Clinical and Biochemical Investigation of Subclinical Hypophosphatemia in Relation to Indigestion in Egyptian Buffaloes

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Abstract:

The occurrence of hypophosphatemia in Egyptian buffaloes is acknowledged as a significant metabolic disorders with a high frequency. This research focuses on highlighting certain clinical and biochemical alterations in buffaloes experiencing subclinical hypophosphatemia(SCH). A clinical examination was conducted on 35 affected buffaloes and additional 10 buffaloes that appeared to be healthy. The results of clinical examination revealed that SCH buffaloes were suffered from rectal temperature of 38.9^{o}c, slightly increased Heart rate, decrease body weight, anorexia, stiffness in gait, pale mucous membrane of vulva and conjunctiva, decreased milk production, ruminal hypo motile and constipation and Biochemical analysis showed that the buffaloes with hypophosphatemia had significantly lower average values of total protein (TP) and albumin in contrast to the apparently healthy group. They also had a significantly higher mean values in comparison to the control group in liver enzymes as aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Furthermore, the buffaloes with hypophosphatemia had significantly lower mean values of glucose 6 phosphate (G-6-P), calcium (Ca2+), phosphorus (P-), potassium (K+), and chloride (CL-) compared to the control group.

This conclude that a result of increased liver enzymes brought on by liver illnesses, phosphorus shortage results in defects in G-6-P and

cellular energy metabolism of adenosine triphosphate (ATP). These defects cause ruminal hypo motility and ruminal stasis, which in turn cause economic losses owing to weight loss and a decrease in milk output.

Keywords: Buffaloes, G-6-P, Hypophosphatemia, Indigestion, Phosphorus.

Introduction:

One of the minerals present in all body cells, phosphorus is essential for various body functions such as the synthesis of cell membranes, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), bone, and critical energy pathways (ATP). As a result, phosphorus is now recognized as a master mineral in many different contexts. Moreover, it is the second most common mineral in the body after calcium. ***(Singh et al., 2018).***

The prevalence of hypophosphatemia in buffaloes increased to 78.57% as a result of feeding cruciferous plants, sugarcane tops, and sugar beets, which worsen phosphorus deficiency either due to their low phosphorus content or specific inhibitors that impede the appropriate absorption of phosphorous from food ***(Yadav et al., 2023).***

One well known metabolic diseases that affect cattle and buffaloes is hypophosphatemia that resulting in deficiency of phosphorus or imbalance in the calcium phosphorus ratio leading to hypophosphatemia and hemoglobinuria by altering the process of glycolysis and ATP synthesis in RBCs ***(Rashid et al., 2021).***

Furthermore, because of the quick and continuous phosphate losses in milk production during the first stage of lactation and the low phosphorus content in diet, particularly during the later stages of gestation, the high incidence was linked to late pregnancy and the early milking phase. Appetence, pale mucous membranes, decreased milk production, abnormalities in locomotion, lameness, and hemoglobinuria with recumbence in severe and complex cases are the chief symptoms that are known ***(Kataria et al., 2022).***

Clinical observations, however, revealed that many buffaloes with hypophosphatemia do not exhibit overtly traditional clinical symptoms. Therefore, it is necessary to distinguish between hypophosphatemia that is clinical and subclinical. It is challenging to diagnose phosphorus shortage precisely. Even while plasma phosphorus levels fall when there is a phosphate shortage, the lack of a strict homeostatic control mechanism limits the diagnostic use of this finding ***(Randhawa et al., 2018).***

When grazing animals are kept in areas with low soil phosphorus content and fed an inadequate diet consisting of cabbage, barley, wheat, or straw, they can develop moderate hypophosphatemia (subclinical cases), which is characterized by poor growth, stiffness in gait, weight loss, and anorexia ***(Nasr El-Deen et al., 2021).***

Consequently, the purpose of the current research was to characterize the medical examination and biochemical analysis linked to subclinical hypophosphatemia and its relation to digestive function in buffaloes in Egypt.

Material and methods:

Ethical approval

The Committee on Scientific Research Ethics on creatures Researches, Faculty of Veterinary Medicine, Suez Canal University, Egypt (2021017) authorized all techniques utilized in this study.

Study period and location

From January to June of 2023, the research was conducted at the Veterinary Teaching Hospital of Zagazig University in Egypt.

Animal and study design

A total of 45 buffaloes in all were brought into the clinic and grouped into:

First Group (G1): include 10 apparently healthy buffaloes.

Second group (G2): include 35 cases with a history of decreased milk production and anorexia.

