POSSIBLE INTERRELATION BETWEEN PHOSPHOROUS DEFICIENCY AND VITAMIN D IN BUFFALOES (Bubalus bubalis)

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Abstract: This investigation was conducted to appraise the interrelation between the influences of phosphorus deficiency on vitamin D and parathyroid hormone and assessment its effect on the liver and muscular markers. These investigations were carried on forty adult female water buffaloes divided into three groups according to serum phosphorus level. The first group contained ten apparently clinically healthy buffaloes and used as a control. The second and third groups were fifteen buffaloes for each suffered from moderate or severe signs of phosphorus deficiency (signs include anorexia, emaciation, weakness and paleness mucous membranes in the two groups in addition to red urine in the third one). Phosphorus level showed a significant decline in sub-clinical cases and highly significant decrease in clinical cases. Parathyroid hormone was significantly decline in sub-clinical and clinical cases, while active form of vitamin D wasn’t changed in sub-clinical cases and significantly decline in clinical cases when compared with normal control. Creatine phosphokinase significantly rise in clinical cases. Malondialdehyde was significantly increased while glutathione peroxidase was significantly decreased in both moderate and severe cases. This conclude that phosphorous deficiency causes lessening in parathyroid hormone, 25 hydroxycholecalciferol (25 (OH₂) D₃ and increasing in the markers of oxidative stress and muscular damage which exaggerated by increasing in the deficiency. So, addition of vitamin D supplementation and antioxidant should be conservative treatment regime.

Key words: buffaloes; vitamin D; parathyroid hormone; antioxidant; LDH; CPK; liver

Introduction

Inorganic phosphorous (Pi) is essential for many biological processes as well as cellular energy metabolism (ATP), phospholipid membranes and bone formation. In addition to phosphorous complexes containing phosphate ions, as (DNA, RNA). It is an important buffer of the blood and urine aiding to preserve acid-base balance so the change in serum level of Pi has an imperative conclusion (1).

Phosphorous homostasis depends on problematical hormonal regulation which embraces parathyroid hormone (PTH), calcitonin, fibroblast growth factor 23, phosphatine and active form of vitamin D also parenchymatous organs as liver and kidney are involved (2). Parathyroid hormone is the utmost important hormone regulates renal phosphate transport. Parathyroid hormone diminishes reabsorption of bicarbonate, calcium, sodium and phosphate in the proximal tubules of the kidneys, while it augments calcium reabsorption from the distal tubules. So, the net effect occasioned in an increased serum calcium concentration and phosphate urea (3). The in-
crease in extracellular phosphate originate to proliferation fibro-growth factor 23 and PTH secretion while the decrease in serum phosphorous level found to decrease serum PTH level (4). Parathyroid glands directly respond to the modification in serum calcium through Ca-sensing receptors, resultant increasing PTH manufacture. It is not directly responds to intravenous phosphorous infusion but indirectly respond to extracellular phosphorous concentration by its effect on mRNA stability coding for PTH which destabilizes in cases of hypophosphatemia (5).

In the kidney, the proximal tubules is the most major site of 1,25 dihydroxycholecalciferol \(\{1,25\text{(OH)}_2\}\text{D}\), vitamin D\(_3\) synthesis as well as the main site of Pi absorption (6). Vitamin D\(_3\) (cholecalciferol) is produced in the skin depend on the intensity of ultraviolet irradiation and exposure (7) then it imparted to liver by vitamin D binding protein (DBP) and hydroxylated by 25 hydroxylase enzyme and 25-hydroxy vitamin D\(_3\) (25 (OH) D\(_3\)) yielded (8). 25 (OH) D\(_3\) is the biologically inactive formula and requirements to be hydroxylated to the active one 1, 25 (OH)\(_2\). So, it bounded to DBP and imparted to the kidney where it is filtrated and taken by proximal tubules under the effect of \(1\alpha\) hydroxylase (9). It has been verified that phosphorous directly regulate the manufacture of 1, 25 (OH)\(_2\) vitamin by the kidney. Also, the little level of serum 1,25 (OH) vitamin D may reflect the reply to direct effect of phosphorous on renal synthesis of 1,25 (OH) vitamin D (10-11).

