Trace Elements Status and Antioxidants Profile in Ill-thrift Buffalo Calves

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ABSTRACT:
The goal of the present study was to evaluate some trace element concentrations and the activity of antioxidant systems in ill-thrift calves. A total number of (52) buffalo-calf was subjected to study. Out of them, (37) buffalo-calves had poor coat, poor general body condition score which constituted the ill-thrift group. The remained animals (15) were clinically healthy and kept as control group. Complete blood picture, trace elements profile, serum calcium, phosphorus & magnesium , blood urea nitrogen (BUN) , creatinine, some liver function parameters and total antioxidants capacity were evaluated. Results showed a significant reduction in the blood serum concentration of copper, cobalt, iron, selenium and zinc associated with normocytic normochromic anemia. Also a significant reduction in the activity of total antioxidants value was evident in ill-thrift calves. While levels of serum calcium, phosphorus and magnesium, blood urea nitrogen, creatinine and liver function parameters showed non-significant changes. In conclusion, a state of sub-optimal growth (ill-thrift) in buffalo-calves was largely attributed to trace element deficiency, in particular copper, cobalt, iron, selenium and zinc deficiency that may cause reduction in the total antioxidant capacity, with a lower ability to reduce oxidative compounds.

1. INTRODUCTION
Poor growth and unthriftness in calves are consider the widely mentioned complain among farmers in the world especially in the developing countries as Egypt, Calf ill-thrift is a vaguely defined condition with a variety of causes however ill-thrift and suboptimal growth are terms often used interchangeably (Radostits et al. 2007). Failure to gain weight is the main feature of this condition and chief complain of livestock producers. It has a drastic economic impact on livestock production as it affects animal’s rate of body weight gain, marketing, day to the first calving, herd survivorship and future productivity (Radostits, et al.1994; Underwood and Suttle, 1999). Ill-thrift occurs when calves grow slower than expected on a known amount of food (Radostits et al., 2007). Ill-thriftiness appear as a failure of some animal to make satisfactory weight, in spite of the presence of ample food and absence of signs of any primary disease, at the time all other classes appear in satisfactory health and body condition. It has multiple etiological factors involving marginal trace elements deficiencies, intestinal parasitism and coccidiosis. A number of stress factors including environmental and management variables such as climatic condition, type of soil and weaning practices have also been reported to be contributing factors to calf ill-thrift (Reid and Howath, 1980; Scibilia, et al. 1987). Stress of trace element deficiencies and hot climatic condition generally increases the production of free radicals, leading to oxidative stress (Elsayed, 2001; Saleh et al. 2008) which has a negative impact on the calves live weight, mortality and health. The present study was designed to evaluate some trace element status and total antioxidant profile in ill-thrift calves. The free radical defense system in ill-thrift calves was assessed by measuring total antioxidant capacity (TAC) which are considered the cumulative effect of all antioxidants present in blood and body fluids (Miller and Rice-Evans, 1996) not just the antioxidant capacity of a single compound.

2. MATERIAL AND METHODS:
2.1. Animals:
This study was carried out on 52 buffalo-calves of both sex from different localities in El- Behira governorate, aged from 6-12 months and weighed from 50-150 kg. All calves were proved as normally delivered and had no disease at birth. The feed stuffs available in the areas under investigation were the same. History revealed that there was no evidence of infectious diseases that are causing still birth or chronic weight loss. All calves underwent program of vaccination for infectious diseases including: Bovine
viral diarrhea virus (BVD), Foot and mouth disease (FMD), Clostridial diseases and Pasteurellosis. Thirty seven calves showed general weakness, poor calves vitality, with no apparent diseased condition. Failure to gain was the chief complain, while the rest calves (15) were apparently clinically healthy and used as control group.

