Effect of Copper Deficiency on Ovarian Activity in Egyptian Buffalo-cows


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Abstract: Reproductive disorders and malnutrition are the main problems that determine productivity in buffaloes and cause great economic losses. This study was designed to correlate between copper status and ovarian activity in buffalo-cows. A total number of 1365 buffalo-cows reared at Lower Egypt was included in this study during the period from 2004-2008. Animals were clinically and gynecologically examined and blood samples were collected for carrying out some relevant analyses. Results revealed that 19.12% of the examined animals showed clear clinical signs of copper deficiency (hypocuprosis) and had a poor body condition score (BCS). Also, 21.84% of these hypocupremic buffalo-cows suffered from ovarian inactivity. In comparison with control cows, hypocupremic animals showed low serum progesterone level, especially during the luteal phase of the estrous cycle; anemia with no marked changes in leukogram; increased malondialdehyde (MDA) and nitric oxide (NO) and decreased, catalase (CAT), ascorbic acid (ASCA), superoxide dismutase (SOD), reduced glutathione (GSH-R) and total antioxidant capacity (TAC); zinc (Zn), iron (Fe), copper (Cu) and ceruloplasmin. It could be concluded that copper deficiency causes oxidative stress to buffalo-cows resulting in cessation of ovarian activity. Ceruloplasmin could be used as a good indicator for copper deficiency. It is recommended to supplement animal feeds with balanced mineral mixture to avoid retarded growth, anemia and cessation of ovarian activity.

Key words: Buffaloes • Ovarian activity • Copper • Oxidative status • Blood • Progesterone

INTRODUCTION

Buffaloes are considered as the black gold of Egypt due to these animals are the main source for good quality animal proteins. Also, buffaloes have high disease resistance and can efficiently convert poor roughages into meat and milk, despite, these animals are reputed for certain reproductive problems, such as late maturity, ovarian inactivity, long calving interval, silent heat and seasonality [1-2] which are major contributory factors towards lower productivity.

Reproductive disorders and nutritional deficiencies are the principal problems that affect buffalo productivity and cause great economic losses. Ovarian inactivity is the main obstacle facing buffalo production, especially in animals kept in small holder farms and exposed to mismanagement as well as some stress conditions such as malnutrition, parasitism, bad hygiene and pollution and [3-4].

Hypocuprosis is one of the most widespread mineral deficiencies. It affects grazing cattle as there are extensive copper deficient areas throughout the world which have adverse effects on the crops and livestock [5]. Also, endoparasitism has a depressing effect on blood copper and hemoglobin contents and can aggravate existing hypocupremia [6].

Copper plays an essential role as a micronutrient. So, hypocuprosis has a severe impact on growth and reproduction in domestic animals [7]. The reproductive efficiency of cattle and buffaloes was lowered due to copper and phosphorus deficiencies accompanied by low level of feeding and management [8]. Copper deficiency affects various physiological functions that may be important in immunological defense to pathogenic challenge [9]. The consequences of hypocuprosis include a failure of copper metalloenzymes, many of which form part of the antioxidant defense system [10].

The prevalence and severity of several important health and reproductive disorders in dairy cows are related to oxidative stress, whereas, the accumulated reactive oxygen metabolites (ROM) are toxic to cells, the body has developed a sophisticated antioxidant system...
that relies on antioxidant nutrients. Several trace minerals (as part of enzymes) and some vitamins are integral components of this antioxidant system [11]. Animals having Cu or Zn deficiency are usually suffering from general weakness, stunted growth and infertility [12]. Heifers that received no supplemental copper had low pregnancy rate [13] and more infected quarters during the next lactation [14].

The purpose of this study was to investigate the relationship between copper deficiency and ovarian activity in buffalo cows reared at villages of Lower Egypt and to examine some relevant blood constituents, especially, oxidant/antioxidant markers, in relation to copper deficiency.

**MATERIALS AND METHODS**

The present study was carried out during the period extended from 2004-2008 as a part of the National Research Centre Project No. 7120106.

