EVALUATION OF SOME MINERALS IN BUFFALOES IN BASRA PROVINCE

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ABSTRACT

The present study was carried out to evaluate the levels of copper, zinc and iron in buffaloes from different regions in Basra province. Serum samples were taken from (255) buffaloes and (20) soil samples from pastures as well as (20) samples of green forage grazed by the animals.

Results divided the buffaloes into three groups, the first one was healthy 38 (14.9%) of total buffaloes, second 17 (6.6%) those were subclinically affected and the third group 200 (78.4%) were clinically affected and revealed deficient values in comparison with other groups.

Values of copper, zinc and iron were (70.3 ± 0.867, 154 ± 4.459, 320 ± 2.844) µg/dl, respectively for healthy control buffaloes, while the values of subclinical group were (59.7 ± 0.384, 94.7 ± 1.943 and 291.8 ± 4.214) µg/dl respectively, and those of deficient group were (44.6 ± 0.442, 78.4 ± 1.069 and 229.1 ± 4.098) µg/dl, respectively.

The soil levels of copper, zinc and iron were (0.25 ± 0.039, 0.72 ± 0.032 and 5.9 ± 0.301) µg/g respectively, and in forage (2.3 ± 0.269, 23.8 ± 0.486 and 25.5 ± 0.641) µg/g, respectively. The main clinical signs of deficient group were; Alopecia, depigmentation, emaciation, parakeratosis and pale mucous membranes.

In conclusion, the study exhibited values of minerals in buffaloes those were higher in healthy buffaloes than those of subclinical and deficient groups. Soil of Basra, was deficient in copper and zinc but normal in iron, while forages were deficient in those minerals.

INTRODUCTION

Buffaloes are important farm animals, spread in various parts of the world (98%) of about (150) million buffalo heads populated all over the world where originated from Asia. In Iraq[1], (60%) of buffaloes inhabit in southern areas and
marshes" the rare aquatic landscape in the desert[2], and it contributes significantly to the food supply in the form of milk(58%) and meat(1.3%) [3,4]. Most buffalo owners nourish their animals on wheat bran and cotton seeds, and in most cases on only small amounts of green forages [5,6]. Nutritional imbalances and deficiencies due to erratic management or feeding programs, nutrients interactions and factors related to the animals or its environment create various types of problems those have a negative effect on the buffalo productive and reproductive efficiency[7,8], that by the time result in a pronounced decline in the population of Iraqi buffaloes in the last years [2,9,10]. Minerals are the essential nutrients bearing a significant role in the animal production and reproduction, because their excess or deficiency produce a detrimental effect on the performance of livestock [11]. Copper, zinc and iron as trace elements are found to be very essential for normal livestock growth and body functions [11,12]. If mineral concentration of serum or tissues was consistently higher or below the 'normal' ranges, it will provide a suggestive evidence of dietary deficiency or excess of particular minerals [13].

Mineral status of soils and forage influences or may govern mineral profile of grazing livestock [13]. Under pasture systems, animals depend on forages to satisfy all of their nutritional requirements, which are unfortunately, often do not provide all of the needed level of minerals, those required by the animals therefore many incidences of mineral inadequacies in animals have been reported which are principal causes of reproduction failure and low production rate [14]. Bone abnormalities; tetany, anemia, infertility, alopecia and pica are some of the clinical signs often suggestive for mineral deficiencies in livestock [15]. Despite of the devastating economic results of mineral deficiencies [14], mineral nutrition of buffalo is an area that has not been sufficiently investigated [16], and there are limited research works done on this topic [17], therefore this study was designed to investigate the concentration of Copper, Zinc and Iron in the serum of Buffaloes, soil of pasture and their forage in Basra province.

**MATERIALS AND METHOD**

During a period from 3 /9 /2012 to 16 /3 /2013, the study was carried out on (255) buffalos from both genders in different ages, green forage they graze and soil at which it arise, in Basra province south of Iraq.
Jugular blood samples (10ml), where collected by evacuated tubes and serum was separated after centrifugation (3000 rpm/minute) (25) minutes, then freeze until the digestion as in [18], by adding (2ml) of nitric acid (HNO3) and one ml of perchoric acid (HClO4) to (0.5 ml) of serum, then heated in oil path to 160° C for one hour, then cooled and finally completed to 10 ml with (0.3) Hydrochloric acid (HCl). Twenty samples of soil were collected from where the animals were grazing as in [19].