2.2. Sampling:

2.2. a. Blood Sample:
Two blood samples were collected from each animal. First blood samples (coagulated blood) were used for measuring serum total proteins, albumin, liver enzymes, BUN, some trace elements (Copper (Cu), Cobalt (Co), Iron (Fe), Selenium (Se) and Zinc (Zn)), total antioxidant capacity, serum calcium, magnesium and phosphorus, while second blood samples (non-coagulated blood) were used for hematological analysis.

2.2. b. Fecal Sample:
About 30 gm of feces were collected from each calf in special air-tight containers for subsequent macroscopic and parasitological examination by standard floatation, sedimentation technique (Coles, 1986).

2.3. Methods:
2.3.a. Hematological analysis :- Red blood cell count (RBCs T/ l), hemoglobin concentration (Hbg/l), packed cell volume (PCV%), mean corpuscular volume (MCV fl), mean corpuscular hemoglobin (MCH pg), and mean corpuscular hemoglobin concentration (MCHC g/dl) were determined by using of fully automated veterinary hematology analyzer, Exigo, Boule medical AB, Sweden in the central laboratory, faculty of veterinary medicine, Alexandria University.

2.3.b. Biochemical analysis:
Concentrations of Cu, Se, Co, Fe, and Zn (mg/l) in the serum were determined with Flame emission atomic absorption spectrophotometer – model 210 vgp, Buck scientific, USA, in the central laboratory, Faculty of Agriculture, Zagazig University.

Biochemical analysis of calcium, phosphorus, magnesium, AST, ALT, Albumin, Total proteins and Creatinine were carried out by using commercial test kits supplied by (Bio-labo, France) while analysis of BUN, total and direct bilirubin were carried out by using commercial test kits supplied by (Vitro Scient., Egypt) following standard methods mentioned in the leaflet of the manufacturer.

Oxidants/Antioxidant status: the following markers were analysed using test kits supplied commercially by (Bio-Diagnostic, Cairo, Egypt) as follows;

2.3.b.1. Total antioxidant capacity (TAC): Total antioxidant react with a defined amount of hydrogen peroxide provided and the residual hydrogen peroxide is determined calorimetrically at 505 nm (Koracevic et al., 2001).

2.3.b.1. Hydrogen peroxide (H2O2): H2O2 was measured by colorimetric method in the presence of peroxidase (HRP), H2O2 reacts with 3,5-dichloro-2-hydroxybenzensulfonic acid (DHBS) and 4-aminophenazone (AAP) to form a chromophore, Quinoneimine dye (Aebi, 1984).

2.4. Parasitological examination:- Faecal samples were examined physically for color, odor and consistency before being subjected to thorough parasitological examination for internal parasites using standard floatation, sedimentation technique according to methods described by (Coles, 1986).

2.5. Statistical analysis:
Data collected were subjected to analysis by T-independent student test to assess significant differences between groups with the aid of SAS (2004).

3. RESULTS:

Clinical course: No specific signs of ill-thrift were observed. Poor growth, wasting and weakness were the only obvious clinical signs. Also, poor coat and hair condition were noticed.

Fecal analysis: Analysis of fecal sample by standard sedimentation floatation technique revealed no evidence of parasitic infestation.

Haematological analysis: reveals that ill-thrift calves had significantly low red cell count (p<0.01), with normal packed cell volume and mean corpuscular volume indicating the presence of normochromic normocytic anaemia in ill-thrift calves as compared with the control group (Table 1).

Biochemical analysis:
Serum albumen, globulins, total protein and liver enzymes, AST and ALT, showed no significant change in comparison with control group (Table 2).
Serum Creatinine and BUN showed no significant change in comparison with the control group (Table 3).

**Mineral concentrations:** Serum calcium, magnesium and phosphorus showed no significant change in comparison with the control group (Table 4).

**Trace element concentrations:** Concentrations of trace elements in serum such as Zn (p<0.01), Cu (p<0.0001), Fe (p<0.0001), Co (p<0.05) and Se (p<0.0001) were significantly decreased in ill-thrift calves as compared with the control group (Table 5).

