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Comparative relationship between copper–zinc plasma concentrations and superoxide dismutase activity in camels and cows

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Abstract – On an experimental farm, five camels and five cows were fed with a similar basal diet over a 6-month period. They received an oral trace element supplement for 3 months (days 22–112). This supplement contained zinc and copper sulphate, and corresponded to twice the daily requirement generally recommended for cows. Plasma zinc and copper concentrations were significantly lower in camels (44 pg/100 mL for copper and 38 pg/100 mL for zinc) than in cows (106 and 83 pg/100 mL, respectively). The supplementation had no effect on the plasma zinc concentration in the camels in spite of the low observed values. The mean erythrocyte SOD activity was also significantly higher in the cows (2 404 ± 21 IU/100 gHb) than in the camels (1 720 ± 31 IU/100 gHb). In both species, no correlation was found between copper plasma concentration and erythrocyte SOD activity. In cows, a positive relationship was observed between plasma zinc concentration and SOD activity (r = 0.396). In contrast, a negative relationship was found in camels (r = -0.369). These results are discussed in relation to the physiological peculiarities of the camel. © Inra/Elsevier, Paris.

copper / zinc / superoxide dismutase / camel / cow / erythrocyte


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Les concentrations plasmatiques en cuivre et zinc étaient plus faibles chez les dromadaires (44 pg/100 mL pour le cuivre et 38 pg/100 mL pour le zinc) que chez les bovins (106 et 83 respectivement). La complémentation orale n’a aucun effet sur le zinc plasmatique du dromadaire en dépit des faibles valeurs observées. En moyenne, l’activité de la SOD est également significativement plus élevée chez la vache (2 404 ± 211 IU/100 gHb) que chez le dromadaire (1 720 ± 312 IU/100 gHb). Dans les deux espèces, aucune corrélation n’a été observée entre le cuivre plasmatique et l’activité érythrocytaire de la SOD. Chez la vache, une relation positive a été observée entre zinc plasmatique et activité de la SOD (r = 0.396). À l’inverse, une relation négative est relevée chez le dromadaire (r = -0.369). Ces résultats sont discutés en relation avec les particularités physiologiques du dromadaire. © Inra/Elsevier, Paris.

cuivre / zinc / superoxyde dismutase / dromadaire / vache / érythrocyte

1. INTRODUCTION

Copper and zinc play essential physiological and biochemical roles by functioning as cofactors of metalloenzymes and other metalloproteins. Through their relationships with dependent enzymes, they are important for the maintenance of optimal health, normal cellular homeostasis and in the structure and function of various tissues and immune systems. Superoxide dismutase (SOD, E.C. 1.15.1.1.) is a copper- or zinc-dependent intracellular enzyme. Zinc stabilizes the enzyme and copper is necessary for catalysis [20]. SOD acts as an antioxidant by oxidizing free radical oxygen and protects against lipid peroxidation [15]. It is the major copper-dependent enzyme found in the erythrocytes. Positive relationships are generally found between plasma levels of copper and zinc, and SOD activities in humans [25] and rats [21]. Few data are available concerning cattle SOD [13, 14, 24] and to our knowledge, there is no data concerning camel SOD reported in the literature [22].

The purpose of the present study was to investigate the relationship between the erythrocyte SOD activity and the copper and zinc concentrations in the plasma of camels as compared to that in cows under similar mineral supplementation and feeding conditions and to evaluate if superoxide dismutase can be used as an efficient biochemical indicator of copper or zinc status in camels and cows.

2. MATERIALS AND METHODS

The study was carried out at the experimental station of the Institut Agronomique et Vétérinaire Hassan II (Gharb farm), 80 km north of Rabat (Morocco).

2.1. Animals

The study included five adult camels originating from south Morocco, and five black-foot Friesian cows born at the experimental station. All the animals were non-lactating and non-pregnant mature females. They were subjected to the same management regimes 2 months before the trial. The approximate mean weights were 400 kg for the camels and 600 kg for the cows at the start of the experiment and did not vary significantly during the trial. The animals were treated for external and internal parasites using ivermectine (Ivomec N.D.) and were clinically healthy during the whole experiment.

