Effect of Supplementation with Trace Elements (Copper, Cobalt, Zinc and Selenium) on Blood Cell Count in Camels (Camelus dromedarius)

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Abstract

The goal of this study was to examine influence of Cu, Co, Zn, and Se supplementation on hematological parameters, and to set up provisional reference range for cellular blood constituents in supplemented and non-supplemented camels. Animals were fed on grass and wheat bran with known concentrations of trace elements. Zn, Cu and Co supplementation had positive effect on RBC and Hgb (0.32 and 0.38 for Zn; 0.37 and 0.3 for Cu; and 0.74 and 0.69 for Co). Se moderately negatively correlated both with RBC and Hgb (-0.37 and -0.5, respectively).

Keywords: Camel; Cellular blood constituents; Supplementation; Copper, Cobalt, Zinc, Selenium, ICP-MS

1. Introduction

Camels play an important role in livestock economy and tradition of the United Arab Emirates (UAE), since camel racing is a significant element of local tradition. For the best health condition and racing performance, camels are fed as per various diet protocols and supplemented with variable amounts of trace element supplements.

Cu, Co, Zn, and Se are essential trace elements since they participate in different reactions of biotransformation and have specific functions indispensable for life such as respiration, normal skeletal growth and development, glucose utilization, prevention of sterility, protein and nucleic acid metabolism, activation of antioxidative enzyme functions (Tapiero et al., 2003; Stefanidou et al., 2006).

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Cobalt is mainly present in the body as Cobalamin, or Vitamin B12, and has effects on erythropoiesis. Cobalamin deficiency caused by poor diet is characterized by anemia, and can be prevented by consumption of low amounts of this micronutrient (Jelkmann, 2012). Zinc deficiency affects macrophage and neutrophil performance, and has been associated with increased susceptibility to infections. (Fraker, 2004; Osredkar and Sustar, 2011). Along with its antioxidant action, Selenium deficiency in animals has been associated with “white muscle disease”, while higher Se plasma concentrations have antiviral effects, and are essential for successful reproduction, as well as reduction of the risk of autoimmune thyroid disease. (Nazifi et al., 2009).

Due to complex kinetics of metals (absorption, distribution, metabolism, elimination, deposition and excretion) and their mutual interactions, it is almost impossible to predict metal effects or tissue concentrations based on their concentrations in feed, water, and environment.

Trace mineral requirements depend on age, sex, breed, and intended use of the animal. Variations in animal selection, time and place of sampling, conditions of sample preservation (temperature etc.), can significantly affect study results and make obtained analytical data non-comparable. Additionally, the biological availability of an element and its ultimate effect on an organism may depend on a chemical form in which the element is encountered, and it's interactions with other toxic or essential elements (Zwolak and Zaporowska, 2011).

Several studies on Cu, Co, Zn, and Se in camels, conducted in past decade in the Gulf region, have shown big variation in plasma concentration range (Al Busadah, 2003; Badiei et al., 2006; Seboussi et al., 2009; Faye et al., 2008). Some of them discuss effect of mineral supplementation on heavy metals status, activity of antioxidative enzymes, deposition and distribution of minerals in tissues and body fluids (Seboussi et al., 2009; Abdelrahman et al 2011). Similarly, some research papers report changes in CBC count in camel as an effect of different physiological and pathological conditions (Ayoub et al., 2003; Kamal, 2008; Al Busadah et al., 2007). Published data on reference hematological values in camel have shown large variation, due to diversity in animal selection, time and place of sampling, environmental setting, conditions of sample transportation and preservation, as well as choice of applied analytical techniques (Al Busadah, 2007, Farooq et al., 2011).

The goal of this study was to examine influence of Cu, Co, Zn, and Se supplementation on CBC count in racing camels fed on different diets.

2. Materials and methods

2.1. Location of the study

Camels were selected from different camps, owned by Department of President’s Affairs in the Abu Dhabi Emirate, United Arab Emirates.

