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SERUM COPPER LEVELS IN DROMEDARIES AFTER LONG TERM EXOGENOUS COPPER SUPPLEMENTATION

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ABSTRACT

Thirteen dromedary calves (6 to 12 month old) and three 2 year old females were kept at CVRL for a copper supplementation experiment. The animals received a daily dose of 50 mg/animal of copper over a period of 42 days. After this period, the dose was doubled to 100 mg copper/ animal per day for another 57 days. Blood samples were collected on different days and analysed for copper using the UNICAM 969 Flame Atomic Absorption Spectrometer (AAS) at 324.8 nm. Before starting supplementation, 4 out of 16 camels (25%) possessed normal basal serum copper concentration (above 60 µg/dl) and 12 animals (75%) showed copper deficiency (less than 60 µg/dl). Neither 50 nor 100 mg/day of additional copper supplementation had any significant effect in camels with normal serum levels. However, in copper deficient camels, the lower dose (50 mg/day) induced already a slight, temporary increase in serum copper concentration. However, the higher dose significantly elevated the serum copper concentration to the level of reference values.

Keywords : AAS, copper values, dromedary, exogenous supplementation

On the Arabian Peninsula, copper deficiency in livestock is very common and contributes to a range of diseases (Ivan *et al*, 1990). It occurs both in New World and Old World camelids (Abu Damir, 1998; Faye and Grillet, 1984; Faye *et al*, 1986; Wensvoort, 1992).

Physiological copper levels in camel plasma or sera are reported by several authors (Abdel-Moty *et al,* 1968; Tartour, 1975; Whabi *et al*, 1980; Hussein *et al*, 1982; Faye *et al,* 1986; Abdalla *et al,* 1988; Faye and Mulato, 1991; Zong-Ping *et al*, 1994) and are comparable to those in sheep, goats and cattle. A serum copper value of 70.4 ± 10.8 µg/dl has been observed in 2100 UAE racing dromedaries (Wernery *et al*, 2002a). Wernery *et al* (2002a) consider copper values of 70-120 µg/dl in dromedary plasma or serum as normal. Values below 60 µg/dl indicate a copper deficiency (Abu Damir, 1998).

A secondary copper deficiency with copper blood values as low as 0.28 ± 1.7 µg/g (28 µg/dl) was described by Zong-Ping *et al* (1994) in Bactrian camels in Gansu province, China due to high molybdenum content in soils and fodder. The camels suffered hypochromic microcytic anaemia, swayback, emaciation, limb deformities and fractures. Wernery *et al* (2002a) found very low liver and kidney copper mean values of 12.43 mg/kg wet weight and 2.74 mg/kg wet weight, respectively in 10 necropsied dromedary calves. These neonates did not develop any anaemias, sway backs, leg deformities or paralysis as it was described by Wensvoort (1992). However, these calves suffered from secondary immunodeficiency due to failure of passive transfer (Barrington *et al*, 1999; Walker and Tibary, 1999; Wernery and Kaaden, 2002) of maternal immunoglobulins to the neonate leading to ill thrift and septicaemia.

To increase the copper levels in deficient animals, extra supply of copper is necessary. In a copper feeding trial with 2 dromedary calves it took 5 months of daily application of 50 mg of copper to reach reference values (Wernery *et al*, 2002b).

The aim of this study was to investigate the effect of exogenous copper supplementation both to copper deficient and to copper normal camel calves.

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Materials and Methods

Thirteen dromedary calves (6 to 12 month old, 8 males and 5 females) and three 2 year old females were selected for this study. Calves were kept in one group separated from their dams at the Central Veterinary Research Laboratory, Dubai, U.A.E. The calves had access to their mothers for suckling two times a day for half an hour, and were fed hay and concentrate two times a day. The 2 year old female camels were kept together with the calves and had no access to their dams. Camels also had free access to salt blocks (Frank Wright, UK, copper 400mg/kg, manganese 200 mg/kg, zinc 120 mg/kg, cobalt 124 mg/kg, iodine 190 mg/kg, selenium 10 mg/kg, sodium 36 %, calcium 1.3 %, phosphorus 0.23 %, magnesium 0.3 %, Vitamin D3 40,000 IU/kg) before and throughout the entire study.

The experimental design of this study is summarised in Table 1. At the beginning of the study (day 0), blood samples were collected from all animals to determine basal (pre-treatment) serum copper concentration. For 42 days, camels were then fed daily 50 mg/animal of copper in the form of copper-trition (Equine products, UK). One scoop of copper-trition contains 50 mg copper, 10 mg zinc, 5 mg manganese and 1.25 mg selenium. After this period, camels were given double dose, 100 mg of copper daily for further 57 days. In order to avoid peak values after immediate absorption from the gastrointestinal tract, copper supplementation was suspended 2 days before sample collection.