2.2. Experimental procedure

During the whole trial, the animals were kept in individual boxes. The camels received a basal diet including 3 kg of wheat straw, 1.5 kg of rice meal and 1.5 kg of molasses. The composition of basal diet for cows was similar but the quantities were doubled to take into account the weight of the animals and their normal food intake. The diet was fed individually. There were no refusals at any time. The animals were watered ad libitum. The trace element composition of the water used was negligible. The diet was considered to satisfy the maintenance requirements for energy and crude protein of the animals from the two species.
After an adaptation period of 2 weeks to balance the mineral status of the animals, the experimental period (195 days) consisted of the following three phases.

1) A control period (days 1–21). During this stage, the animals received only the basal diet. They did not receive any mineral supplementation.

2) A supplementation period with mineral additives (days 22–112). A mineral mixture including 9.5 g copper sulphate and 44 g zinc sulphate was prepared. This additive corresponded to a daily supply for each animal of 240 mg of copper and 1 000 mg of zinc. These quantities were estimated to be double the requirements usually proposed for cows [17] and suggested for camels [10]. The mineral supplementation was mixed with the molasses, then with the rice meal and distributed individually each morning. The copper and zinc composition of the mineral supplement has been verified.

3) Post-supplementation period (days 113–195). During this last period of the experiment, the supplementation was discontinued.

2.3. Blood sampling procedure

Blood was collected from the jugular vein with vacutainer tubes containing anticoagulant (Heparin N.D.), free of copper and zinc salts. The blood was centrifuged immediately and the plasma removed. The samples were identified and kept frozen at −20 °C until analysis was performed. Blood sampling was carried out in the morning before feeding.

During the first stage of the trial, blood sampling was performed on days 1, 7 and 17. Twelve samplings were carried out during the course of the supplementation stage on days 24, 31, 38, 48, 55, 62, 69, 80, 87, 94, 101 and 108. In the post-supplementation period, blood sampling was performed once a week during the first month (days 115, 122, 129, 136), then once every 2 weeks until the end of the trial (days 150, 164, 178, 195).

2.4. Laboratory analysis

In each component of the basal diet, copper and zinc were measured by atomic absorption spectrophotometry according to the Bellanger method (1971) [1]. Plasma copper and zinc concentrations were determined by the method of Bellanger and Lamand (1975) using an atomic absorption spectrophotometer [2]. The accuracy of copper and zinc determinations for feed was assured using two reference materials: meal (NIST 1567a from the National Institute of Standard and Technology) and milk (IAEA A11 from the International Atomic Energy Agency). For plasma, seronorm trace elements (ref. 5337, NYCOMED AS., Pharma diagnostics, Oslo, Norway) were used. Fifteen replicate assays of these reference materials were used and the precision was below 5%.

The role of superoxide dismutase is to accelerate the dismutation of the toxic superoxide radical (O2•−), produced during oxidative energy processes, to hydrogen peroxide and molecular oxygen. The analytical method used employed xanthine and xanthine oxidase to generate superoxide radicals which reacted with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. The SOD activity was then measured by the degree of inhibition of this reaction. The blood sample was centrifuged at 3 000 g for 15 min and the plasma discarded. The erythrocytes were then washed four times with 3 mL of 0.9% NaCl solution and centrifuged after each wash. The washed centrifuged erythrocytes were made up to 2 mL with cold redistilled water and the lysate diluted with 0.01 M phosphate buffer pH 7. A 50-fold dilution of lysate was recommended for bovine samples. Similar dilutions were proposed for camel samples. Each prepared sample was mixed with xanthine oxidase and the final absorbance was read after 3 min. Haemoglobin concentration was performed by colorimetry (Boehringer Mannheim, ref. 124 729). The SOD activity was expressed in international units per 100 g of haemoglobin (IU/100 g Hb). The precision of the results was controlled using a RAN-SOD control (ref. CAT.NO.SD 126, Randox laboratories, Crumlin, North Ireland).

2.5. Statistical analysis

Variance analysis was carried out using SYSTAT software. For each variable, the effect of the species (two levels: cow or camel), the mineral supplementation period (three levels: before, during and after) and the day of sampling (23 levels) were tested. Previously, normality of distribution and the interactions between time and animals were tested. Correlations between two variables were studied using the Spearman
method. The multiple comparison test of the linear regression model was used to compare camels to cows.

3. RESULTS

3.1. Mineral composition of the basal diet and trace element intake

The mineral analysis of dietary components is given in Table I, and shows that the basal diet was low in copper and zinc.