2.2 Animals

Apparently healthy female racing camels, aged between one and four years. Animals were kept under same conditions with free access to water, divided into two groups, according to the diet they were fed on.
2.3 Exclusion criteria

Clinically and laboratory confirmed infection, or positive findings for blood or fecal parasites.

Group I consisted of 30 camels fed on mix of Saboos wheat bran racing camel feed 2 kg/day, and dry Rhodes grass 6-8 kg/day.

Group II consisted of 28 non-supplemented female camels, fed on dry Rhodes grass *ad libitum*, in an approximate quantity of 8-10 kg/day.

Both groups had free access to water.

This study was conducted with approval of the Committee for the Welfare of Camels, Management of Scientific Centers and Presidential Camels.

2.4 Chemicals and reagents

Chemicals listed below were used for sample preparation: Milli Q water, resistivity 18 MΩcm, Millipore USA;

Trace Metal Grade Nitric Acid, Fisher Scientific, UK; Trace Metal Grade Hydrochloric Acid, Fisher Scientific, UK;

Hydrogen peroxide solution ≥30%, TraceSELECT® Ultra, for ultratrace analysis, Sigma Aldrich

Calibration of ICP-OES and ICP-MS was performed by Accu Trace Reference Standard for Trace Metals I and ICP-MS Internal Standard Mix1, while tuning was performed by aspirating ICP-MS Tuning Solution I, all manufactured by AccuStandard, USA,

2.5 Sample collection and preparation

Venous blood was withdrawn from jugular vein into the BD Vacutainer Purple top K2EDTA for hematological analysis; into the BD Vacutainer Royal Blue K2EDTA (Becton Dickinson, USA) trace element free tube for elemental analysis: into the BD Vacutainer tube with silica clot activator.

2.6 Hematological analyses

Hematological analyses were performed immediately upon arrival of blood to the laboratory. Three level hematology controls (e-check, Sysmex, Japan) were analyzed prior to sample analysis. Satisfactory results for veterinary proficiency testing samples (VETQAS, UK) confirmed proper performance of the equipment.

2.7 Elemental analysis of serum samples

Plasma was separated after centrifugation at 3700 RPM for 10 minutes, and kept in acid washed cryo vials at –80°C, until analysis. Prior to Cu, Co, Zn, and Se analysis in plasma, Seronorm Trace
Elements in Serum Level 1&2 (SERO AS, Billingstad, Norway) and retained PT samples for elemental analysis (Interlaboratory Comparison Program for Metals in biological matrices, Centre de Toxicologie du Quebec/INSPQ, Canada) were analyzed with each batch of samples by an accredited method (A2LA Cert. No.3118.01). Satisfactory proficiency testing results (CTQ, Canada) confirmed validity of the applied method. Iron was analyzed from serum obtained after centrifugation of blood at 3700 RPM for 10 minutes. Serum samples and controls (Randox Human Assayed Serum Level 2&3, Randox, UK) were analyzed using a bichromatic endpoint technique and ascorbic acid/Ferene reagent, applying an accredited method.

2.8 Analysis of feed raw materials, forages, camel feeds and water

Elemental analysis of feed raw materials and forages was performed by ICP-OES applying an accredited method. Prior to analysis, samples were digested using following procedure: About 0.50 grams of prepared sample was accurately weighed and carefully transferred into PTFE microwave digestion vessel. High purity acids (1.5 ml HCl, 4.5 ml HNO₃) and 1 ml of Hydrogen Peroxide were added. Reagent blank was prepared in a same manner, omitting the sample. Vessels were capped and digested in the microwave digester using the optimized power program (Power: 1600 W, Power: 100%, Ramp: 20 min., Temp., 180°C, pressure: 800 psi, Hold time: 10 min. and Cooling time: 30 min.). After cooling time, content of vessels was quantitatively transferred into a 25 ml volumetric flasks, volume made up to 25 ml with deionized water and filtered. Filtered solutions were analyzed using ICP-OES.