**Animals:** A total number of 1365 buffalo-cows kept in small holder farms at villages of Lower Egypt was included in this study. Buffalo-cows were fed on Egyptian clover (Barseem; *Trifolium alexandrinum*), few amounts of concentrates, crop residues and rice straw. A full case history, owner complains and general health conditions of each animal were recorded. Gynecological examination was carried out by rectal palpation for two successive weeks and ultrasonography using an Ultra sound apparatus (PiaMedical Falcs e’Saote, Netherlands) with an endorectal linear array transducer (6-8 MHz). Animals did not show estrous signs at least 6 months after calving during the breeding season (September-March) and have small nonfunctioning ovaries have been considered to suffer from ovarian inactivity. The condition was confirmed by ultrasonography and later on by monitoring progesterone level.

**Samples Collection:** Samples of blood with and without EDTA were collected from all animals. Uncoagulated blood samples were used for performing complete blood picture as well as determination of GSH-R and selenium (Se) values. Serum was separated from coagulated blood samples by centrifugation (x 3000g, 15 minutes, 40°C) and kept at -20°C until use for assaying progesterone level as well as some oxidant/antioxidant markers, trace elements and ceruloplasmin.

**Analyses**
- Complete blood picture including erythrogram and leukogram were carried out as outlined by Jain [15]
- Serum progesterone level was assayed by ELIZA microwell technique using kits from DIMA (Germany). The kit had a sensitivity of 2.0 pg/ml with inter-and intra-run precision coefficient of variations of 2.9 and 4.85, respectively [16].
- Oxidant/antioxidant markers including MDA [17], NO [18], CAT [19], SOD [20], ASCA [21], R-GSH [22] and TAC [23] were colorimetrically assayed.
- Ceruloplasmin activity in serum was determined using a colorimetric enzyme assay [24].
- Trace elements including Zn, Cu and Fe concentrations in diluted serum samples and Se in whole blood samples were determined using atomic absorption spectrophotometer (Perkin Elmer, 2380) as outlined by Varley et al. [25].

**Statistical Analysis:** Data were computed and statistically analyzed using Student’s t-Test [26].

**RESULTS**

**Prevalence of Hypocuprosis in Buffalo-cows:** Out of 1365 examined buffalo cows, 261 animals (19.12%) showed clear clinical signs of copper deficiency manifested by hair discoloration. The changes in hair color ranged from red to yellow color with rough texture on small to large body areas (Fig. 1). Digestive disturbance, mainly intermittent diarrhea and deprived appetite with poor BCS (P<0.05) were observed as compared to control animals (2.08±0.21 Vs 2.88±0.28).

**Ovarian Inactivity:** Out of 261 examined hypocupremic buffalo cows, 57 buffalo-cows (21%) showed ovarian inactivity. These animals had small sized uteruses and small hard ovaries in texture with no physiological structures on their surface. Moreover, these animals showed no signs of heat after calving since at least 6 months during the breeding season.

**Serum Progesterone Level:** Serum progesterone level was low in hypocupremic animals, especially during the luteal phase of the estrous cycle as shown in Table 1.

**Blood Picture:** Blood picture of hypocupremic buffalo-cows revealed anemia indicated by decreased red cell
Fig. 1: Mild (Left) to severe (Right) hair discoloration in buffaloes suffered from hypocuprosis

Table 1: Effect of hypocuprosis on serum progesterone level (ng/ml) in non pregnant buffalo-cows (Mean±SE)

<table>
<thead>
<tr>
<th>Phase</th>
<th>Control group</th>
<th>Hypocuprosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular</td>
<td>0.50±0.11</td>
<td>0.37±0.08</td>
</tr>
<tr>
<td>Luteal</td>
<td>2.94±0.36</td>
<td>2.51±0.73</td>
</tr>
<tr>
<td>Inactive ovaries</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
</tr>
</tbody>
</table>