Blood samples were collected from the jugular vein on different days to monitor changes in serum copper levels. Blood was centrifuged shortly after collection for 10 minutes at 3000 rpm and the serum stored at -20°C until tested. Copper levels were measured using the UNICAM (Cambridge, UK) 969 Flame Atomic Absorption Spectrometer (AAS) at 324.8 nm as described by Wernery *et al* (2002a).

For the statistical analysis, camels were grouped according to pre-treatment (basal) serum copper concentration. Camels with low levels (< 60 µg/dl) were put to group 1 (12 camels) and camels with normal levels (> 60 µg/dl) were put to group 2 (4 camels). To test the effect of treatment, analysis of variance was performed on serum copper levels. The group and the time of blood sampling were the 2 main factors in the model. The difference between blood sampling periods within each group was tested with Duncan multiple range test at $P<0.05$ level. T-test was used to compare mean serum copper concentration of the groups at different blood samplings.

Results

At the start of the study, four out of 16 camels (25 %) possessed normal serum copper concentration of 73.3 ± 5.79 µg/dl, and 12 animals (75 %) had serum copper levels of 29.9 ± 5.41 µg/dl indicating copper deficiency. Analysis of variance showed a significant effect of both factors, pre-treatment serum copper concentration ($P<0.01$) and time of blood sampling ($P<0.001$) on serum copper levels. There was also a significant interaction ($P<0.01$) between the two factors. In case of group 2 (camels with normal serum levels at the beginning of the study), additional copper supplementation of 50 or 100 mg/day did not increase the serum copper concentration ($P=0.261$, Table 2, Fig 1). However, in copper deficient camels (group 1), the lower dose of 50 mg/day induced a slight and temporary increase in serum copper concentration. After 42 days of administration of 50 mg/day, serum levels were not significantly higher than before treatment (Table 2, Fig 1). The higher dose of 100 mg/day significantly elevated serum copper concentration and the values reached reference levels. Even one hundred days after the end of copper treatment,

Table 1. The experimental design of the copper supplementation study in dromedaries.

Days of the study	Events of the study
Day 0	Blood sampling
Days 3 to 15	50 mg copper supplementation daily
Day 17	Blood sampling
Days 18 to 46	50 mg copper supplementation daily
Day 48	Blood sampling
Days 49 to 80	100 mg copper supplementation daily
Day 82	Blood sampling
Days 83 to 107	100 mg copper supplementation daily
Day 109	Blood sampling
Day 116	Blood sampling
Day 137	Blood sampling
Day 172	Blood sampling
Day 207	Blood sampling

Table 2. Changes in serum copper concentration after two doses of 50 and 100 mg copper/day supple-mentation over a period of 97 days in dromedaries.

Days of the study	Low (< 60 µg/dl)***	Normal (> 60 µg/dl)***
0	27.9 ± 5.41[a]	73.3 ± 5.79
17*	46.8 ± 5.52[bc]	75.5 ± 4.52
48*	41.0 ± 3.10[ab]	55.3 ± 3.47
82**	73.3 ± 3.57[de]	75.0 ± 6.86
109**	57.5 ± 3.02[cd]	65.5 ± 2.22
116	67.0 ± 2.34[d]	69.0 ± 5.03
137	84.6 ± 6.17[e]	77.8 ± 11.91
172	59.8 ± 7.18[cd]	49.5 ± 12.72
207	60.9 ± 8.25[cd]	58.5 ± 15.12

Different superscripts in the same column mean significant difference at P<0.05 level.
* 50 mg copper daily for 42 days
** 100 mg copper daily for 57 days
*** Pre-treatment serum copper levels (mean ± SEM)

serum concentration still remained elevated in the reference range and did not return to pre-treatment levels (Table 2, Fig 1).

