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Clinical, haematological and some biochemical variations hypophosphataemia in buffaloes before and after treatment at Assiut Government

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A total number of 28 buffaloes aged between 5-7years, body weight ranged between 400-500 kg, and belonged to private farms at Assiut Governorate constituted the materials of this study. Twenty of them showed the classical signs of hypophosphataemia while the other eight buffaloes were proved to be healthy by both clinical and laboratory investigations, used as control group. Biochemical analysis of blood sera showed a highly significant hypophosphataemia, hypocalcaemia and hypomagnesaemia in diseased buffaloes either pre or post treatment when compared with the healthy control ones. Meanwhile, the examination of blood showed marked decreases in erythrocytic count, haemoglobin concentration and packed cell volume in diseased animals. All parameters improved in affected buffaloes after 10 days of treatment. The chemical analysis of agronomical samples of soil and drinking water were done. The statistical analyses between the studied parameters were carried out in buffaloes before and after treatment.

Hypophosphataemia is a well-known metabolic disorder of cattle and buffaloes resulted from deficiency of phosphorous or imbalance in Ca:P ratio. Phosphorus deficiency leading to hypophosphataemia may play a part in haemoglobinurea by decreasing red cell glycolysis and ATP synthesis. It is a sporadic disease affecting high ratio of buffaloes, and considered to be of dietary origin, as a result of prolonged feeding on barseem (Nassif, 1995; Selim *et al*., 1998; Rizk *et al*., 1999).

Hypophosphataemia affects recently calved and heavey lactating buffaloes. Buffaloes at the 3 rd to 6th lactations are most commonly affected. The disease usually appears in the period from 2nd to 4th weeks after calving, it was considered that there was much more drainage of this element through milk (Chugh *et al*., 1998 and Abdul Samad, 1997). The pregnancy stress indicates that buffaloes from the 7th month of pregnancy have a significant wider ratio of blood Ca:P than those the earlier stages of pregnancy (Pirzada *et al*., 1998).

Many authors pointed that the higher incidence of hypophosphataemia in buffaloes occurs from May to January which coincides with the late pregnancy and early post-parturient periods as the majority of buffaloes either in late stage of pregnancy or early post parturient stage during these months.

The mechanism by which the interavascular hemolysis occurs has not fully defined. Several predisposing factors are included such as recent parturition, inadequate dietary phosphorous intake and/or high calcium ratio, copper deficiency, drinking of cold water or exposure to extreme coldness (Smith, 1996 and Chugh *et al*.,1998). Faqing *et al*., (2002) speculated that the constituents of phospholipids in the erythrocyte membrane, the protein in the membrane skeleton and the form of erythrocyte changes when haemolysis occurs in the cow with low phosphorus intake.

The deficiency of phosphorus causes loss of appetite, referred as 'Pica`, which symptomized by eating bones, manure, placenta and other materials like clothes and wood. This commonly occurs in animals grazing on soils low in phosphorus as a results of which the forage grown with low in

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phosphorus (Pirzada *et al*.,1998). The other most prominent clinical symptoms related to phosphorous deficiency were haemoglobinuria (coffe-coloured urine), pale mucous membranes of conjunctiva and vulva, sharp decrease in milk yield and constipation with constricted sphincter ani accompanied with either normal or subnormal body temperature (Selim *et al*., 1998 ; Rizk *et al*.,1999). The most prominent symptoms of hypophosphataemia in buffaloes include hemoglobinuria, inappetance, depression of milk yield, the mucous membranes are palled, weakness associated with osteomalacia, muscles weakness, lameness, locomotor disturbances, staggering gait. Recumbency occurs later in severe hypophosphatemic animals (El-Magawry *et al*., 1995; Rizk *et al*., 1999; Hoda, 2006). There is also, labored respiration with acceleration of pulse and jugular pulsation .Finally the affected animals with severe hypophosphataemia may die from anemic hypoxia or anoxia (Smith, 1996). The phosphorus deficiency in buffaloes is one of the most important causes of ovarian inactivity among Egyptian cows and buffaloes (Nassif, 1995).

The level of serum inorganic phosphorus in buffaloes with haemoglobinuria was very low (0.9mg/100ml) with a decrease in serum level (Pandy and Misra, 1987).