Other Twenty samples of plant including leaves and stems (eaten by the buffaloes and other grazing animals) were also collected beside the holes from which soil was taken, as in [20].

Samples of soil and plants were saved in clean dry plastic bags and stored in room temperature in relation to soil and in the refrigerator at 5C° for plants [20], until they were prepared to digestion.

After air-drying of soil for four days in a well-ventilated shed, samples were crushed, sieved and re-stored in a clean, closed container [21], then digested as in [22], by adding (7ml) of hydrofluoric (HF) acid and (1ml) of perchloric acid (HClO4) to (0.05 g) of soil in a platinum crucible and heated gently by an electro-thermal at 200°C for 2 hours without covering the crucible, in a good ventilated space, after complete evaporation of white fumes, mixture was cooled, then (3ml) of nitric acid and (0.5ml) of sulphuric acid (H2SO4) were added and re-heated at (200°C) for (15) minutes then re-cooled. Thenafter (30 ml) of distilled water were added and doubly filtrated with Whatman -42 filter paper, then finally it was completed to (50) ml with distilled water in a volumetric flask to be ready for estimation.

Plant samples firstly cleaned by washing with a pure water, dried, then ground [20], to be digested according to [23]. (0.5 g) of sample was placed in a (250ml) digestion tube and (3.5ml) of sulphuric acid (H2SO4) was added. The mixture was allowed to stand for (30) min at room temperature. Then (3.5ml) of 30% Hydrogen peroxide (H2O2) was added to the digestion tube then heated at (200°C) for (45) minutes, then the digestion tube was removed from the electro-thermal and cooled down and filtered twice through Whatman -42 filter paper and transferred to a (25) ml volumetric flask and completed by adding distilled water.

Values of minerals in all samples were estimated by the strategy of Atomic absorption spectrophotometry [24].

**Statistical analysis according to SPSS**.
RESULTS

Values of copper in serum
The mean values of copper in the serum of control group were (70.3µg/dl±0.867), whilst mean values of subclinical group were (59.7µg/dl±0.384), and it was (44.6µg/dl±0.442) in deficient group. (Table 1).

<table>
<thead>
<tr>
<th>Groups of Buffaloes</th>
<th>No. of examined</th>
<th>(% of examined</th>
<th>Cu µg/dl</th>
<th>Zn µg/dl</th>
<th>Fe µg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean±SE</td>
<td>Mean±SE</td>
<td>Mean±SE</td>
</tr>
<tr>
<td>Control</td>
<td>38</td>
<td>14.9</td>
<td>70.3±0.867</td>
<td>154±4.459</td>
<td>320±2.844</td>
</tr>
<tr>
<td>Subclinical</td>
<td>17</td>
<td>6.6</td>
<td>59.7±0.384*</td>
<td>94.7±1.943*</td>
<td>291.8±4.214*</td>
</tr>
<tr>
<td>Deficient</td>
<td>200</td>
<td>78.4</td>
<td>44.6±0.442*</td>
<td>78.4±1.069*</td>
<td>229.1±4.098*</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>255</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*= P<0.05

The copper in serum of control buffaloes was significantly higher (p<0.05) than that of subclinical and deficient groups.

On the other hand, values of copper in serum of subclinical group was significantly higher (p<0.05) than the values of deficient group.

Values of zinc in serum
The mean values of zinc in serum of control group were (154 µg/dl±4.4.59), whereas it was (94.7µg/dl±1.943)and (78.4 µg/dl±1.069),in subclinical and deficient groups respectively as in (Table 1).

Values of zinc in serum of control group were significantly higher(p<0.05) than values of both subclinical and deficient groups.

Likewise ,values of zinc in subclinical group were significantly higher(p<0.05) than those of deficient group.

