CLINICAL, HEMATOLOGICAL AND BIOCHEMICAL STUDIES OF SOME MINERALS DEFICIENCY IN BUFFALOES IN BASRAH, IRAQ

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ABSTRACT
Clinical, hematological and biochemical studies were applied on Eighty one local buffaloes breed 3-6 years old of both sexes during the period started from September 2013 to April 2014. The present work were carried out in Basrah province (Basrah-Iraq). Ten clinically healthy buffaloes breed were used as controls. Diseased buffaloes were divided into two groups, First group (51) local buffaloes breed were grazed during the day light most of the time (out door feeding group) However the others (30) local buffaloes breed were kept indoor all of the time (Indoor feeding group). Diseased animals show signs of partial or complete loss of appetite, pale mucus membranes of the eyes, depigmentation of hair, decrease milk production, parakeratosis of ears and withers, partial alopecia, generalized weakness and Interrupted diarrhea. Furthermore, on clinical examination diseased buffaloes show significant increase (p<0.05) heart and respiratory rate with normal body temperature. Results of hematological examinations indicated significant decrease (p<0.05) in TRBC, Hb and PCV reflected normocytic normochromic type of anemia. Biochemical investigation reveal significant decrease (p<0.05) in Zinc, Cobalt, Copper and sulfur values in all diseased buffaloes. However the deficiency were more evident in out door feeding group those whom spend most of day times in grazing out door in comparison with indoor feeding group and controls. It have been concluded that most Iraqi buffaloes breeds in Basrah, Iraq were suffer from minerals deficiency due mostly to poor grazing fields.

INTRODUCTION
Trace minerals are those minerals which required in small amounts. For the fact that such small daily quantities of trace minerals are needed, Therefore dietary requirements were generally expressed and measure in parts per million (ppm), rather than percent (1,2).

Proper mineral and vitamin nutrition will contributes to good and strong immunity, reproductive performance, and proper animal health (3). An accepted balanced mineral program requires consideration of past animal nutrition history include hay or pasture forage intake, the percent concentration of the mineral, and mineral supplement intake (4). Mineral requirements might depend on the age of the animal and the stage of production, However the knowledge of the animal’s demands is only the way in assessment an animal’s mineral status. Moreover the most important economic results of trace mineral deficiencies are low reproductive rates of animals and late puberty time of heifers associated with prolong parturition time (5).

Trace elements such as zinc, copper, and cobalt are essential nutrients for animals and are required in small amount for different body functions including immune and antioxidant function, growth and reproduction (1). Their deficiencies were related to
different metabolic mechanisms and cause different types of diseases. The mechanisms of its effects are incompletely clear and in spite of different research, the role of this microelement needs further studies (4).

The more common clinical signs of trace element deficiencies seen in diseased animals are anorexia, pica, diarrhea, anemia, alopecia, depigmentation of hair. Moreover seborrhea, weakness, hyperkeratosis and/or parakeratosis, infertility, retard growth rate, decreased production, tetanic convulsions, decrease in protein synthesis, insufficiencies in the immune system, abortions not related to infections (6) are all detected. Therefore the present work were amide to study the clinical, hematological and biochemical changes of some minerals deficiency in buffaloes in Basrah, Iraq.

MATERIALS AND METHODS

Animals:
Ninety one male and female local buffaloes breed 3-6 years old were used in this study during the period started from September 2013 to April 2014. The present work were carried out in Basrah province (Basrah-Iraq). Among these animals Ten clinically healthy buffaloes breed were used as controls. Diseased buffaloes were divided into two groups, First group (51) local buffaloes breed were grazed during the day light most of the time (out door feeding group) However the others (30) local buffaloes breed were kept indoor all of the time (Indoor feeding group).

Blood collection and laboratory methods:
Ten milliliter of blood were drained from each animal by jugular vein-puncture, from these (2.5) milliliter of blood mixed with EDTA used to determine Total erythrocyte count (TRBCs), Hemoglobin concentration (Hb), Packed cell volume (PCV), Furthermore values of MCV and MCHC were also calculated (7). Blood serum samples were tested spectrophoto-metrically using flame photometer (PYE Unicam spg) for evaluation of Zinc, Cobalt, Copper and sulfur according to (8).

Statistical analysis:
Significance of variations in values of disease buffaloes and those of normal control animals were analyzed statistically using student t-Test and one way analysis of variance (SPSS program) (9).