Selim *et al*., (1998) proved that the injection of tonophosphan i/m 25cc once daily in the first two days with s/c of 20% sodium acid phosphate every 12 hours for 5 successive days, in addition with 120gm disodium phosphate orally once daily for 10 days give good results.

Rizk *et al*., (1999) treated hypophosphataemic buffaloes with i.v injection of Ringer solution, (1L) together with i.m injection of AD3E (5ml/animal) and the dose was repeated after one week. In addition 5ml Pregazole followed i.v. slow injection of 1L of dextrose solution (5%) containing 30gm dihydrogen phosphate were also used. In fatal cases, blood transfusion is recommended (Hotter and Buchner, 1995).

Magnesium is considered to be the major intracellular divalent cation and it is an essential element among a number of enzymatic activities in the body including phosphates and enzymes that involve ATP. The action of magnesium extends to all major anabolic and catabolic processes. Magnesium plays a vital role in muscle contraction, protein, fat and carbohydrate metabolism, function properties and immune responses (Kaneko *et al*., 1997; Radostitis, *et al*., 2003).

The absorption of phosphorous from the reticulorumen is severely reduced as in dietary phosphorous deficiency. The absorption of both Ca and Mg from the reticulorumen becomes impaired (Care, 1994). Kaneko *et al*., (1997) added that magnesium interacts with extra cellular Ca by binding to many shared ligaments. In general Mg acts as a Ca antagonist. Magnesium binds to chief cells in the parathyroid gland and inhibits secretion of parathyroid hormones. Goff, (1999) noticed that the majority of the calcium, phosphorous and magnesium balance disorders are due to low blood concentrations of these minerals.

Analysis of blood in hypophosphataemic buffaloes showed marked decrease in erythrocytic count, hemoglobin concentration and packed cell volume. Observed improvement in these parameters occurs in affected buffaloes after 10 days of treatment due to increased level of blood phosphorus resulting into cessation of haemolysis. (Abdou *et al*., 1999; Mobark and El-Bealawy, 2002)

Preventive measures could be taken to reduce the incidence of hypophosphataemia in buffaloes. Ration in the beginning days of lactation must be quantitatively sufficient in phosphorus content. The minimum daily requirement of buffaloes (450kg.) for phosphorus as phosphate is 22gm/day producing about 7.5 kg milk/day is adequate (Harris *et al*., 1990; Radostitis *et al*., 2003).

This study amid to throw light upon clinical, haematological and some biochemical alteration in buffaloes suffered from hypophosphstaemia associated with trials of treatment.

Materials and Methods

Animals. A total number of 28 buffaloes aged between 5-7 years old with body weight ranged between 400-500 kg. Animals are belonged to private farms at Assiut Governorate. The study extended between October 2006 to January 2007. Twenty of examined buffaloes showed the classical signs of hypophsphataemia and divided to three groups, each one took different treatment. Another eight clinically and healthy buffaloes were used as a control. These animals were subjected to careful clinical and laboratory examination to ensure their healthy status and used as control.

Blood samples. Two blood samples were obtained from each animal by a jugular vein puncture one without anti-coagulant before and after treatment to obtain clear non-haemolysed sera, which used for biochemical studies. The obtained sera were used for determination of magnesium, calcium and phosphorus using test-kits after the methods described by Bohuon (1962), Gindler (1972) and Goldenberg (1966) respectively. Another blood samples with anti-coagulant (before and after treatment), were used for haematological studies (total erythrocytic count, hemoglobin concentration and packed cell volume) according to the methods described by Schalm (1986).

Soil samples. Samples were collected from the top 20cm of soil. 500gm of the soil was bagged in clean dry nylon bag (Page, 1982). For chemical analysis of agronomical samples, the soil samples were washed by using ammonium bicarbonate for extraction of inorganic phosphorus (Soltan, 1985). Ammonium acetate (Normal) was used to extract calcium (Lindsay and Norvell, 1969).

Water samples. Two samples are taken, one is from tap water (supplied by the General Organization for Drinking Water and Sanitary Drainage) and another from the underground water. Water samples were subjected to collected estimation of their phosphorous, calcium and magnesium levels.