Clinical examination:

All animal had a clinical assessment using the methodology of ***(Constable et al., 2016).***

Sampling

A total of 45 blood samples in total were taken in the manner described below. 35 samples from the buffaloes under investigation and 10 samples from the control group. Every buffalo had a jugular vein puncture to yield about 5 milliliters of blood. Samples of blood serum were collected into plain tubes, allowed to clot for thirty to sixty minutes, centrifuged for ten minutes at 3000 rpm, and then stored at -20°C until additional analysis was performed ***(Kaneko et al., 2008).***

Biochemical analysis

The liver enzymes, AST and ALT, as well as albumin and serum total proteins were tested calorimetrically using commercial kits supplied by Biomerieux, Egypt. In the meantime, albumin was subtracted from total proteins to determine globulin concentration, and albumin value was divided by globulin value to determine A/G ratio. In the meantime, the assy gerie sandwich ELISA kit assessed serum glucose 6-phosphate dehydrogenase (G-6-P) in accordance with the manufacturer's instructions. Using an autoanalyzer (Aeroset; Abbot Toshiba, Japan), serum Ca++, P-,

Cl-, and K+ were measured calorimetrically.

Statistical Analysis:

The description of every quantitative data set was mean ± typical error. The SCH group was compared using the Student's *t*-test with control groups. Plotting significance at $p \leq 0.05$ and $p \leq 0.01$ was used.

Results:

Clinical findings:

Clinical results for patients with SCH were displayed in Table 1. The examined animals had pale mucous membranes, anorexia, decreased body weight, stiff stride, hypomotility in the rumen, constipation, non-significant changes in rectal temperature, and significant increases in heart and respiration rates.

Serum biochemical analyses:

As indicated in Table 2, highly significant decrease in the average values of TP at ($P \leq 0.01$) and a significant decrease in the mean values of albumin at ($P \leq 0.05$) of buffaloes with SCH compared with control group, as well as there was a non-significant change in the mean values of globulin and A/G ratio at ($P < 0.05$) of buffaloes with SCH compared with control group.

Table 3 illustrates a highly significant decrease in the mean values of G-6-P at ($P \leq 0.01$) and a highly significant increase in the mean values of AST and ALT at ($P \leq 0.01$) of buffaloes with SCH compared with control group.

Table 4 revealed a substantial decrease in the mean values of K+ at ($P \leq 0.05$) and a highly significant decrease in the mean values of Ca++, P, and CL- at ($P \leq 0.01$) of buffaloes with SCH compared with control group.

Table 1. *Clinical finding of subclinical hypophosphatemia in buffaloes*

Groups / Parameters	Healthy Buffaloes (control)	SCH
Rectal temperature °c	**39.03 ± 0.09**	**38.8 ± 0.1**
Heart rate (beat/min)	**58.33 ± 0.33**	**79.33* ± 0.33**
Respiratory rate (cycle/min)	**18.33 ± 0.88**	**23.0* ± 0.57**
Appetite	**normal**	**In appetence**
Mucous membrane	**Bright red**	**pale**
Ruminal movement	**3.67 ± 0.33**	**1.67** ± 0.33**

Table 2. *Serum level of proteinogram of subclinical hypophosphatemia in buffaloes*

Groups / Parameters	Healthy Buffaloes (control)	SCH
Total protein (g/dl)	7.53 ± 0.03	6.27** ± 0.15
Albumin (g/dl)	3.47 ± 0.12	2.5* ± 0.2
Globulin (g/dl)	4.07 ± 0.13	3.77 ± 0.09
A/G ratio	0.86 ± 0.06	0.67 ± 0.065

In the same row, means with distinct superscripts exhibit significant differences ($P \leq 0.05$) or extremely significant differences ($P < 0.01$). The means in the same row that have the same superscripts differ not substantially at ($P < 0.05$).

Table 3. *serum activity of Liver enzymes and G-6-P of subclinical hypophosphatemia in buffaloes*

Groups / Parameters	Healthy Buffaloes (control)	SCH
AST(U/L)	123.33 ± 0.88	245.33** ± 0.33
ALT (U/L)	26.33 ± 0.88	57.67** ± 0.88
G-6-P (pg/ml)	105.8 ± 0.16	93.3** ± 0.56

In the same row, means with distinct superscripts exhibit significant differences ($P \leq 0.05$) or extremely significant differences ($P < 0.01$). The means in the same row that have the same superscripts differ not substantially at ($P < 0.05$).

Table 4. *Serum level of minerals of subclinical hypophosphatemia in buffaloes*

Groups / Parameters	Healthy Buffaloes (control)	SCH
Ca^{++} (mg/dl)	10.6 ± 0.61	6.53** ± 0.33
P^{-} (mg/dl)	6.03 ± 0.53	2.43** ± 0.73
K^{+} (mmol/L)	4.1 ± 0.1	3.1* ± 0.57
CL^{-} (mmol/L)	106.3 ± 1.67	91.67** ± 0.88

In the same row, means with distinct superscripts exhibit significant differences ($P \leq 0.05$) or extremely significant differences ($P < 0.01$). The means in the same row that have the same superscripts differ not substantially at ($P < 0.05$).