RESULTS

Diseased buffaloes show signs of Partial or complete loss of appetite (95%), pale mucous membranes of the eyes (77%), depigmentation of hair (64%), decrease milk production (55%), parakeratosis of ears and withers (41%), partial alopecia (38%), generalized weakness (37%), interrupted diarrhea (22%). Table 1.

| Table (1) Clinical sings of diseased buffaloes affected with minerals deficiency |
|-------------------------------------------------|----------|
| Clinical sings                                  | %        |
| Partial or complete loss of appetite            | 95       |
| Pale mucous membranes of the eyes                | 77       |
| Depigmentation of hair                          | 64       |
| Decrease milk production                        | 55       |
| Parakeratosis of ears and withers               | 41       |
| Partial alopecia                                | 38       |
| Generalized weakness                            | 37       |
| Interrupted diarrhea                            | 22       |
| Total number of diseased animals                | 81       |
Moreover, on clinical examination diseased buffaloes show significant increase (p<0.05) heart and respiratory rate with normal body temperature. Table 2.

**Table 2: Body temperature, respiratory and heart rate of diseased buffaloes affected with minerals deficiency and controls.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control Buffaloes</th>
<th>Diseased Buffaloes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body temperature °C</td>
<td>38.6 ± 0.77</td>
<td>39 ± 0.11</td>
</tr>
<tr>
<td>Respiratory rate/min</td>
<td>21.38±4.58</td>
<td>44.38±7156**</td>
</tr>
<tr>
<td>Heart rate/min</td>
<td>72.53±5.32</td>
<td>104.13±8.21**</td>
</tr>
</tbody>
</table>

**(P<0.05), Values are mean ± standard error of mean**

Results of hematological examinations indicated significant decrease (P<0.05) in TRBc, Hb and PCV. However MCV and MCHC results were reflected normocytic normochromic type of anemia Table 3.

**Table (3): Hematological changes in diseased buffaloes affected with mineral deficiency and controls**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control buffaloes</th>
<th>Diseased buffaloes</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC count (×10^6)</td>
<td>7.32 ± 1.14</td>
<td>4.6 ± 1.26 **</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>12.57 ± 0.37</td>
<td>8.7 ± 1.37 **</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>37.38 ± 2.31</td>
<td>26.36 ± 2.87 **</td>
</tr>
<tr>
<td>TLC (×10^3)</td>
<td>11.58 ± 2.56</td>
<td>11.91 ± 1.47</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>57.19</td>
<td>57.52</td>
</tr>
<tr>
<td>MCHC (gm/100ml)</td>
<td>33.05</td>
<td>32.16</td>
</tr>
</tbody>
</table>

**(P<0.05), Values are mean ± standard error of mean**

Results of minerals investigation revel significant decrease (P<0.05) in Zinc, Cobalt, Copper and sulfur values in all diseased buffaloes. However the deficiency were more evident in outdoors feeding group those whom spend most of day times in grazing outdoors in comparison with indoor feeding group and controls. Table 4.
Table 4: Zinc, Cobalt, Copper and sulfur values in diseased buffaloes and controls

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control buffaloes</th>
<th>In door feeding group</th>
<th>Out door feeding group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum zinc (μg/dL)</td>
<td>151.8±12.2 a</td>
<td>93.44±52 b</td>
<td>53.07 ± 3.61 c</td>
</tr>
<tr>
<td>Cobalt(μg/dL)</td>
<td>112.±3.82 a</td>
<td>98.67±5.21 b</td>
<td>79.2±3.67 c</td>
</tr>
<tr>
<td>Copper(μg/dL)</td>
<td>70.93±5.53 a</td>
<td>62.12±5.32 b</td>
<td>51.34±6.45 c</td>
</tr>
<tr>
<td>Sulfur(μg/dL)</td>
<td>391.4±22.11 a</td>
<td>388.34±26.36 a</td>
<td>277.86±14.73 c</td>
</tr>
</tbody>
</table>

Values are mean ± standard error of mean. Values with different letters mean the presence of significant differences (p<0.05).

DISCUSSION

Micro minerals are those whom devoted in several biological processes, such as component of different enzymes and co enzyme factors, they work always either as activator of enzymes related in intracellular detoxification mechanism or in stability of secondary molecules (10), However some of these minerals considered as a component of hormones thereby directly regulates activities of some endocrine system (1), and because of its involvement in metabolism of different chemicals such as proteins, carbohydrate, and nucleic acid therefore any alterations in its level may change the production of reproductive and other hormones (2).

It have been postulated that micro minerals deficiency impairs productivity in livestock, Moreover immunity and reproductive functions are directly affected where copper, zinc, cobalt, iodine and others likely to influence these functions (11) However late puberty of heifers and low reproductive rates of animals associated with prolong parturition intervals were also negative results of mineral deficiency (12).