Examination of urine. Voided urine samples from diseased buffaloes were examined for detection of dark red brown to black in color (coffee colored urine of varying intensities). Hemoglobinuria were noticed by allowing urine to stand and settle according to the methods described by Schalm, 1986.

Treatment trials. The diseased animals were treated by using different lines of treatment to evaluate the best line that could improve the clinical, haematological and biochemical parameters. In the first group 60gm of dibasic sodium phosphate (El-GomhouriaCo.) were dissolved in 300ml normal saline and given i/v. in addition to 60gm of dibasic sodium phosphate dissolved in 300ml normal saline given s/c for the first three days followed by 60gm orally for seven days. In the second group, 50ml tonophosphan solution 20% (Intervet Egypt Co.) was given i/v plus 25ml i/m and 25ml s/c for five days. In third group, 60gm of monobasic sodium phosphate (El-Gomhouria Co.), dissolved in 300ml normal saline given i/v. in addition to 60gm of monobasic sodium phosphate dissolved in 300ml normal saline given s/c for five days, followed by 60gm orally for seven days.

Statistical analysis. The obtained results were analyzed statistically according to Selvin, (1996).

Results

Clinical symptom. Clinical examination of buffaloes suffering from hypophosphataemia showed voiding of coffee coloured urine, sharp decreased in milk yield, partial to depraved appetites, anemia (manifested by pale and icteric mucous membranes), weakness, accelerated pulse and respiratory rates with decreased in normal to slightly increased in body temperature, dullness and decreased ruminal stasis, and constipation with straining appears.

The clinical symptoms of haemoglobinuria disappeared within two days in the first group of treatment. Within 4-5 days, the symptoms disappeared in the second group of treatment and the symptoms disappeared after 6 days in the last treated group of diseased buffaloes and animals return to normal.

Biochemical analysis of blood serum phosphorous, magnesium, and calcium. Blood serum inorganic phosphate, calcium and magnesium levels showed significant decrease in hypophosphataemic buffaloes and these parameters reached to near the normal values after treatment in all treated group (Table, 1, 2, 3).

The haematological picture of RBCs, Hb and PCV. The examination of blood samples in hypophosphataemic buffaloes revealed a highly significant oligocythaemia in red cell count, haemoglobin concentration and packed cell volume when compared with healthy ones. Marked improvement began gradually following treating trials which reached near to the normal values after treatment (Table 1, 2, 3).

Urine analysis. Examination of the urine revealed passing of coffee coloured urine usually clear after centrifugation. Sediment retained uniform clear red colour without blood clotted or pus cells and no strong ammoniacal odder.

Chemical analysis of agronomical samples. Phosphorous, calcium and magnesium

Table (1): Biochemical analysis of blood serum phosphorus, magnesium and calcium levels and some haematological parameters in hypophosphataemic buffaloes before and after treatment with dibasic sodium phosphate.

Parameters	Control	Hypophosphataemic buffaloes before treatment	Hypophosphataemic buffaloes after treatment
Phosphorous mg%	5.82±0.08 (5.80-6.00)	4.92±0.80* (4.60-5.40)	5.82±0.08 (5.80-6.00)
Magnesium mg%	6.12±1.03 (6.50-7.80)	4.97±0.60** (4.50-5.57)	6.12±1.03 (6.50-7.80)
Calcium mg%	9.96±0.14 (8.10-10.99)	8.42±0.26* (7.61-8.70)	9.96±0.14 (8.10-10.99)
RBCs T/L	9.34±0.14 (8.90-10.15)	5.04±0.11** (4.60-5.70)	9.34±0.14 (8.90-10.15)
Hb gm%	12.05±0.2 (11.40-13.10)	8.32±0.18** (7.60-9.40)	12.05±0.2 (11.40-13.10)
PCV %	32.85±1.15 (30.00-38.00)	26.60±1.10** (25.00-28.00)	32.85±1.15 (30.00-38.00)

* Significant at $p<0.05$.
** Highly significant $p<0.01$.
T/L = 10^{12}/L

Table (2): Biochemical analysis of blood serum phosphorus, magnesium and calcium levels and some haematological parameters in hypophosphataemic buffaloes before and after treatment with Tonophosphan.