Table 2: The effect of hypocuprosis on the blood picture in buffalo-cows (Mean±SE)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Hypocuprosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrogram</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red cell count(10^6/µL³)</td>
<td>5.81±0.12</td>
<td>4.98±0.08**</td>
</tr>
<tr>
<td>Hemoglobin content (g/dl)</td>
<td>13.80±0.31</td>
<td>11.02±0.29**</td>
</tr>
<tr>
<td>Packed cell volume (%)</td>
<td>35.82±0.16</td>
<td>33.48±0.8*</td>
</tr>
<tr>
<td>Leukogram</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cell count (10^³/µL³)</td>
<td>6.47±0.28</td>
<td>6.77±0.31</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>62.14±1.86</td>
<td>63.17±0.98</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>38.31±1.72</td>
<td>36.22±1.61</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>1.83±0.55</td>
<td>1.55±0.73</td>
</tr>
<tr>
<td>Basophils (%)</td>
<td>0.23±0.12</td>
<td>0.21±0.10</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>1.23±0.61</td>
<td>1.23±0.48</td>
</tr>
</tbody>
</table>

* P<0.05** P<0.01

Oxidant/Antioxidant Markers: Concentrations of some oxidant/antioxidant markers in the blood of buffalo-cows in relation to copper deficiency are shown in Table 3. Both MDA and NO values increased (P<0.01), while, CAT, ASCA, SOD, GSH-R and TAC values decreased (P<0.01) in the blood of hypocupremic buffalo-cows as compared to normal animals.

Table 3: The effect of hypocuprosis on oxidant/antioxidant markers and ceruloplasmin in buffalo-cows (Mean±SE)

<table>
<thead>
<tr>
<th>Markers</th>
<th>Control</th>
<th>Hypocuprosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA (mmol/ml)</td>
<td>0.98±0.08</td>
<td>2.99±0.15*</td>
</tr>
<tr>
<td>NO (mmol/L)</td>
<td>15.51±1.61</td>
<td>23.31±1.80**</td>
</tr>
<tr>
<td>Antioxidants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAT (U/ml)</td>
<td>2.31±0.06</td>
<td>0.79±0.17**</td>
</tr>
<tr>
<td>ASCA (µ/dl)</td>
<td>128.16±4.13</td>
<td>81.79±3.87**</td>
</tr>
<tr>
<td>SOD (U/ml)</td>
<td>337.12±5.16</td>
<td>283.2±13.17**</td>
</tr>
<tr>
<td>GSH-R (mmol/L)</td>
<td>5.84±0.13</td>
<td>1.63±0.11**</td>
</tr>
<tr>
<td>TAC (mmo/L)</td>
<td>1.46±0.06</td>
<td>0.48±0.08**</td>
</tr>
<tr>
<td>Ceruloplasmin</td>
<td>44.10±3.75</td>
<td>12.63±1.32**</td>
</tr>
</tbody>
</table>

* P<0.05** P<0.01

Table 4: Effect of hypocuprosis on some trace elements concentrations in the blood of buffalo-cows (Mean±SE)

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Control</th>
<th>Hypocuprosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper (µg/dl)</td>
<td>88.45±3.33</td>
<td>57.17±1.98**</td>
</tr>
<tr>
<td>Zinc (µg/dl)</td>
<td>138.17±1.80</td>
<td>113.91±1.77**</td>
</tr>
<tr>
<td>Iron (µg/dl)</td>
<td>172.43±2.13</td>
<td>133.71±9.11**</td>
</tr>
<tr>
<td>Selenium (µg/dl)</td>
<td>132.66±0.41</td>
<td>126.71±7.12</td>
</tr>
</tbody>
</table>

** P<0.01

Concentration of Some Trace Elements: Table 4 shows decreased trace element concentrations in the blood of hypocupremic buffalo-cows as compared to normal animals, especially in Zn, Cu and Fe values (P<0.01).

DISCUSSION

Reproductive efficiency in buffalo is alarmingly low, causing severe economic losses to breeders. Ovarian inactivity is still one of the most prevalent reproductive disorders in this species [4, 27]. Underfeeding, or unbalanced feeding as well as deficiencies in minerals, vitamins or trace elements compounded with other factors are mainly responsible for the occurrence of reduced fertility in buffalo [28, 29]. A poor BCS at calving affects...
fertility, characterized by prolonged post-partum intervals, reduced conception rates and more services per conception [30-32].