Water samples were analyzed without previous pretreatment.

2.9 Instrumentation

1. Sysmex XT 2000i hematology analyzer for veterinary use, equipped with veterinary hematology software package (Sysmex Corporation, Kobe, Japan).
2. ICP-MS instrument Varian 820 MS, controlled by Bruker Quantum software Version v3.0 b797, (Varian /Bruker, Australia), and equipped with SPS3 autosampler (Varian /Bruker).
3. Siemens RXL-Max clinical chemistry analyzer, Siemens, Germany
4. ICP-OES, Varian 720 ES (Varian /Agilent).
5. Microwave digestion system MARS- CEM Corporation, USA

2.10 Statistical Analysis

Results are presented as mean ± standard deviation (SD). Statistical analysis was performed with the unpaired t-test, Wilcoxon’s rank sum test, and linear regression using the least-squares method. Differences were considered significant at p<0.05.

3. Results

Performance characteristics of the ICP-MS method applied for analysis of Cu, Co, Zn, and Se are summarized in Table 1.
Table 1 Summary of ICP-MS method characteristics for elemental analysis

<table>
<thead>
<tr>
<th>Element</th>
<th>Cu</th>
<th>Co</th>
<th>Zn</th>
<th>Se</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOD µg/L</td>
<td>1.11</td>
<td>0.09</td>
<td>2.0</td>
<td>1.5</td>
</tr>
<tr>
<td>LOQ µg/L</td>
<td>6.0</td>
<td>0.3</td>
<td>7.3</td>
<td>5.9</td>
</tr>
<tr>
<td>Accuracy (%)</td>
<td>98</td>
<td>103</td>
<td>99</td>
<td>101</td>
</tr>
<tr>
<td>Within day precision (CV%)</td>
<td>1.4</td>
<td>3.4</td>
<td>6.4</td>
<td>2.5</td>
</tr>
<tr>
<td>Intermediate precision (CV%)</td>
<td>3.6</td>
<td>7.8</td>
<td>8.8</td>
<td>6.8</td>
</tr>
</tbody>
</table>

As a part of the study, Cu, Co, Zn, and Se were measured in feed raw materials and forages. Results are presented in Table 2.

Table 2 Trace element levels in Feed Raw Materials, Forages and Water

<table>
<thead>
<tr>
<th>Description</th>
<th>No. of samples</th>
<th>Cu Range (mg/kg)</th>
<th>Cu Mean (µg/L)</th>
<th>Co Range (mg/kg)</th>
<th>Co Mean (µg/L)</th>
<th>Zn Range (mg/kg)</th>
<th>Zn Mean (µg/L)</th>
<th>Se Range (mg/kg)</th>
<th>Se Mean (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>10</td>
<td>2.62-5.47</td>
<td>3.61</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>16.4-28.6</td>
<td>19.6</td>
<td>&lt;LOD-&lt;LOQ</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>Wheat Bran</td>
<td>12</td>
<td>8.75-11.1</td>
<td>9.58</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>57.8-70.2</td>
<td>65.9</td>
<td>&lt;LOD-&lt;LOQ</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>Grass/Hay/Green shrub</td>
<td>12</td>
<td>4.54-17.3</td>
<td>8.27</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>15.4-92.3</td>
<td>39.2</td>
<td>&lt;LOD</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>Racing camel Feed</td>
<td>18</td>
<td>30.0-49.9</td>
<td>36.6</td>
<td>1.13-2.22</td>
<td>1.60</td>
<td>123-155</td>
<td>140</td>
<td>&lt;LOD-0.53</td>
<td>0.18</td>
</tr>
<tr>
<td>Water</td>
<td>11</td>
<td>&lt;LOD-0.24</td>
<td>0.03</td>
<td>&lt;LOD-0.01</td>
<td>&lt;LOD-0.01</td>
<td>&lt;LOD-0.08</td>
<td>0.02</td>
<td>&lt;LOD-0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Results of plasma trace elements in are presented in Table 3. As expected, animals on trace element rich diet have shown significantly higher all examined elements.

