST and GGT in PHU cattle in our study could be attributed to the fatty liver changes associated with the negative energy balance occurring in the peripartum period. **(Kaneko, 1989).**

Increased blood urea levels in PHU-affected cattle could be attributed to the endogenous release of corticosteroids, starvation, and tubular epithelial necrosis **(Latimer et al., 2003).** Additionally, dehydration usually occurs with PHU, which is a source of decreased renal perfusion, resulting in a reduced glomerular filtration rate and increased blood urea level **(Finco and Duncan, 1976**; **Benjamin, 1978; Latimer et al., 2003**, ; **Stockdale et al., 2005).** 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 circulates in the circulatory system and remains unutilized (**Stockdale et al., 2005**). In the present study, creatinine was significantly increased in PHU cattle.

In this consider, **Benjamin (1978)** considered that concentrations over 2 mg/dl lead to a reduced glomerular filtration rate, which affects creatinine in a manner similar to that of blood urea **(Latimer et al 2003)**. Both urea and creatinine levels were elevated and positively correlated to each other in PHU-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 (**Latimer et al., 2003**), these substances build up in the body, and elevated blood levels of urea and creatinine are indications of pathological kidney function (**Latimer et al., 2003**). It was concluded from the present study that phosphorous deficiency plays a key role in causing haemoglobinuria, anemia disturbed liver and kidney function and reduced G6PD in erythrocytes of affected cattle. Moreover, control of feeding program is very important in the control of such clinical problem.

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دراسات إكلينيكية وبيوكيميائية عن نقص الفسفور في الأبقار بعد الولادة

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أجريت هذه الدراسة بهدف دراسة التغييرات التي تح دث في الدم والتغييرات الكيميائية المصاحبة لحالات نقص الفسفور بعد الولادة في الأبقار . تم إجراء الدراسة علي عدد 30 بقرة مصابة بنقص الفسفور و 20 بقرة سليمة من الناحية الإكلينيكية . وقد أظهرت النتائج وجود انخفاض في العدد الكلي لكرات الدم الحمراء ومستوي الهيموجلو بين مع ارتفاع في نسبة سرعة الترسيب. كما لوحظ ارتفاع في نسبة كريات الدم البيضاء ومستوي إنزيمات الكلي مع انخفاض في مستوي الجلوكوز . كما لوحظ أن هناك ارتفاع ملحوظ في مستوي إنزيمات الكبد وانخفاض ملحوظ في نسبة البروتين الكلي والالبيومين والجلوبيولين G6PD & في الأ بقار المصابة بنقص الفسفور عند مقارنتها بمثيلتها الضابطة . وقد خلصت الدراسة إلي أهمية التغييرات الدموية والكيميائية في تشخيص حالات نقص الفسفور إضافة إلي أهمية هذا المرض كمسبب لحالات الأنيميا في الأبقار.