Values of Iron in serum
In control group, values of iron in serum were (320.3µg/dl±2.844) and in subclinical group it was (291.8µg/dl±4.214) while values were (229.1µg/dl±4.098) in deficient group. (Table 1)

Values of iron in serum of both control and subclinical groups were significantly higher (p<0.05) than those of deficient group. While no significant variation (p>0.05) was obtained between control and subclinical groups.

Values of minerals in soil

The mean values of total copper, zinc and iron in soil of Basra province were (0.25µg/g±0.039, 0.72µg/g±0.032, 5.9µg/g±0.301), respectively. The values of iron were significantly higher (p<0.05) than values of both copper and zinc. Likewise, values of zinc were significantly higher (p<0.05) than those of copper. (Table 2).

<table>
<thead>
<tr>
<th>Samples</th>
<th>Cu µg/g Mean ±SE</th>
<th>Zn µg/g Mean ±SE</th>
<th>Fe µg/g Mean ±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td>0.25±0.039</td>
<td>0.72±0.032</td>
<td>5.9±0.301</td>
</tr>
<tr>
<td>Forage</td>
<td>2.3±0.269</td>
<td>23.8±0.486</td>
<td>25.5±0.641</td>
</tr>
</tbody>
</table>

Values of minerals in plants

The mean values of total copper, zinc and iron in green forages grazed by animals in pastures of Basra province were (2.3µg/g±0.269, 23.8 µg/g±0.486, 25.5 µg/g±0.641), respectively.

Iron level in forage was significantly higher (p<0.05) than level of each of copper and zinc.

Level of zinc in forage was significantly higher (p<0.05) than that of copper. (Table 2).

DISCUSSION

Values of minerals in serum

Values of copper

The mean values of copper in serum of control group in this study were (70.3µg/dl±0.867), to be coincidente with [25], those recorded values
of (76 µg/dl±3.08) in healthy buffaloes. But it was less than each of [26,27], those recorded (117.9 µg/dl±54.1) and (120.78 µg/dl±4.05), respectively in healthy buffaloes.

On the other hand, copper values were higher than (60.3 µg/dl±2.66 and 62.23 µg/dl±2.20) those found by [28,29], in healthy buffaloes. These results were in line with [30] who referenced that value of copper in serum considered to be (70-120) µg/dL and.

In deficient group of this study, mean values of copper were (44.6 µg/dl±0.442), which are in agreement with [30], who discussed that values below 60 µg/dL in serum of cattle indicate copper deficiency. And also in line with [27], who find (50.7 µg/dl±3.15), in deficient buffaloes. Moreover [31], reported hypocupremia in cattle based on copper, molybdenum and total sulphur content pasture forage. whilst [32], added that copper deficiency may be first due to shortage intake of the diet or secondary due to presence of competitive amounts of zinc, iron and molybdenum those reduce copper absorption.

The copper in serum of control buffaloes was significantly higher (p<0.05) than that of subclinical and deficient groups.

On the other hand, values of copper in serum of subclinical group was significantly higher (p<0.05) than the values of deficient group.

This significant decrease in serum copper levels of buffaloes was recorded by [33], those suggested that the cause may be attributed to the high concentration of molybdenum in the ration, which, reduce the availability of dietary copper due formation of insoluble cupric – thiomolybdate complex in the rumen of animals as well as the intestinal status of animals.

Values of zinc

The mean value of zinc in serum of control group was (154 µg/dl±4.459), which is in asymptotic range but little lower than that of [29], who found (164 µg/dl±2.01) in healthy buffaloes, but higher than values gained by each of [27] who recorded (80 µg/dl±6.1) in healthy buffaloes, and [34], those recorded level of (106 µg/dl) in healthy cows. whereas value of zinc in serum of deficient group was (78.4 µg/dl±1.069), this value is higher than that of [27] those calculated (59 µg/dl±4.05) in affected buffaloes.
The zinc deficiency can occur in ruminants as a result of consumption of immature grass which affect digestibility and also presence of excessive dietary sulphur and calcium [35,36].

Values of zinc in serum of control group were significantly higher (p<0.05) than values of both subclinical and deficient groups. Likewise, values of zinc in subclinical group were significantly higher (p<0.05) than those of deficient group.