The present investigation was carried out to highlight interrelation between one of the most important reproductive problem affecting buffalo-cows; ovarian inactivity and one of the most widespread minerals deficiency in the field; copper.

In this study, out of examined buffalo-cows, 19.12% showed clinical signs of copper deficiency with a poor BCS during the breeding season. In this respect, it was reported that in each region, at least a third of the cattle was deficient or marginally deficient, whereas, 35.90-40.6% of US beef cattle were classified as copper deficient [33]. Meanwhile, the clinical investigation revealed that 29% of Canadian calves in slaughterhouse were suffering from copper deficiency as indicated by signs of poor hair coat and unthriftiness coupled with low liver copper concentrations [34]. In accordance with the current clinical Cu deficiency symptoms, it was reported that Cu deficiency is linked to a variety of clinical signs, including poor quality coat, anemia, spontaneous fractures, poor capillary integrity, myocardial degeneration, hypomyelination of the spinal cord, impaired reproductive performance, decreased resistance to infectious disease, diarrhea and generalized ill-health causing severe economic losses in buffaloes [35]. It is known that copper is essential for erythrocyte production and the maintenance of their integrity in the circulation. In copper deficiency, there is an impairment of iron release from the reticuloendothelial cells, because of a decreased activity or production of ceruloplasmin and ferroxidase II. Thus, the iron is not available for erythrocyte production resulting in anemia. The affected calves are usually stiff and lame with a marked welling at the distal metatarsal and metacarpal physis. The biochemical lesion is a decrease in amine or lysyl oxidase; enzymes responsible for cross linkages of collagen and elastin and thus the stability and strength of bone. In copper deficiency, there is a breakdown in the conversion of tyrosine to melanin because of reduced amino oxidase activity resulting in achromatricia. This enzymatic activity has been shown to be linked to increased copper intake [36,37]. Limited research has addressed changes in BW and BCS in mature beef cows fed a Cu-depleting diet. In young cattle, a copper depletion phase diet had no affect on ADG or final BW in pregnant beef heifers [38]. In very young calves, ADG decreased substantially after 16 and 20 wk of Mo supplementation in calves, possibly due to decreased feed intake [39].

In this study, 21.84% of Cu deficient buffaloes showed ovarian inactivity. Under field conditions, copper deficiency is actually induced by molybdenum, which reacts with sulphur in the rumen to form thiomolybdates, which in turn bind copper with high affinity. Although subfertility is a multifactorial problem, a considerable proportion of animals has been shown to respond to appropriate copper supplementation [40, 41]. The current investigation agrees with Phillippo et al. [39] who reported reduced conception rates, anovulation and anoestrus, decreased LH levels in molybdenum-induced copper depletion. Moreover, it was reported that deficiency of Cu, Fe, Zn or Se can cause reduced production, especially when a deficiency corresponds to the phases of growth, reproduction, or lactation [8,42]. It was reported that animals having Cu or Zn deficiency are usually suffering from general weakness, stunted growth, increased susceptibility to infection and infertility [43]. The mechanism by which copper deficiency affects reproduction is not well identified. However, [44] showed that ovaries were reduced in size and have a decreased response to FSH-induced superovulation regimen in the molybdenum-induced copper depletion. The later may have central effects via the hypothalamus-pituitary axis on LH secretion leading to reduced ovarian oestradiol secretion and absence of estrus in animals. Moreover, it was also found that LH-induced differentiation of bovine theca cells in vitro can be prevented by thiomolybdates and these effects can be ameliorated by copper supplementation. So, copper responsive subfertility may result from perturbation of the normal pattern of ovarian steroidogenesis [45].

In this work, serum progesterone level was lower in Cu deficient animals, especially during the luteal phase of the estrous cycle. It was reported that pituitary tissue LH concentrations were lower in Mo-induced Cu-deficient calves compared with control Cu-supplemented calves [46]. Pituitary LH concentrations could have been affected by Cu deficiency and/or Mo supplementation. It was reported that GnRH increased LH and FSH release from rat pituitaries when Cu was present in the portal blood [47] possibly by influencing GnRH receptor binding [48] and/or intracellular Ca activity [49].