In cows, the copper intake was 4-fold during the supplementation period, while in camels, this value was almost eight times higher. Similar proportional increases were observed for zinc (Table I).

3.2. Plasma copper and zinc values

The mean plasma copper concentration was significantly lower in the camel (Figure 1). During the three stages of the experiment, the mean copper values were 106, 111 and 113 μg/100 mL, respectively, for cows. Camels presented lower values but with similar evolution: 44, 63 and 67 μg/100 mL. In the camel, the plasma concentration of copper increased significantly ($P < 0.01$) during the supplementation stage. In the cow, there was no significant variation in the plasma concentration over the same period.

Similar to the copper results, the mean value of the zinc concentration was significantly lower in the camels than in the cows. During the three stages of the experiment, the plasma zinc concentrations were 73, 84 and 87 μg/100 mL for cows and 35, 36 and 42 μg/100 mL for camels (Figure 2).

In two cases (plasma zinc or copper), a strong species effect ($P < 0.001$) was observed. The day of sampling was observed to have no effect. A significant effect of the experimental stage was observed for copper only ($P < 0.005$). The ratio cow/camel for copper values had opposite changes to those of zinc.

3.3. SOD activity

On the whole, the SOD activity was significantly higher in the cows than in the camels (Figure 3). During the three stages of the trial, the mean cow SOD activity was 2,254 ± 205 IU/100 gHb in period 1, 2,420 ± 193 IU/100 gHb in period 2 and 2,436 ± 237 IU/100 gHb in period 3. The camel SOD activity was 1,474 ± 252, 1,720 ± 332 and 1,813 ± 352 for the three periods, respectively.

### Table I. Mineral content of the dietary ingredients (mg/kg DM).

<table>
<thead>
<tr>
<th></th>
<th>Straw</th>
<th>Rice meal</th>
<th>Molasses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper</td>
<td>2.9</td>
<td>6.8</td>
<td>17.1</td>
</tr>
<tr>
<td>Zinc</td>
<td>21.3</td>
<td>53.6</td>
<td>14.2</td>
</tr>
</tbody>
</table>

### Table II. Mineral dietary intakes (mg/animal/day).

<table>
<thead>
<tr>
<th>Period</th>
<th>Cows</th>
<th>Camels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Copper</td>
<td>Zinc</td>
</tr>
<tr>
<td>Control</td>
<td>70</td>
<td>289</td>
</tr>
<tr>
<td>Supplementation</td>
<td>310</td>
<td>1289</td>
</tr>
<tr>
<td>Post-supplementation</td>
<td>70</td>
<td>289</td>
</tr>
</tbody>
</table>
Figure 1. Effect of the mineral supplementation on the plasma copper concentration (mean ± SD) in camels and cows. (Significant effect of time compared to the control period: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.)

Figure 2. Effect of the mineral supplementation on the plasma zinc concentration (mean ± SD) in camels and cows. (Significant effect of time compared to the control period: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.)
In both species, the SOD activity slightly increased when copper–zinc supplementation occurred, but the difference was only significant ($P < 0.05$) in the cows between periods 2 and 1. During the depletion period, the mean SOD activity continued to increase but only slightly significantly ($P < 0.05$). The camel SOD activity was characterized by a very high individual variability.

### 3.4. Relationship between copper–zinc plasma and SOD activity

There were no correlations between the plasma copper levels and SOD activity, both in camels and in cows. A positive relationship was observed between cow plasma zinc concentration and cow SOD activity. The correlation was low but significant ($r = 0.396, P < 0.05$). Surprisingly, this relationship was reversed in camel ($r = -0.369, P < 0.05$). The biological relationships between zinc status and SOD activity were revealed to be quite different between the two species.

### 4. DISCUSSION

#### 4.1. Plasma copper concentration

In the present study, the plasma copper concentration was higher in the cow than in the camel which was in contrast to previous observations reported by the review of Faye and Bengouni [7]. Indeed, these authors reported camel copper plasma values between 83 and 107 µg/100 mL and cow copper plasma values between 64 and 83 µg/100 mL in comparative field studies. In our study, plasma copper level for the camels were generally under 70 µg/100 mL. Such values were above the deficiency threshold admitted for cows [16,17]. In the previous studies performed in field conditions, the diet for the camels was quite different than for the cows. In contrast, the feeding conditions were similar in the present study. This study showed that the camel can maintain a copper plasma concentration at a lower level than the bovine. Thus, the
difference observed between the cow and the camel in our experiment could be attributed to a physiological difference between species. In fact, camels store less copper in the liver than cows [8, 10]. The camels seem to show a better use of copper intake during the post-supplementation period by increasing the absorption and slowing the liver release [8]. This mechanism of regulation shows a latent period, similar to that observed in cases of dehydration [3].