Discussion:

One significant macro mineral is phosphorus. When it bonded with an oxygen atom, phosphate (PO4) was formed. The body uses both organic (PO) and inorganic (PI) phosphate to establish this. This particular kind of phosphate is essentially free—it isn't attached to any molecules that contain carbon. It is assessed in serum or plasma and is based on extracellular fluid. The proper absorption of P ions, mostly in the small intestine, the level of Na+ ions, the proper excretion in urine, and the mobilization of bone all affect the body's inorganic phosphate level ***(Grünberg, 2008).***

Buffaloes are susceptible to a seasonal, sporadic metabolic disease called hypophosphatemia. Long-term use of green fodder (barseem) with insufficient phosphorus content is the cause of it ***(Rashid et al., 2021).***

Subclinical hypophosphatemia is a disease marked by poor growth, weight loss, and diarrhea that can occur in grazing animals in areas with low soil phosphorus content. It can also occur when animals are fed an inadequate diet, such as cabbage, berseem, wheat, or straw ***(El-Deen et al., 2021)***

The current investigation found that low body weight, anorexia, stiffness in gait, pale mucous membranes, and low serum phosphorus levels were the clinical signs of SCH. The identical clinical symptoms were reported by ***(Forrester and Moreland, 1989).***

When in contrast to the normal apparently healthy group, the proteinogram of both groups showed a substantial decrease in the amounts of albumin and total serum proteins. The fall in albumin globulin ratio was caused by a decrease in serum albumin, and the hypoalbuminemia may have resulted from a decrease in feed intake and decreased production by injured liver our results come in agreement with ***(Rashid et al., 2021).***

The present research found that SCH groups had higher activities of AST and ALT than the normal control group. This could be explained by fatty liver changes that occur concurrently with negative energy balance and cause hepatocellular damage. Additionally, SCH groups had significantly lower levels of G-6-P activity than the healthy control group, which could be explained by enzymatic abnormalities brought on by the rise in ALT and AST activity, Similar results were recorded previously in ***(Nasr El-Deen et al., 2021)*** and the elevation in the activity of transferase especially activity of AST can be associated with the alterations of skeletal, cardiac muscles and hepatic cells ***(Jayanthi et al., 1997).***

A highly significant decrease in serum calcium levels compared to the normal control group. Hypoalbuminemia, decreased feed intake, and/or decreased intestinal calcium absorption could all be the cause of the decline. Nearly similar

results were obtained by ***(El-Deen et al., 2021).***

Serum electrolyte analysis showed a highly significant reduction in the levels of calcium, phosphorus, and chloride and significant decrease in potassium. Hypochloremia may occur due to sequestration of hydrochloric acid in the rumen caused by rumen atony. Furthermore, hypokalemia and hypophosphatemia and hypocalcemia is caused primarily by anorexia ***(Reddy et al., 2014)***.

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فحوصات اكلينيكية وكيميائية لنقص فوسفات الدم غير الظاهري وعلاقته بعسر الهضم في الجاموس المصري

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الملخص العربي

يعتبر نقص فوسفات الدم من الاضطرابات الايضية الرئيسية التي تحدث بشكل كبير في الجاموس في مصر. هذه الدراسة تسلط الضوء على بعض التغيرات الاكلينيكيه والكيميائية في الجاموس المصاب بنقص فوسفات الدم والتي لم يظهر عليها اعراض. تم إجراء الفحص الاكلينيكي لـ 35 جاموسة مصابة و عشر حالات أخرى سليمة ظاهريًا. أظهرت نتائج الفحص الاكلينيكي أن الجاموس المصاب يعاني زيادة طفيفة في معدل ضربات القلب، وانخفاض وزن الجسم، وفقدان الشهية، وتيبس في المشية، وشحوب الغشاء المخاطي للفرج والملتحمة، وانخفاض إنتاج الحليب، وقلة حركة الكرش والإمساك ، وأظهر التحليل الكيميائي انخفاضًا كبيرًا في متوسط قيم البروتين الكلي (TP) وانخفاضًا كبيرًا في الألبومين في الجاموس المصاب بنقص فوسفات الدم مقارنة بالمجموعة السليمة، وزيادة كبيرة في متوسط نشاط أسبارتات ترانسفيراز (AST) وألانين ترانسفيراز (ALT) في الجاموس المصاب بنقص فوسفات الدم مقارنة بالمجوعات السليمة بالإضافة إلى انخفاض كبير في متوسط قيم جلوكوز 6 فوسفات (G-6-P) والكالسيوم (+Ca2) والفوسفور (-P) والبوتاسيوم (+K) والكلوريد (-CL) في الجاموس المصاب بنقص فوسفات الدم مقارنة بمجموعة التحكم .
ووتوصلت الدراسة إلى أن نقص الفسفور يؤدي إلى خلل في استقلاب G-6-P والطاقة الخلوية (ATP)بسبب زيادة إنزيمات الكبد نتيجة لاضطرابات الكبد، مما يؤدي إلى قلة الحركة وركود الكرش وخسائر اقتصادية بسبب فقدان الوزن وانخفاض إنتاج الحليب .
الكلمات المفتاحية: الجاموس،G-6-P ، نقص فوسفات الدم، عسر الهضم، الفوسفور .