**Total antioxidant capacity and hydrogen peroxide:** The study showed substantial increasing (p<0.001) of $\text{H}_2\text{O}_2$ levels with significant reduction (p<0.0001) in total antioxidants capacity in ill-thrift calves in comparison with the control group (Table 6).

### Table (1) Mean values (±SE) of hematological indices in apparent healthy and ill-thrift calves:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Apparent healthy calves</th>
<th>Ill-thrift calves</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCs (10⁶/µL)</td>
<td>7.99 ± 0.31</td>
<td>6.97 ± 0.15**</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>11.03 ± 0.5</td>
<td>10.34 ± 0.29</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>22.87 ± 1.2</td>
<td>21.27 ± 0.65</td>
</tr>
<tr>
<td>MCV (FL)</td>
<td>29.20 ± 0.6</td>
<td>29.97 ± 0.63</td>
</tr>
<tr>
<td>MCH (pg.)</td>
<td>14.16 ±0.20</td>
<td>14.27 ± 0.19</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>48.63±0.56</td>
<td>48.52± 0.48</td>
</tr>
</tbody>
</table>

**significant at P<0.01**

### Table (2) Mean values (±SE) of liver function tests in apparent healthy and ill-thrift calves:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Apparent healthy calves</th>
<th>Ill-thrift calves</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP (g/dl)</td>
<td>5.87 ± 0.34</td>
<td>5.59 ± 0.20</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.11± 0.16</td>
<td>3.03 ± 0.11</td>
</tr>
<tr>
<td>Globulins (g/dl)</td>
<td>2.76 ± 0.28</td>
<td>2.56 ± 0.14</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>0.23 ± 0.03</td>
<td>0.26 ± 0.01</td>
</tr>
<tr>
<td>Direct bilirubin (mg/dl)</td>
<td>0.10 ± 0.01</td>
<td>0.11 ± 0.01</td>
</tr>
<tr>
<td>AST (IU/l)</td>
<td>88.24 ± 9.94</td>
<td>93.69 ± 5.72</td>
</tr>
<tr>
<td>ALT (IU/l)</td>
<td>37.06 ± 4.69</td>
<td>38.39± 2.91</td>
</tr>
</tbody>
</table>

### Table (3) Mean values (±SE) of renal function tests in apparent healthy and ill-THRIFT calves:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Apparent healthy calves</th>
<th>Ill-thrift calves</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUN (mmol/l)</td>
<td>34.04 ± 2.28</td>
<td>34.16 ± 1.25</td>
</tr>
<tr>
<td>Creatinine (µmol/l)</td>
<td>0.83 ± 0.08</td>
<td>0.82 ± 0.04</td>
</tr>
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</table>

### Table (4) Mean values (±SE) of some serum Macronutrients concentrations in apparent healthy and ill-thrift calves:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Apparent healthy calves</th>
<th>Ill-thrift calves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mg/dl)</td>
<td>8.75 ± 0.35</td>
<td>8.32 ± 0.22</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>6.63 ± 0.28</td>
<td>6.63 ± 0.23</td>
</tr>
<tr>
<td>Magnesium (mg/dl)</td>
<td>2.71 ± 0.17</td>
<td>2.75 ± 0.08</td>
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</tbody>
</table>
A significant correlation between ill-thrift and iron deficiency was proven in this study. These results were in concern with those reported by Nockles et al., (1994) and Borghese, (2005) who concluded that deficiency of copper results in poor growth, Unthriftiness and coat and skin abnormality.