Authors such as [27], reported that decrease in zinc and iron in serum may be attributed to disturbance in their absorption from gastrointestinal tract or may be due to loss of appetite. However, [37], found that high calcium content of the diet raised the zinc requirement substantially in cattle and reduced zinc absorption in calves. [38], explained that zinc absorption was depressed when iron was added to the low phosphorus diet. In fact, the iron was being bound by the phosphorus.

**Values of Iron**

In control group, values of iron in serum were (320.3µg/dl±2.844), those in same range but relatively lower than values obtained by [39], who recorded (366.051µg/dl±42.85), and [29], who found (358 µg/dl±3.46), in healthy buffaloes, and more higher than (87 µg/dl±4.1), found by [27]. while values were (229.1µg/dl±4.098), in deficient group.

Values of iron in serum of both control and subclinical groups were significantly higher (p<0.05) than those of deficient group. While no significant variation (p>0.05) was obtained between control and subclinical groups.

The significant differences were found by [27,40], those turned that to an intestinal condition interfere with absolute absorption, this appointment agrees with [41], who stated that dietary iron deficiency rarely has been observed in grazing cattle.

The mean values of copper, zinc and iron in subclinical group in the present study were (59.7µg/dl±0.384, 94.7µg/dl±1.943, 291.8µg/dl±4.214), respectively that was supported by [42,43], when they ensured that the incidence and importance is probably underestimated because subclinical forms of deficiency can occur and go unnoticed for a prolonged period.
Values of minerals in soil

The mean values of total copper and zinc in soil of Basra province were (0.25 µg/g ± 0.039 and 0.72 µg/g ± 0.032), respectively, those level were lower than levels of [44], who fixed the range (0.30-0.50 µg/g) for copper and (0.90-1.50 µg/g), for zinc, in normal balanced soil, while the iron level was (5.9 µg/g ± 0.301), which is in up level of [44], who signed to the range (3-5 µg/g) to be the approximate iron level in balanced soil. Adequate soil iron values were also reported by [45,46] from Florida and similar findings have been reported by [47-49]. These results are supportive to the report of [50] in which it was indicated that iron deficiency is rare in grazing animals due to generally adequate contents in soils. However, [51] reported iron deficiency in animals grazing on sandy soils in Florida. [52], related copper deprivation of soil to three different soil anomalies, inherently low copper status, as in the calcareous sands, low available copper status, as in peat soils rich in organic matter and those enriched with molybdenum.

Zinc and copper have been found to be affected by low pH and cultivation. Zinc may be more soluble and susceptible to leaching in low pH soils and high rainfall areas [53].

The values of iron were significantly higher (p<0.05) than values of both copper and zinc.

Likewise, values of zinc were significantly higher (p<0.05) than those of copper. [54] added that the most important factors in this respect being the nitrogen and phosphorous concentrations in the soil, as fertilization with P and N increases this will increase the risk of Zn deficiency.

Values of minerals in plants

The mean values of total copper, zinc and iron in green forages grazed by animals in pastures of Basra province were (2.3 µg/g ± 0.269, 23.8 µg/g ± 0.486, and 25.5 µg/g ± 0.641), respectively. All values are lower than the normal level in green grasses that recorded by [55], who reported the values (8 µg/g, 30 µg/g and 30 µg/g), respectively.

Copper availability to plants seems to be affected by soil pH. [53,56] suggested that available copper decreases with increase in soil pH. At higher pH, copper adhere to soil components and thus it may have led to decrease in copper in soil solution as cupric ions, which is the available form for plants.
related the zinc deficiency in plants to degree of maturation, so immature grasses have low level of zinc and the same for copper.

Iron level in forage was significantly higher\((p<0.05)\) than level of each of copper and zinc.

Level of zinc in forage was significantly higher\((p<0.05)\) than that of copper. this significant variation may be due the antagonistic relationship between copper and molybdenum which was recorded by [57], those reported that forage with molybdenum concentration greater than copper concentration often lead to copper deficiency, even when forage copper is adequate.
REFERENCES

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