4.2. Plasma zinc concentration

As with copper, the plasma zinc concentration was 2-fold greater in cows than in camels. Bovine values were in the normal data range (70–120 μg/100 mL) [16]. Present results concerning camels confirm recent observations in Djibouti [10], Morocco [5] and France [11]. Camels appear to have a normal lower level of the plasma zinc concentration and a deficient threshold below 40 μg/100 mL has been suggested [8]. Indeed zinc supplementation did not increase the plasma zinc concentration, in comparison to the cow, which can be explained by the higher fecal zinc excretion in camels [8].

4.3. Relationships between copper and SOD activity

Although erythrocyte superoxide dismutase activity was detected, the extracellular activity level is a less efficient biomarker for copper or zinc status [19]. The activity of erythrocyte SOD of domestic animals is about the same as or higher than that in man [16]. Our results showed the camel SOD activity nearer that of the human [20] than the bovine [16].

A depressed SOD activity is considered to indicate a severe and prolonged deficiency of copper or an inflammation [12, 18, 21]. However, the functional significance of these observations is not clear. Panemangalore and Bebe [20] observed a significant linear relationship between dietary copper levels and SOD activity. However, in humans, a relationship between the SOD activity and serum copper was not observed in the case of hypertension [26].

The lack of correlation between copper or zinc and SOD cannot be attributed to the short supplementation period. Several authors have shown that copper supplementation of copper depleted humans for 3 or 4 weeks can restore SOD activity to normal [20]. However, a longer duration of oral copper supplementation may be needed to effectively increase erythrocyte SOD activity. In our study, the depleted period occurred after 3 months of mineral supplementation. Plasma copper level was maintained after cessation of supplementation by the mobilization of copper stored in the liver during the previous period [8]. The variability of plasma copper was probably not sufficient in both species to observe a significant change in SOD activity. Elsewhere, a possible negative interaction between copper and zinc could act in the erythrocyte [23] as was observed for intestinal absorption in both cows [25] and camels [5].

4.4. Relationships between zinc and SOD activity

A moderately high zinc diet has been shown to slightly increase SOD activity in female rats given a normal copper diet [20]. High dietary zinc level could reduce the copper plasma concentration and thus alter the erythrocyte enzyme activity. Co-existing ionic copper level in the erythrocytes inhibits the binding of zinc to SOD. Elsewhere, ionic copper can be transferred to both copper- and zinc-binding sites of SOD efficiently while ionic zinc is only transferred to the zinc-binding site at a comparable efficiency [23]. Complex relationships therefore seem to exist between copper, zinc and SOD.
activity. In our study, the supplemented diet included both copper and zinc. The camel was characterized both by a high individual variability in SOD activity values and a negative relationship with plasma zinc concentrations, which was opposite to what we observed in the cows. In camels, the zinc plasma levels did not change when the copper plasma levels increased. Since the competition between copper and zinc on SOD binding sites is favourable to copper, the relationship between zinc and SOD will tend to be reduced. In cows, zinc and copper plasma concentrations tended to increase simultaneously. Thus, the competition between copper and zinc on SOD binding sites was less efficient. However, the interaction between copper and zinc could partly explain the weak relationship with cow SOD activity.

It was noteworthy that the plasma zinc concentration in the camel was not influenced by the dietary zinc concentrations. This lack of variation in camel plasma zinc levels was previously observed in different field or experimental conditions [3, 4, 10]. It appears that plasma zinc concentrations in the camel are not a good indicator of dietary zinc status or SOD activity.

5. CONCLUSION

Our results show that the camel differs from the cow with regard to copper and zinc metabolism. The results clearly showed a different biochemical response between the two species. Similar observations have been made concerning the relationships between copper and ceruloplasmin [9] or between selenium and glutathione-peroxidase [6]. Zinc requirements in the camel are probably lower than in the cows. A low plasma zinc concentration does not affect their SOD activity. These results tend to indicate that the camel is able to assume its intracellular enzymatic function in spite of a reduced copper and zinc concentration in the diet.

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