In the current investigation, anemia was clear in Cu-deficient buffalo-cows compared to normal animals. Meanwhile, no significant changes were observed in leukogram of these animals. These results were in agreement with the findings of Heidarpour et al. [50] who noted improved RBC parameters and MCV in calves injected with Cu and Fe and with that of Cerone et al. [51].
who noted that total leukocyte numbers were not affected by Cu deficiency. However, in Cu-deficient goat, only few minor changes were detected in the hematological parameters [52].

Investigating the oxidative status in hypocupremic buffalo-cows herein indicated that these animals were under stressful condition as indicated by high MDA and NO and low CAT, ASCA, SOD, GSH-R and TAC values. In a accordance with this result, liver thiobarbituric acid-reactive substances (TBARS) was reduced by the addition of vitamin E and Cu to the diet fed to pigs [53]. TBARS were significantly higher in liver and heart from copper-deficient rats compared with control rats after exposure of tissue homogenates to iron-induced lipid peroxidation. Copper deficiency has a harmful effect on lipid peroxidation in the cardiovascular system [54]. The lipid peroxidation (LPO) level of the Cu-deficient chicken was markedly increased [55]. It was reported that in vivo, erythrocytes are possible targets of oxidative damage because they are exposed to high concentrations of oxygen and contain heme iron that can autooxidize, which results in the formation of superoxide anions which can interact with NO leading to decreased NO concentrations in Cu deficient mice [56]. Cu, Zn - SOD Activity decreased markedly in erythrocytes during copper deficiency [57]. Leucocyte and erythrocyte SOD significantly decreased in hypocupremic ewes [58].

Serum ceruloplasmin concentration decreased in hypocupremic buffalo-cows as compared to normal animals. These findings coincided with Arthington et al. [59] who found that such copper-dependent acute-phase protein (ceruloplasmin) was not increased after challenge in Mo-induced Cu-deficient heifers. The clinical chemistry investigations made by Sas [60] showed anemia, minimal ceruloplasmin activity in the blood and high molybdenum concentrations in the rumen contents, liver and kidney. Also, it was recorded that ceruloplasmin concentration was low in blood serum of Cu-deficient goat [52]. Moreover, Calves fed Cu-supplemented diet had higher plasma Cu concentrations and plasma ceruloplasmin activities [61]. However, in copper-deficient cattle, the serum ceruloplasmin activity and Cu, Zn and SOD activities were low. Copper deficiency alters the activity of several enzymes, which mediate antioxidant defenses and ATP formation. These effects may impair the cell immune functionality, affecting the bactericidal capacity and making the animals more susceptible to infection [51].

In this study, serum Cu, Zn and Fe values showed marked decreases in copper deficient buffalo-cows compared to normal animals. It was reported that serum levels of Cu, Fe and ceruloplasmin activity decreased in Cu-deficient animals [62]. Zinc insufficiency was strongly associated with low milk production and impaired locomotion in dairy herds and was also associated with diarrhea and poor growth in calves [63]. Decreased Cu status has been shown to reduce holo-ceruloplasmin production and impair ferrioxidase activity, leading to decreased tissue Fe release and the generation of anemia that is responsive to dietary supplementation with Cu but not Fe. Dietary Fe absorption also requires the presence of a multicopper ferrioxidase, Hephaestin, a caeruloplasmin homologue, works in concert with the IREG1 transporter to permit Fe efflux from enterocytes for loading onto transferrin [64].

In conclusion, the present study shows that there is a possible association between ovarian inactivity and copper deficiency in buffalo-cows. Hypocupremic animals suffer from a poor body condition score, anemia, oxidative stress and decreased antioxidant defense. Ceruloplasmin could be used as a good indicator for copper deficiency. It is recommended to supply animal feeds with balanced mineral mixture to avoid retarded growth, anemia and cessation of ovarian activity.

REFERENCES