Parameters	Control	Hypophosphataemic buffaloes before treatment	Hypophosphataemic buffaloes after treatment
Phosphorous mg%	5.82±0.08 (5.80-6.00)	4.34±0.06* (4.00-4.80)	6.26±0.29 (5.60-7.50)
Magnesium mg%	6.12±1.03 (6.50-7.80)	5.65±0.21* (4.51-6.36)	6.10±0.93 (5.04-6.98)
Calcium mg%	9.96±0.14 (8.10-10.99)	8.18±0.05* (7.11-9.25)	9.50±0.27 (9.24-10.50)
RBCs T/L	9.34±0.14 (8.90-10.15)	4.60±0.15** (3.80-5.20)	6.16±0.16* (5.20-6.80)
Hb gm%	12.05±0.2 (11.40-13.10)	7.40±0.25** (6.80-8.50)	8.30±0.01* (11.40-13.10)
PCV %	32.85±1.15 (30.00-38.00)	24.00±1.50** (2300-26.00)	27.00±1.95* (26.00-29.00)

* Significant at $p<0.05$.
** Highly significant $p<0.01$.
T/L = 10^{12}/L

Table (3): Biochemical analysis of blood serum phosphorus, magnesium and calcium levels as well as some haematological parameters in hypophosphataemic buffaloes before and after treatment with monobasic sodium phosphate.

Parameters	Control	Hypophosphataemic buffaloes before treatment	Hypophosphataemic buffaloes after treatment
Phosphorous mg%	5.82±0.08 (5.80-6.00)	3.60±0.60** (3.50-3.83)	4.95±0.25* (3.90-4.60)
Magnesium mg%	6.12±1.03 (6.50-7.80)	4.08±0.19** (3.60-4.95)	6.03±0.23 (5.20-6.80)
Calcium mg%	9.96±0.14 (8.10-10.99)	8.60±0.24* (7.66-9.56)	9.60±0.55 (8.66-10.80)
RBCs T/L	9.34±0.14 (8.90-10.15)	4.48±0.17** (4.10-5.20)	7.82±0.17* (6.20-9.10)
Hb gm%	12.05±0.2 (11.40-13.10)	7.82±0.17** (6.20-9.10)	9.22±0.32* (7.90-9.90)
PCV %	32.85±1.15 (30.00-38.00)	23.80±1.35** (22.00-25.00)	27.40±1.15* (30.00-38.00)

* Significant at $p<0.05$.
** Highly significant $p<0.01$.
T/L = 10^{12}/L

Table (4): Chemical analysis of agronomical samples.

Parameters	Soil	Tap water	Ground water
Phosphorus mg/dl	1.27±0.6 (1.09-1.45)	3.50±0.25 (3.25-3.75)	4.00±0.35 (3.65-4.35)
Magnesium mg/dl	1.88±0.93 (1.86-1.91)	0.00±0.00 (0.00-0.00)	0.00±0.00 (0.00-0.00)
Calcium mg/dl	15.79±0.55 (15.26-16.32)	11.95±0.90 (11.05-12.85)	8.40±0.95 (7.55-9.75)

concentration are determined in soil, tape water and underground water in the area of study (Table 4).

Discussion

Phosphorus is considered the major important divalent cation, and constituent of bones and teeth of animal body. It is an essential component of nucleic acid DNA and RNA, nucleotides and for enzymatic activities in the body. Phosphate plays a critical role in every cell, tissue and organ and plays more varied role in the chemistry of living organisms.