Discussion

This copper feeding trial was initiated to study the effect of copper supplementation in both, deficient and normal camel calves. Before the experiment, 4 out of 16 camels (25%) had normal basal serum copper concentration and 12 animals (75%) showed copper deficiency. Even so, both groups received the same amount of copper of 50 or 100 mg daily, serum copper levels only increased in the group with low copper serum levels. Copper supplementation of 50 or 100 mg/day had no significant effect in camels with normal serum levels. However, in copper deficient camels, the lower dose of 50 mg/day induced a slight, temporary increase in serum copper concentration, but only the higher dose of 100 mg/day elevated significantly the serum copper concentration to the reference range. Wernery *et al* (2002b) demonstrated in a copper feeding experiment that it took 5 months of treatment with 50 mg/day to reach copper reference values in 2 camel calves. In our study, the same dose given for 42 days only, did not

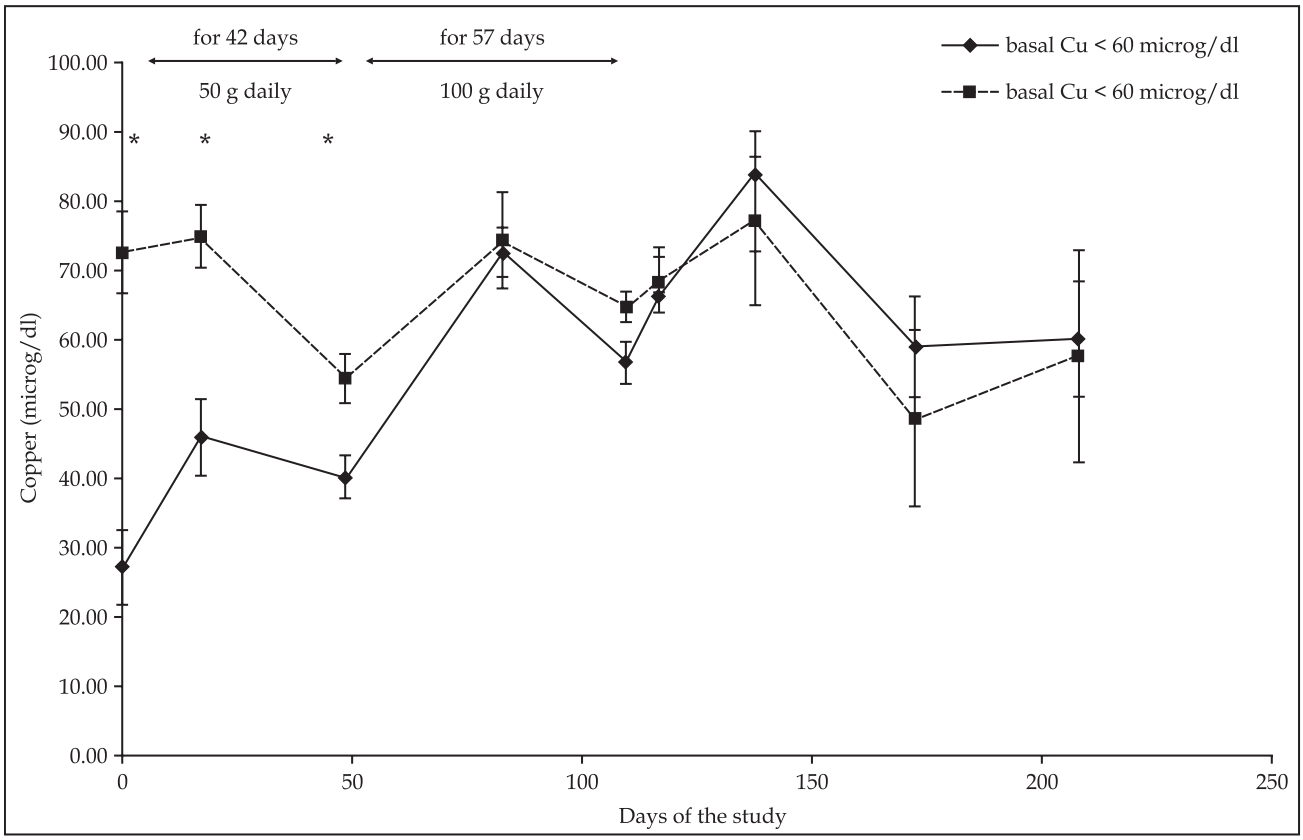

Fig 1. Mean (± SEM) serum copper concentration after long term oral copper administration in dromedary camels with low and normal pre-treatment serum values. 50 mg/animal and 100 mg/animal of copper were given daily for 42 and 57 days, respectively. Arrows indicate the period of copper supplementation. * indicates significant difference at P<0.05 level between the 2 groups at a given time.

significantly increase serum copper levels in deficient animals.

From our experiment it can be concluded, that 100 mg/day of copper supplementation is necessary to increase low copper levels to reference values in 4 to 6 weeks. These values remain in the normal range for at least 3 month after supplementation. However, in camel calves with normal copper serum values, copper supplementation had no increasing effect on serum copper levels and no toxic effect on the animals. For this reason, supplementation of such animals is not necessary, also from the economical point of view.

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