Table 3 Trace element levels in camel plasma µg/L (mean ±SD)

<table>
<thead>
<tr>
<th></th>
<th>Cu</th>
<th>Co</th>
<th>Zn</th>
<th>Se</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (n=30)</td>
<td>758±168*</td>
<td>1.56±0.33*</td>
<td>539±125*</td>
<td>152±26*</td>
</tr>
<tr>
<td>Group II (n=28)</td>
<td>602±118</td>
<td>0.96±0.18</td>
<td>380±47</td>
<td>90±23</td>
</tr>
</tbody>
</table>

* P<0.05

Results of analysis of basic cell blood constituents presented in Table 4 show significantly higher red blood cells, hemoglobin, PCV, and neutrophils.

In order to exclude potential influence of Iron on hematopoiesis, we measured serum Iron. Iron concentration did not differ significantly between two groups (936±190 and 887±141 µg/L, respectfully).
Table 4 Hematological parameters mean ± SD in supplemented and non-supplemented camels

<table>
<thead>
<tr>
<th></th>
<th>RBC (M/µL)</th>
<th>Hgb (g/L)</th>
<th>MCV (fl)</th>
<th>PCV (%)</th>
<th>MCH (pg)</th>
<th>WBC (K/µL)</th>
<th>Neu (%)</th>
<th>Lymp (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (n=30)</td>
<td>10.6±1.4*</td>
<td>139±12*</td>
<td>28.5±1.9</td>
<td>30±2.8*</td>
<td>12.4±0.6</td>
<td>10.4±1.7</td>
<td>56.8±13.3*</td>
<td>39.9±13.2*</td>
</tr>
<tr>
<td>Group II (n=28)</td>
<td>9.9±0.9</td>
<td>125±8</td>
<td>28.1±1.8</td>
<td>27.8±1.9</td>
<td>12.8±0.5</td>
<td>11.8±1.8</td>
<td>42.4±7.4</td>
<td>46.1±7.3</td>
</tr>
</tbody>
</table>

* P<0.05

4. Discussion

In order to examine influence of trace element supplementation on CBC count, we analyzed Cu, Co, Zn, and Se in plasma of camels fed on different diets. As expected, ICP-MS analysis of plasma samples has shown that group I had significantly higher all four added micronutrients.

4.1 Copper

Literature data report Cu values in plasma or serum of different camel breeds in the range 567 µg/L (Faye et al 2003) to 1340 µg/L (Badiei et al., 2006). Extremely low values between 323.3 µg/L and 450 µg/L were reported in plasma of lactating camels before supplementation (Dell'Orto et al., 2000), and in camel serum samples from sub-Saharan Africa (Faye and Bengoumi, 1994), respectively. Copper deficiency is considered when Cu serum values are 400 µg/L or below, and occurs when diet is low in Copper or very high in other constituents such as Sulphates or Molybdenum. Since there are no clear clinical signs of Cu deficiency, it is hypothesized that Cu mainly plays role in prevention of oxidative damage and Fe metabolism. Copper toxicity, with values higher than 1200 µg/L is manifested through intravascular hemolysis, liver necrosis and sudden death (Maas 2009). In our study, measured values for Cu in plasma were in the range of values found in the UAE and Oman (Faye et al., 2008; Eltahir et al., 2010).

4.2 Cobalt

Although Co levels were significantly higher in the group receiving supplementation, there were no signs of deficiency in animals fed on grass. Literature data for Co blood levels in camel are very limited; Faye and coworkers (2008) reported 0.4 µg/L of Co, in plasma of animals of the same sex and age group, receiving no supplementation.