Material and methods

Buffaloes

This study was conducted on forty adult female water buffaloes (2-5 years) and distributed into three groups. The first group was 10 apparently healthy buffaloes kept as normal control group. These buffaloes were collected from dissimilar localities in Sharkia governorate through the period from January 2020 till April 2021 and the buffaloes were fed on seasonal green fodder (barseem). The second group was (15 buffaloes) suffering from moderate hypophosphatemia (sub-clinical cases), by clinical examination the buffalo’s publicized anorexia, emaciated, weak and have paleness conjunctiva and vulvar mucous membranes also the buffaloes had stiffness in gait (12-14). The third group was (15 buffaloes) suffering from severe hypophosphatemias (clinical cases) some of these buffaloes were 3rd-5th week’s post-partum. The clinical examination revealed red urine, anorexia, paleness mucous membranes, labored breathing, jugular pulsations and the buffalo may be in late-stage recumbent (14-16). All these signs suspect hypophosphatemia were confirmed with measurement of serum phosphorus level.

Blood sampling

Throughout jugular vein puncture, the blood samples were drawn from each buffaloes in clean tubes without anticoagulant for serum separation to be used in biochemical assessment spectrophotometrically using special kits.

Biochemical analysis

Phosphorus and calcium were assessed using special kits (17-18). Activities of transferases either; (ALT) or (AST) were estimated (19). Serum glucose 6- phosphate dehydrogenase (G-6-P) was appraised by the assay sandwich ELISA kit according to the manufacturer’s instructions. Serum malondialdehyde (MDA) and reduced glutathione peroxidase (GPX) were estimated using ELISA kits (20-21). Serum active form of vitamin D (25hydroxy choliciferol), serum parathyroid hormone (PTH), lactate dehydrogenase (LDH) and creatine kinase (CPK) were estimated using Roche/Hitachi 902, Roche Diagnostics, Basel, Swiss (22-24).

Statistical Analysis

All buffalo’s values were presented as mean ± standard error. The significant variance between the mean of the buffaloes groups were statistically explored by one way analysis of variance (ANOVA) by SPSS 20.0 program (25). The significance between the buffaloes groups established as P < 0.05

Results and discussion

Hypophosphatemia is one of the most common metabolic disorders of bovines. It’s mainly caused due to the decrease in serum inorganic phosphorous level. The most common causes for deficiency are incorrect P/Ca ratio in food, low absorption of phosphorous compounds and disorders of hormonal system (2).
Possible interrelation between phosphorous deficiency and vitamin D in buffaloes (*Bubalus bubalis*)

Severe (acute) hypophosphatemia (clinical cases) usually affects adult buffaloes immediately after parturition (1-60 days) post-partum (14). It's commonly seen during third to sixth lactation (26) and it can also appear during 6th-8th month of pregnancy (27). The disease is characterized by rapid intravascular hemolysis, hemoglobinuria, weakness, anorexia, anemia, marked decrease in milk production in lactating animals and may be obvious with recumbence (28).

Moderate hypophosphatemia (subclinical cases) can be seen in grazing animals in regions with low phosphorous content in the soil and also feeding the animals on inadequate ration as feeding on cabbage, Barseem, wheat or straw (27) and the disease characterized by poor growth, stiffness in gait, loss weight and anorexia (29).

The present study was carried out on female water buffaloes suffering from the clinical signs suggested to be hypophosphatemia. The subclinical cases (moderate hypophosphatemia) revealed poor growth, loss of weight, anorexia, stiffness in gait (Figure 1), pale vulvar and conjunctival mucous membranes and serum phosphorous level was 2.6±0.14 mg/dl (Table 1). The same clinical signs were reported by previous authors (12-14 and 29). While in acute cases (severe hypophosphatemia) the buffaloes were suffering from all of the previous symptoms in addition to red urine (Figure 2) and the buffaloes may be recumbent and serum phosphorous level was 1.40±0.43 mg/dl. These clinical signs were in the agreement with those reported (14-16, 27, 30).

The present study revealed a significant decrease in serum phosphorous level in both 2nd and 3rd groups. This decrease could be ascribed to prolonged serving on phosphorous deficient diet as Barseem. Also, high calcium to phosphorous ratio diet results in declining phosphorous absorption from intestine (31).