The most important clinical signs in diseased buffaloes were passing of coffee coloured urine, sharp decreased in milk yield, decreased to depraved appetites. Anaemia was manifested by pale and icteric mucous membranes, weakness accelerated pulse and respiratory rates with decreased to normal or slightly increased in body temperature, dullness and decreased to ruminal stasis, constipation with straining appears. These clinical signs coincided with that previously mentioned by (Udall, 1992; Smith, 1996; Radostitis *et al*., 2003). Such signs may be attributed to haemolysis of RBCs and decrease in haemoglobin concentration and PCV from increased fragility of erythrocytes leading to haemolysis. Inadequate phosphorus in the plasma leads to decrease ATP synthesis which is essential in maintaining the shape and deformability of RBCs. These results are in agreement with (Nassif 1995; Abdul Samad, 1997; Kaneko *et al*., 1997; Radostitis *et al*., 2003). Improvement in affected buffaloes after treatment due to increased level of

blood phosphorous resulted in cessation of haemolysis from increased fragility of erythrocytes. The reduction of ATP caused the cells being spherical with increased liability to osmotic fragility with subsequent shortening in RBCs life span (Bhikane *et al*, 1995; Abdul Samad, 1997).

Biochemical analysis of blood serum phosphorous, magnesium, and calcium of buffaloes suffering from hypophosphataemia showed a significant decrease in pre-treated if compared with the treated ones, Similar findings were recorded by (Udal 1992; Smith, 1996; Radostitis *et al*., 2003), The authors pointed out that a concurrent hypocalcaemia with hypomagnesaemia may have a contributing effect. In many instances rations given to such animals may have very high Ca but much lower P contents as in barseem or due to drainage of phosphorus in milk (Smith, 1996; Radostitis *et al*., 2003). Moreover magnesium deficiency may influence calcium homeostasis. Hypomagnesaemia associated with hypocalcaemia which are contributing factors in many instances may even be the dominant factor. Declined blood serum calcium during pregnancy as a result of large increase in the rate at which foetal skeleton needs Ca for mineralization and after parturition due to colostrums, needs high level of calcium. All these factors may decreased free blood calcium levels. Similar findings were previously reported by (Pandey and Misra, 1987; El-Magawery *et al*., 1995).

Biochemichal analysis of soil and tape water as well as ground water revealed a significant decrease in phosphorous and magnesium levels, such decrease may be attributed to the severity of the clinical signs. These results are in agreement with (Mark and Mark 2003).

Attention to phosphorus content in the ration beginning of lactation period. Bone meal, dicalcium phosphate, disodium phosphate and sodium pyrophosphate provided in supplementary feed or allowing free access to their mixture salt. Adding to the pasture super-phosphate in adequate methods to correct the deficiency and advantage the phosphorous content in the plants.

Finally, the study revealed the influence of hypophosphataemia upon the studied parameters. Also trials of treatment of diseased buffaloes alleviate the clinical signs of hypophosphataemia and elevated the levels of the studied parameters in treated animals to be around the normal obtained levels in buffaloes.

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الأعراض الإكلينيكية والدموية والتغيرات فى بعض العناصر البيوكيميائية قبل وبعد العلاج في الجاموس المصاب بنقص الفسفور فى محافظة أسيوط

تم إجراء هذه الدراسة على عدد ٢٨ جاموسة تراوحت أعمارها من ٥-٧ سنوات وأوزانها بين ٤٠٠-٥٠٠ كيلو جرام وهي تابعة لبعض المزارع الخاصة بمحافظة أسيوط. تم تقسيم الحيوانات حسب الأعراض الإكلينيكية إلى مجموعتين , الأولى تعانى من البول المدمم وعددها ٢٠ جاموسه , والثانية عددها ٨ حالات ثبتت بالفحص الإكلينيكى والمعملى أنها بصحة جيدة واستخدمت كضابط للبحث.

أوضحت التحاليل البيوكيميائية عن حدوث انخفاض معنوى فى معدلات كلا من الفسفور والكالسيوم والماغنسيوم فى دم حيوانات المجموعة المريضة بالمقارنة بالمجموعة السليمة. أوضحت الصورة الدموية حدوث انخفاض معنوى فى عدد كريات الدم الحمراء وتركيز الهيموجلوبين ونسبة الخلايا المصمتة فى الحيوانات المريضة بالمقارنة بالسليمة. تم إجراء التحاليل الكيميائية للتربة ومياه الشرب شاملة العناصر السابقة. وقد أدى علاج الحيوانات المريضة الي التحسن التدريجي في مستوي جميع العناصر التي تم قياسها. تم إجراء التحاليل الإحصائية والمقارنة للنتائج قبل وبعد محاولات العلاج.