The minerals availability of the natural feeds is determined firstly by the mineral content from the soil and then by the actual mineral composition of soil (1) some factors were influence the mineral composition of the soils such factors are the origin of the rocks, filtration, surface erosion, the application to the soil of pesticides, evaporation, fertilizers, sludges and manures and may be industry residues, In addition soil factors, such as acidity, moisture or drainage conditions, temperatures and seasonal effects influence mineral uptake by crops and pastures (13).

It have been found that some minerals have specific mechanism in the body and their deficiency might created deterioration effects on the body as whole such, minerals like copper which considered as one of the most commonly encountered nutritional problems in ruminants for the fact that animals will actually deplete their own body reserves to ensure neonatal adequacy, since copper being an essential component of the immune function, this maternal deficiency likely results in poor colostrum quality and inadequate neonatal protection even in calves that get adequate volumes of colostrum (14,15) Moreover, Zinc is an essential mineral that is required by all cells in animals as well zinc plays a clear and effective roles in numerous enzymatic reactions, Nevertheless deficiency of zinc are associated with reduced
growth rate, poor immune function, decrease reproductive performance, as well as affecting skin in severe cases. Furthermore, Cobalt is an important component of vitamin B12 which produced by ruminal organisms and require a regular supply of cobalt in the animal’s diet. In addition, Vitamin B12 is secreted in milk which provides an early source to suckling neonates and its deficiency might show signs of lack of appetite, anemia, emaciation, decreased production and decreased estrus time.

Sulfur is essential for synthesis of methionine and cystine, as well as the thiamin and biotin, its also required by ruminal microorganisms for normal growth and metabolism. Since ruminal microorganisms are capable of synthesizing all organic sulfur containing compounds required by the animal from inorganic sulfur, Although sulfur deficiency is very uncommon in domesticated animals, however, signs of its deficiency might include, anorexia, weakness, dullness, emaciation, excessive salivation, and in rare cases death.

In the present study, loss of appetite was the main clinical sign exhibited by diseased buffaloes, as well partial or complete loss of appetite has been also reported in buffalo calves affected with zinc and other minerals deficiency. It has been thought that reduced appetite had been attributed to reduced ability to taste and smell foods. Whereby changes in appetite are associated with changes in the concentration of amino acid derived neurotransmitters in the brain, thus some mineral deficiency may reduce the appetite by impairing the taste because it postulated that the sense of taste is mediated through the salivary zinc dependent therefore low salivary zinc concentration leads to a reduction of taste and reduced appetite. The reduced appetite in deficient animals could be the reason for paleness of mucous membranes which detected in diseased buffaloes in the present study.

In the present study, parakeratosis, depigmentation of hair are an expectable and common signs of minerals deficient animals. Moreover partial loss of hair was the second most frequent findings in diseased animals. Those finding are in accordance with others.

Results indicated significantly increases in the respiratory and heart rates in buffaloes with minerals deficiency compare with normal controls. These differences could be due to the fact that some minerals such as zinc is a component of the enzyme carbonic anhydrase, which is located in the erythrocytes and gastric parietal cells, and is related to the transport of respiratory carbon dioxide and the secretion of hydrochloric acid by the gastric mucosa.

Hematological parameters show significant decrease in TRBc, Hb and PCV reflected normocytic normochromic type of anemia, same results were reported by which could be explained on the basis that some trace minerals deficiency leads to impairment of cell replication and protein synthesis and thus the generation of blood cells. Moreover, The known importance of copper in the formation of hemoglobin, since copper is necessary for the reutilization of iron liberated from the normal break down of hemoglobin.

Results of biochemical analysis of serum samples indicated a significant decrease in the level of serum Zinc, Cobalt, Copper and sulfur in outdoor feeding buffaloes group compare with indoor feeding group and controls, same results were also seen by. Due to the fact that large variations occur in the serum levels of minerals in both beef and dairy animals and are commonly correlated with the rainfall, the higher the rainfall the lower the of some minerals level. Moreover the
incidence of secondary mineral deficiency may be highest at other times, depending upon the concentration of the conditioning factor in the soil, mineral composition of feed, season of the year, soil characteristics and its mineral composition, specific geographical area. Concentration of minerals, such as molybdenum, sulfur and others, which can interfere with the presence of copper forage. In contrast, animals receive regular feedings and concentrates most of the year (in door) might affect fewer with minerals deficiency effects (28).

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