The transition between late pregnancy and early lactation from calving till 3rd to 6th weeks

Table 1: Effect of sub-clinical and clinical hypophosphatemia on serum minerals, vitamin D and PTH in buffaloes (mean values ± SE)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Phosphorus deficiency</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Sub-clinical</td>
<td>Clinical</td>
</tr>
<tr>
<td>Ph (mg/dl)</td>
<td>6.94 ± 0.31a</td>
<td>2.65 ± 0.14b</td>
</tr>
<tr>
<td>Ca (mg/dl)</td>
<td>10.53 ± 0.13a</td>
<td>10.40 ± 0.10a</td>
</tr>
<tr>
<td>Vitamin D (ng/ml)</td>
<td>27.38 ± 1.20a</td>
<td>25.48 ± 1.80a</td>
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<tr>
<td>Ph: Ca ratio</td>
<td>1:1.52</td>
<td>1:3.92</td>
</tr>
<tr>
<td>Ca: Vitamin D ratio</td>
<td>1:2.60</td>
<td>1:2.45</td>
</tr>
<tr>
<td>Ph: Vitamin D ratio</td>
<td>1:5.95</td>
<td>1:9.62</td>
</tr>
<tr>
<td>PTH (pg/ml)</td>
<td>11.66 ± 1.03a</td>
<td>1.94 ± 0.23b</td>
</tr>
</tbody>
</table>

Significant at P ≤ 0.05; Ph = phosphorus; Ca = calcium; PTH = parathormon

Table 2: Effect of sub-clinical and clinical hypophosphatemia on serum transferases, LDH and CPK in buffaloes (mean values ± SE)

<table>
<thead>
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<th>Parameters</th>
<th>Control</th>
<th>Phosphorus deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sub-clinical</td>
<td>Clinical</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>19.40 ± 0.54c</td>
<td>31.80 ± 1.44b</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>66.40 ± 0.10c</td>
<td>80.29 ± 4.30b</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>238.00 ± 3.20c</td>
<td>343.00 ± 3.80b</td>
</tr>
<tr>
<td>CPK (U/L)</td>
<td>137.20 ± 3.30b</td>
<td>149.00 ± 4.20b</td>
</tr>
</tbody>
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Significant at P ≤ 0.05; ALT & AST = transferases; LDH = lactate dehydrogenase; CPK = creatine phosphokinase

Table 3: Effect of sub-clinical and clinical hypophosphatemia on glucose-6-phosphate, and oxidative stress markers in buffaloes (mean values ± SE)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Phosphorus deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sub-clinical</td>
<td>Clinical</td>
</tr>
<tr>
<td>G-6-P (pg/ml)</td>
<td>107.97 ± 3.90a</td>
<td>95.00 ± 2.50b</td>
</tr>
<tr>
<td>MDA (nmol/ml)</td>
<td>0.64 ± 0.11c</td>
<td>4.01 ± 0.64b</td>
</tr>
<tr>
<td>GPx (U/ml)</td>
<td>202.00 ± 0.490a</td>
<td>149.00 ± 3.50b</td>
</tr>
</tbody>
</table>

Significant at P ≤ 0.05; G-6-P = glucose-6-phosphate; MDA & GPx = Oxidant markers
Figure 1: Buffalo suffering from sub-clinical hypophosphatemia showing loss weight and stiffness in gait with clear lameness

Figure 2: Buffalo suffering from acute hypophosphatemia showing dark red urine

post-partum (28) lessened absorption from gut due to vitamin D deficiency and gastrointestinal tract illnesses (32). Also, advanced gestation may cause hypophosphatemia as more calcium and phosphorous are requisite by the fetus (33). Similar results were obtained by previous authors (2, 16, 28, 34-35). The reduction in serum inorganic phosphate level in dairy cattle is one of the most mutual metabolic disorders. This drop is considerably subsequent a sudden loss of P as it arises at the onset of lactation through mammary gland also hypophosphatemia can be a result of shifting P from extracellular fluid into intracellular fluid (36).

The serum calcium level (Table 1) was not significantly altered in the 2nd group. Similar results were gotten (15, 37). While in the 3rd group there was a significant decrease in serum calcium level when matched with normal control group. The decrease could be ascribed to decline feed intake, lessened calcium absorption from the intestine and or hypoalbuminemia. Approximately alike outcomes were obtained (2, 13, 36).

Phosphorus (P) is an important macro-mineral. It united with oxygen atom forming phosphate (PO$_4$). This is established in the body in two forms either in organic (PO) and inorganic (PI) phosphate (38). This form of phosphate is fundamentally free (not bound to any carbon-containing molecules). It is founded in the extracellular fluid and measured in serum or plasma (13). The inorganic phosphate level in the body depends on the correct absorption of P ions, mainly in small intestine, level of Na ions, correct excretion in urine and mobilization in bone.

The endocrine regulation of extracellular phosphate hinge on the active form of vitamin D (1-25 dihydroxycholecalciferol) and parathyroid hormone (PTH) also parenchymal organs such as kidney and liver are involved (39). Apropos to serum level of parathyroid hormone both sub-clinical and clinical cases showed a significant decrease in serum level of PTH when compared with normal control group. This decrease in serum PTH level could be attributed to the decrease in serum phosphorous to which parathyroid gland respond by decreasing PTH mRNA level, decreasing gene expression and cell proliferation of the gland. Also, decreasing serum phosphorous level resulted in decreasing production of arachidonic acid in parathyroid gland that decreasing PTH secretion (4). Nearly similar results were obtained (4-5) who reported that the decrease in serum phosphorous level found to decrease serum PTH level.

Regarding to the active form of vitamin D, the sub-clinical cases in the present study bared non-significant change in serum level of 25 hydroxycholecalciferol at paralleled with normal control group. While the clinical cases showed a significant diminution in serum level of 25 hydroxycholecalciferol. This lessening might be due to the
decrease in serum PTH level which stimulates the conversion of vitamin D to its active form in the kidney (40). Also, it may be attributed to liver damage which occasioned in decrease production of 25 hydroxycholecalciferol that converted to the active form 1, 25 dihydroxycholecalciferol in the kidney (41). Also, the decrease in serum calcium level may occasion vitamin D lessening.

The present study showed a substantial increase in transferases activities (ALT and AST) (Table 2) in the 2nd and 3rd groups when compared with normal control group. This could be accredited to fatty liver changes concomitant with negative energy balance that cause hepatocellular damage (42). The same results were obtained (2, 15, 26, 30). Also, there is a connotation of hypophosphatemia with liver injury instigated by hepatic lipidosis are common in highly producible dairy cows in initial lactation and occur alongside with hypophosphatemia (43). Relations between hypophosphatemia and biochemical markers of disturbed liver dysfunction in early lactating dairy cattle have been reported (44).

Lactate dehydrogenase (LDH) is an enzyme originates in the most nucleated and non-nucleated cells including skeletal and liver, cardiac muscles and erythrocytes (45). The present work showed that significant raise in LDH activity in subclinical cases while the clinical cases revealed a highly significant increase. The escalation in LDH activity in equivalent with increasing AST and ALT activities can sustenance liver dystrophy instigated by severe hypophosphatemia (46). The increase in the activity of LDH together with AST activity and CPK is an indicator for necrosis of the skeletal muscle. Also, the increase in LDH may be escorted with the hemolysis of RBCs leads to outflow of LDH into the circulation (45).

Creatine kinase (CPK) is the utmost sensitive and specific indicator of muscular impairment and necrosis (42). Our study reported non-significant change in serum CPK level in subclinical group when compared with normal control group. While severe (clinical) cases showed a significant increase in serum CPK level when compared with normal control group. The same results were reported (12, 41). The elevation in the activity of transferase especially activity of AST can be associated with the alterations of skeletal, cardiac muscles and hepatic cells (47). So, the increase in serum level of CPK together with AST indicates muscle damage and necrosis.

The present study revealed a substantial diminution in the activity of erythrocytic G-6-P in both 2nd and 3rd groups when compared with healthy control group. This diminution could be owed to the mutation which is the utmost mutual reason for enzymatic abnormalities (48). Also, low activity of G-6-P may be accredited to the rise in ALT and AST activity (49). Similar results were recorded previously (15, 27, 34).

About to the oxidative stress markers there was a significant diminution in serum glutathione peroxidase (GPx) activity in both moderate and severe hypophosphatemic buffaloes in comparison with normal control group. This may be accredited to decline activity erythrocytic G-6-P which, liable to decrease the reduced glutathione and so instigating oxidative stress to erythrocytes leads to hemolytic syndrome (49). Nearly similar results were obtained (30-31, 50).

The present study showed a significant increase in serum malondialdehyde (MDA) in subclinical and clinical cases when paralleled with normal control group. This rise could be attributed to lessening serum phosphorous level which estop ATP production prompt oxidative stress as a consequence of high creation of reactive oxygen species that damage to RBCs structure (50).

Conclusion

It could be concluded that phosphorous deficiency causes lessening in parathyroid hormone, 25 hydroxycholecalciferol (25 \( \text{(OH)}_2 \) D\(_3 \)) and increasing in the markers of oxidative stress and muscular damage which exaggerated by increasing in the deficiency. So, vitamin D supplementation and antioxidant recommended to be added in conservative treatment regime

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Competing Interests. The authors declare that they have no competing interests.
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