4.3 Zinc

In our study, Zn concentrations in animals receiving no supplementation were in the lower range of published data, corresponding with values reported by Faye and coworkers (Faye et al., 2008) for camel population the same region (256 µg/L). There is no published evidence on Zinc deficiency in camel, although in other ruminants signs of Zn deficiency include growth retardation, disorder in keratin synthesis, and swelling and lesions in joints, while Zn toxicity causes anemia and digestive disorders (Hosnedlová et al., 2007). Wide range of Zn plasma values in camel can be found in
published data; Tajik and coauthors (2010) reported 680 µg/L of plasma Zn in Iranian camels, while values reported from Saudi Arabia and Oman exceed 1000 µg/L (Al-Busadah, 2003; El Tahir, 2010).

4.4 Selenium

Selenium in camel’s diet and results of Se supplementation were subject of numerous studies. However, published data for Selenium plasma concentrations vary extensively, possibly due to several critical pre analytical and analytical factors, such as animal and feed selection, sampling time, and sample preparation and analysis. Selenium plasma concentrations obtained in our study confirmed values reported earlier for camels in the United Arab Emirates (Faye et al., 2008; Faye and Seboussi, 2009). It has been widely accepted that value of around 100 µg/L plasma Se is minimum requirement for normal metabolic function in large animals. Hall (2011) recommended normal camel Se serum concentrations in the range 100-200 µg/L. Se depots in the body are relatively limited, and we assume that even slightly reduced plasma Se, which is normally present in µg/L in plasma, is a good marker of Se deficiency.

4.5 Cellular Blood Constituents

Our results of CBC analysis have revealed significant difference in RBC count and Hgb concentrations between two examined groups. In our study, we found that RBC count and Hgb concentrations were in line with values earlier published by for healthy female camels in the United Arab Emirates (Saeed and Hussein, 2008). Al Busada, however, reported around 12.7 g/dL of Hgb and 7.7± 0.5 M/µL RBC respectively, in female camels fed on natural pasture and supplemented with mineral salt sticks (Al Busada, 2007). Hgb values around 11.34 g/dL along with RBC count of 7.3 ± 0.6 M/µL were found in one-hampered “well fed” camels in Pakistan (Farooq et al., 2011). Contrary to RBC and Hgb, MCV (mean cell volume) and MCH (mean corpuscular hemoglobin) did not differ significantly between groups. This indicates that supplementation had no effect neither on average volume of a red blood corpuscle, nor on average Hgb content in RBC. Although there was no significant difference in WBC count between two groups, our results have shown that supplemented animals had significantly higher percentage of neutrophils, compared to non-supplemented group. Potential explanation of this finding could be a key role of Zn in normal development of neutrophils, natural-killer cells, macrophages and B-cells (Prasad, 2008; Freitas et al., 2010). Moreover, in vitro and in vivo studies on Se influence on bovine neutrophil function have shown that addition of Se was effective at enhancing the chemotactic migration and ability to kill bacterial pathogens (Sordillo, 2013). Analysis of influence of Zn supplementation on cellular blood constituents has revealed moderate positive linear relationship with RBC and Hgb (0.32 and 0.38, respectively). Se moderately negatively correlated both with RBC and Hgb (-0.37 and -0.5, respectively), which is in accordance with findings for pregnant and lactating camels, published by Seboussi et al (2009). Contrary to Se, Cobalt exerted stimulative effect on erythropoesis.RBC count and Hgb concentration highly correlated with plasma Co concentrations (0.74 and 0.69, respectively).
5. Conclusions

Zn, Se, Cu and Co is essential for normal growth and development of camels. Supplementation with these trace elements increased their plasma levels, and had a significant influence on RBC count, Hgb levels, PCV and Neutrophils. Since numerous clinical parameters strongly depend on hormonal status, geographical location, physical activity, or show diurnal and seasonal variations, further studies are needed on samples collected under controlled conditions. Once reference range is created based on listed criteria, it can be used as a guideline in veterinary clinical pathology.

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