Zinc is a critical nutrient of immunity, being involved in so many immune mechanisms including cell mediated and antibody mediated immunity, thymus gland function and thymus hormone action. Zinc is also important as an antioxidant. So zinc deficiency is implicated in the occurrence of ill thrift in association with copper, manganese and iron deficiencies (Sadiek et al., 1994 and Borghese, 2005). A significant correlation between ill-thrift and zinc deficiency was proven in this study. These results were in concern with those reported by Nockles et al., (1993) who attributed these results to the role of zinc in reducing transportation, feed and handling stress which affects animal growth rates. Our data findings was in agreement with Abou El-Amaiem (2012) who showed, a significant association between zinc (p < 0.05), copper (p< 0.05) and iron (p<0.05) deficiencies and ill-thriftiness in calves so, indicate that nutritional deficiency has a significant impact on growth of buffalo calves. Also there were a proven relationship between ill-thrift in buffalo calves and iron deficiency. These results could be attributed to the role of copper deficiency in decreasing the absorption of iron which has a great role in synthesis of certain enzymes related to oxygen utilization (Sadiek et al., 2007). Our data findings are compatible with those obtained by Eassa, (1987) who mentioned that there were a significant decrease in copper, iron, manganese and zinc in ill-thrift buffalo calves.

Also Our data findings was in agreement with Sadiek et al., (1994) who stated that there were a significant decrease in copper, zinc, iron and...
manganese in unthrifty buffalo calves. Selenium has a notorious history that places it in a unique situation relative to feeding recommendations and regulations. Our data was compatible with (Van Saun, 2007) who reported that selenium deficiency leads to ill-thrift via its effect on thyroid gland function.

Our results were in concern with Sikka et al. (2002) who reported that micronutrients such as Zn, Cu, Fe and Mn improve the efficiency of antioxidant system in lipid peroxidation prevention, as occur elevation of serum hydrogen peroxide & reduction of TAC in our research on ill-thrift calves suffering from trace element deficiencies.

Results of this study was compatible with Bedwal et al., (1994) who stated that zinc, copper and selenium have significant roles in maintaining good health condition in farm animals as deficiency of these trace elements in our study results in a case of calf ill-thriftiness, this also was in agreement with Damir et al., (1988) who reported that low blood copper and zinc concentrations in growing animals result in general weakness, stunted growth and anemia.

It is also believed that suboptimal growth in calves is associated with reduction of antioxidants activity resulting from these micronutrients deficiencies. This reduction of antioxidants activity in the hypocupremic calves may allow reactive oxygen species (ROS) such as superoxide (O$_2^-$) to accumulate beyond their capacity to an extent that oxidative damage may occur. A significant increase (P<0.001) of serum hydrogen peroxide (H$_2$O$_2$) in Cu deficient ill-thrift calf strongly support this assumption and in agreement with Fernandez-Urrusumo et al., (1997). Previous studies showed a tendency for leukocytes to release greater amounts O$_2^-$ in hypocupreemic animals (Jones and Suttle, 1981). For instance, 58% increase in O$_2^-$ was reported in hypocupreic rat tissues (Lynch et al. 1997). Also, elevated O$_2^-$ and diversion of nitric oxide (NO) by O$_2^-$ to peroxynitrite (ONOO$^-$), a potent oxidizing agent, were found in the Cu deficient rat embryos (Hawk et al., 2003; Beckers-Trapp et al., 2006).

This study results was in agreement with Ahmed and Ghada (2007) who proved that the real cause of ill-thriftiness in buffalo calves was trace elements deficiency including Cu, Zn, Se and Fe and there is a tight relationship between antioxidant status and state of inferior vitality in buffalo calves as there was elevation of oxidative stress markers, malondialdehyde (MDA) and significant reduction in TAC.

In such case, it may be hypothesized that a state of negative control on total antioxidant capacity occurs due to overwhelmed antioxidant system secondary to copper, zinc and selenium deficiency, release of lipid peroxide, and consuming of total antioxidants capacity by ROS. These may explain why the activity of TAC decreased.

5. CONCLUSION:

In conclusion, a state of sub-optimal growth (ill-thrift) in calves was largely attributed to trace element deficiency, including copper, iron, selenium, zinc and cobalt deficiency that may cause reduction in the total antioxidant capacity, with a lower ability to reduce oxidative compounds which has a negative impact on health and immunity status of ill-thrift calves. Further studies are needed to investigate the association between trace elements & total antioxidant activities on larger sample size.

6. REFERENCES:


