Antioxidant status and immune responses of growing camels supplemented a long-acting multi-trace minerals rumen bolus

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ABSTRACT

The study was conducted to evaluate the effects of a long-acting trance mineral rumen bolus supplementation on enzymatic and haematological profiles, antioxidant status and immune responses of growing camels under natural grazing conditions. Fifteen 6-month-old growing male camels were used in a 150-day trial. Animals were individually housed in a shaded pen and randomly assigned to receive 0 (CON), one (TMB1) or two (TMB2) long-acting trance mineral rumen bolus. Blood samples were collected from all camels on days 1, 30, 60, 90, 120 and 150 to measure enzymatic concentrations in serum, total antioxidant capacity (TAC) in plasma and haematological variables in whole blood. Camels were injected intradermally with 0.25 mg phytohaemagglutinin (PHA) on days 90 and 140, and then the cell-mediated immune response to this antigen was measured at 0 and 24 h after injection. Animals were immunised intravenously on days 90 and 105 with 2 ml suspension of sheep red blood cells to measure total antibody titres in serum. Using TMB supplement (1 or 2 boluses) resulted in an increase in the plasma concentration of TAC ($p < 0.04$) and improvement in immune responses in terms of increased skinfold thickness after 24 h of PHA injection ($p < 0.01$) and total primary serum antibody titres ($p < 0.04$). Different levels, sources, and synergistic combinations of trace minerals can be used in further studies to elucidate the most advantageous regarding productive variables, availability and cost for camel industry.

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Introduction

Camels (Camelus dromedarius) are an important livestock resource in many parts of the world (e.g. Middle East, Africa, and Asia,) and there has been recently a great attention on camel industry based on milk, meat, leather and by-products. The majority of camels in these regions are usually reared on natural grazing conditions that differed on the quality and quantity of diets. However, the nutrient requirements, particularly micronutrients, of grazing animals are often not met, for at least part of the year, under such these conditions. For example, grazing animals have a high risk for the deficiencies of one or more of trace minerals, e.g. zinc (Zn), selenium (Se), copper (Cu) and cobalt (Co), which can cause reductions in growth rate and productivity, as well as an increased susceptibility to various diseases and a higher mortality rate (Khan et al. 2006; Soetan et al. 2010; Herdit & Hoff 2011).

Trace minerals, as essential micronutrients, play critical roles in the regulation of numerous biological functions (e.g. metabolic processes, production, reproduction and immunity) in the body (Underwood & Suttle 2001; McDowell 2003; Spears 2003). Adequate trace minerals status is an important application for both growth and health status of farm animals. Moreover, a long-acting trace mineral rumen bolus (TMB) is reliable and is the most effective administration methods that can provide a variety of trace minerals over a long-term period (Sprinkle et al. 2006; Grace & Knowles 2012). Seboussi et al. (2008) concluded that serum Se and glutathione peroxidase (GPx) increased in response to supplementation of different levels of Se in female camels. Kosanovic et al. (2014) reported that supplementation with trace minerals including Cu, Co, Zn and Se had positive effect on red blood cells count and haemoglobin in racing camels. Husakova et al. (2014) concluded that determination of Se in blood of alpaca is limited and recommended determination of Se as well as GPx in this species. Osman (2012) observed Cu deficiency in Omani camels
maintained on diet containing dates and fresh alfalfa. Athamna et al. (2012) suggested that the status of some essential trace elements such as Se and Cu are linked with the composition of the diet and the enrichment of the basal diet with supplemental agent may improve the health status (Kosanovic et al. 2014).

However, the effects of using TMB on antioxidant status and immune response in growing camels are largely unknown. The objective of this study was to evaluate the effects of using TMB supplements in camels under grazing conditions.

Materials and methods

Animals and experimental design

The study was undertaken at the Experimental Farm Animal Centre, Department of Animal Production, King Saud University, Riyadh, Saudi Arabia (24°42'N and 46°44'E), and all procedures followed the Implementing Regulations of the Law of Ethics of Research on Living Creatures (Saudi Arabia National Committee of Bio Ethics) with the approval of the King Saud University Animal Ethics Committee.

Fifteen health growing male Al-Mejaheem camels (Camelus dromedaries; average bodyweight 139.51 ± 26.49 kg; 6–8 months old; same body condition scores) were purchased from the local livestock market and then transported to the Experimental Station of Animal Production Department, College of Food and Agriculture Sciences, King Saud University, Riyadh. On the arrived day (30 days before study commenced), animals were immediately weighed, ear tagged, vaccinated against clostridial diseases and treated for internal and external parasites. Camels were housed individually in pens (approximately 3.0 m long × 2.5 m wide) for 150 days. Each pen was fitted with a feed trough and a 30 L plastic water bucket. Camels were given a 4-week adaptation period in the pens immediately prior to the beginning of the study.

At the beginning of the experiment (day 1), camels were randomly assigned to one of three treatments (five animals in each treatment on the basis of weight), received 0 (CON; control group; average bodyweight 134.68 kg), one (TMB1; average bodyweight 132.96 kg) or two (TMB2; average bodyweight 150.90 kg) long-acting multi-trace minerals rumen bolus (TMB; smALL-Trace Boluses for cattle, Agrimin Ltd., North Lincolnshire, UK), containing six trace minerals. The active compositions of the TMB used contained: Zn (104.28 mg/g; ZnSO4 and Zn2O), Se (1.085 mg/g; Na2SeO3), Cu (118 mg/g; Cu2O), Co (2.041 mg/g; CoCO3), I (4.893 mg/g; KI) and Mn (71.955 mg/g MnSO4). This product was designed to slowly release daily requirements of these micronutrients for approximately 180 days.

All camels were offered the same basal diet consisting mainly of chopped alfalfa hay (Medicago sativa) and barley, containing 0.78 Mcal of NE, 15.12% CP/kg, 21.86 mg of Zn/kg, 0.03 mg Se/kg, 5.08 mg Cu/kg, 0.08 mg Co/kg and 31.17 mg Mn/kg (DM basis; Table 1), at maintenance level (2.5% of the initial bodyweight) twice daily at 0800 h and 1500 h. This feeding protocol was applied to simulate the feeding system used when animals are under grazing system in Saudi Arabia. Water was available ad libitum.

Sample processing and analysis

Feed analyses

The basal diet was analysed for nutrient composition, including DM, crude protein, neutral detergent fibre, acid detergent fibre and trace mineral contents using AOAC (1990) procedures. Dry matter content was determined by drying samples in an oven at 100 °C for 24 h, and ash content was determined by incinerating samples at 550 °C for 3 h in a muffle furnace. Crude protein content was measured following the Kjeldahl method. Neutral detergent fibre and acid detergent fibre were determined according to methods described by Van Soest et al. (1991) and the AOAC (method No. 973.18 C, 1990), respectively. An inductively coupled plasma mass spectrometry (Vista-PRO CCD; Varian Inc., Walnut Creek, CA) was used to measure the concentrations of trace minerals (Zn, Co, Cu, Se and Mn) in the diet.

Blood sampling and enzymatic, haematological and antioxidant profiles

Blood samples were collected by jugular venipuncture from all the camels before the morning feeding.
(0700 h) on days 1, 30, 60, 90, 120 and 150. Three 10-mL aliquots were taken into Vacutainer tubes (BD Company, Franklin Lakes, NJ), one containing K$_2$-EDTA for whole blood, one containing lithium heparin for plasma, and one without additives for serum. After collection, samples for serum were allowed to clot for at least 2 h at room temperature (25°C), whereas samples for whole blood and plasma were stored on ice for approximately 3 h. Serum and plasma were obtained by centrifugation at 2400 × g for 15 min at 4°C and then frozen at −20°C until analysis. Whole blood samples in K$_2$-EDTA tubes were processed immediately (within 3 h of collection). Serum concentrations of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (ALT), and creatine kinase (CK) were analysed using commercial kits (Randox Laboratories, Crumlin, UK) and determined on a semi-automated analyzer (RX Monza; Randox Laboratories, Crumlin, UK) according to the manufacturer’s instructions. Plasma total antioxidant status was measured using a Trolox-equivalent Antioxidant Capacity kit (TAC; Assay # 709001, Cayman Chemical, Ann Arbor, MI), and determined on a microplate reader (Multiskan EX, Thermo Fisher Scientific, Waltham, MA) according to the manufacturers’ procedures, and the results are expressed in mM Trolox per litre of plasma. Blood samples without the anticoagulant were analysed for numbers of total and differential leukocytes, white blood cells (WBC), total red blood cells (RBC), neutrophils (N), lymphocytes (L), monocytes (M) and platelets, as well as neutrophil-to-lymphocyte ratio, haemoglobin level, haematocrit, mean cell volume (MCV), mean corpuscular haemoglobin value (MCH) and mean corpuscular haemoglobin concentration (MCHC) using a haematology analyser (Cell-Dyn 3000 SL; Abbott, Abbott Park, IL) according to the manufacturer’s instructions.

**Immunocompetence evaluations**

**Cell-mediated immune response**

Camels were a skin-tested using phytohaemagglutinin (PHA; Sigma Chemical, St. Louis, MO) on days 90 and 140 to induce non-specific delayed-type hypersensitivity and to evaluate the cell-mediated immune response to PHA injection. For each animal, 0.2 mg of PHA was dissolved in 1 mL of pH 7.2 sterile phosphate-buffered saline (PBS; Gibco/Invitrogen, Auckland, NZ) and then injected interdermally into a shaved marked area of skin on the upper part of the left shoulder and an equal amount of PBS was also injected at the equivalent location on the contralateral shoulder for comparison. Double skinfold thickness at each shoulder was measured using an electronic digital caliper (Siechert and Wood, Inc, Pasadena, CA) immediately before and 24 h after the injection. The increases in double skinfold thickness 24 h after PHA injection (PHA$_{24}$ minus PHA$_{0}$) and PBS injection (PBS$_{24}$ minus PBS$_{0}$) were calculated.

**Humoral immune response**

Camels were immunized intravenously on d 90 and 105 with 2 ml suspension of sheep red blood cells (SRBC, 20%) to measure the humoral response. Serum total antibody titres were determined prior to immunization and at weekly intervals thereafter using haemagglutination method according to Witlin (1967).

**Statistical analysis**

All the data were analysed using repeated measures and the Proc Mixed model (SAS Institute Inc., Cary, NC). Treatment (number of blouses received), camels within treatment, day of measurement, and the interaction treatment × day of measurement, with the supplementary treatment as the main effect. Camel within treatment was a random variable and the error term for the main effect. Data are presented in tables and figures as the least square mean (day 30, 60, 90, 120 and 150) with standard error (± SE), and differences were considered significant at $p < 0.05$.

**Results**

**Feed trace mineral concentrations**

The chemical composition, including trace mineral concentrations, of the basal diet used in the experiment is presented in Table 1. Concentrations of Zn, Cu and Se in the diets consisting mainly of chopped alfalfa hay and barley were nearly 22, 5 and 0.03 mg/kg DM, respectively, which are equivalent to moderate deficient in most farm animals (less than 25 ppm for Zn, 5.2 ppm for Cu and 0.1 ppm for Se; NRC, 1996; 2000). Conversely, the diets used, particularly chopped alfalfa hay, appeared appropriate source to meet nutritional requirements of Mn, I and Co, which concentrations of these trace minerals in the diets were above the thresholds for their deficiencies (NRC, 2000).

**Serum enzymatic and blood haematological variables**

Differences between treatments in the concentrations of serum enzymatic and blood haematological variables measured in the study are presented in Tables 2
Table 2. Effects of long-acting trace mineral rumen bolus supplement on serum concentrations of enzymatic variables in growing camels.

| Analyte, unit | CON   | TMB1  | TMB2  | SE    | p value |
|---------------|-------|-------|-------|-------|---------|
| ALP, U/L      | 244.50| 232.97| 225.33| 20.33 | 0.76    |
| ALT, U/L      | 20.09 | 20.99 | 21.34 | 1.71  | 0.19    |
| AST, µL       | 236.77| 201.94| 221.01| 35.01 | 0.07    |
| CK, U/L       | 69.20 | 77.12 | 74.89 | 7.22  | 0.15    |

ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; CK: creatine kinase.

*Values are means at days 30, 60, 90, 120 and 150 for growing camels (n = 15) subjected to grazing condition for 150 days and received 0 (CON), 1 (TMB1) or 2 (TMB2) of a long-acting trace mineral rumen bolus. Blood were collected on days 1, 30, 60, 90, 120 and 150.

Table 3. Effects of long-acting trace mineral rumen bolus supplement on haematological variables in growing camels.

| Variable | CON   | TMB1  | TMB2  | SE    | p value |
|----------|-------|-------|-------|-------|---------|
| WBC, × 10^9/L | 14.66 | 16.32 | 14.46 | 2.12  | 0.06    |
| N, × 10^9/L    | 6.73b | 8.17a | 7.13b | 1.54  | 0.03ab  |
| L, × 10^9/L    | 6.02  | 7.26  | 6.43  | 0.69  | 0.20    |
| M, × 10^9/L    | 0.81  | 0.86  | 0.84  | 0.13  | 0.88    |
| N/L ratio     | 1.12  | 1.13  | 1.09  | 0.04  | 0.32    |
| RBC, × 10^12/L | 9.19  | 10.99a| 11.21a| 0.67  | 0.001abc|
| Hemoglobin, g/L| 125.6 | 130.5 | 128.9 | 13.3  | 0.61    |
| Hematocrit, % | 26.31 | 26.56 | 26.69 | 0.71  | 0.89    |
| MCV, fl       | 25.50 | 24.83 | 25.73 | 0.97  | 0.47    |
| MCH, pg        | 12.09 | 12.22 | 12.37 | 0.71  | 0.53    |
| MCHC, g/L      | 47.64 | 49.15 | 48.27 | 2.65  | 0.07d   |
| Platelets, × 10^12/L | 202.75 | 205.94 | 213.00 | 18.27 | 0.59    |

L: lymphocytes; M: monocytes; MCH: mean corpuscular hemoglobin value; MCHC: mean corpuscular hemoglobin concentration; MCV: mean cell volume; N: neutrophils; RBC: red blood cell count; WBC: white blood cell count.

Within a row, means without a common superscript differ (p < 0.05).

*Values are means at days 30, 60, 90, 120 and 150 for growing camels (n = 15) subjected to grazing condition for 150 days and received 0 (CON), 1 (TMB1) or 2 (TMB2) of a long-acting trace mineral rumen bolus. Blood were collected in days 1, 30, 60, 90, 120 and 150.

Interaction of treatment × day of measurement (p < 0.05). *Treatment effect (p < 0.05).

Discussion

Trace minerals, as essential micronutrients, play critical roles in the regulation of numerous biological functions (e.g., metabolic processes, production, reproduction and immunity) in the body (Miller 1981; Underwood & Suttle 2001; McDowell 2003; Spears 2003). Under extensive grazing systems, ruminants have a high risk of marginal trace mineral deficiencies, causing a decreased productive performance of livestock and an increased susceptibility to a variety of diseases (McDowell 1996; Galyean et al. 1999). Supplementation with these micronutrients are widely practiced in the livestock industry to improve the productivity and wellbeing of livestock to alleviate trace mineral deficiencies and to increase profitability for producers. For example, supplementary TMB to growing camels resulted in an increase in the growth rate (14.4%) and an improvement in the feed efficiency (13.7%; Alhidary et al. 2016). TMB is reliable and the most effective supplementation method to provide a
range of trace minerals over a long-term period (up to 12 months) and reduce the labour cost of repeated mustering (Millar & Meads 1988; Sprinkle et al. 2006; Grace & Knowles 2012).

Evidence from numerous studies indicates that several trace minerals (including Zn, Cu, Se and Mg) play important roles in the regulation of the antioxidant-pro-oxidant balance in the body through their participations in several antioxidant enzymes and enzyme reactions (Underwood 1977; Beckett & Arthur 2005; Surai 2006; National Research Council 2007). Such enzymes, superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT), are required to trace minerals for scavenging reactive oxygen intermediates in vivo. For example, SOD requires Zn and Cu for normal enzyme activity (Valentine et al. 2005), and GPx activity is mainly dependent on the Se status of tissues (Surai 2006). A study conducted by Faixova

Figure 1. Mean (±SE) skinfold thickness (mm) in growing camels received either 0 (CON), 1 (TMB1) or 2 (TMB2) of a long-acting trace mineral rumen bolus, and injected intradermally with 0.2 ml of phytohaemaglutinin. Treatment effect ($p < 0.01$); measurement day effect ($p < 0.07$); Treatment $\times$ measurement day interaction ($p < 0.48$). Means within a day without a common letter (a, b) differ ($p < 0.05$).

Figure 2. Mean (±SE) serum titres of total antibody (log$_2$) of growing camels received either 0 (CON), 1 (TMB1) or 2 (TMB2) of a long-acting trace mineral rumen bolus, and injected with 2 ml of sheep red blood cell. Treatment effect ($p < 0.04$); measurement day effect ($p < 0.01$); Treatment $\times$ measurement day interaction ($p < 0.02$). Means within a day without a common letter (a, b) differ ($p < 0.05$).
et al. (2007) reported that the activity of blood GPx in lambs that received diets supplemented with 0.3 mg of Se per kg DM was greater than those in untreated lambs. Moreover, the inclusion of Zn supplementation as a soluble glass bolus (33 g of Zn) to extensively grazed lambs resulted in an increase in the serum activity of SOD when compared with untreated lambs. This suggests that the improved antioxidant status in plasma (increase in TAC) of camels receiving two boluses in the current study was likely caused by the beneficial effects of Zn, Cu and Se on antioxidant system.

Several trace minerals (including Zn, Cu, Se, Mn and Co) play critical roles in regulating the immune system of the body and have been widely used as immunostimulants, showing a variety of beneficial effects on the health status of both humans and domestic animals. Such these nutrients interact with all aspects of the immune function in a multiplicity of mechanisms, which, in turn, results in significant changes in functional indicators of both types (innate and adaptive) of the immune system (Underwood & Suttle 2001; McDowell 2003; Spears 2003; Surai 2006). These changes in immune function include activation and regulation of oxidative tissue damage and inflammation, inflammatory mediators, phagocytic cell proliferation (neutrophils and macrophages), specific immunity (humoral and cellular immunity), and T-cell interactions with B-cells and macrophages (Finch & Turner 1996; McDowell 2003; Spears & Weiss 2008). In the current study, the improvement of both humoral immune response and cell-mediated immunity by TMB supplementation confirmed these beneficial roles of multi trace minerals and is consistent with previous studies using multi-trace mineral rumen bolus or injection (Richeson & Kegley 2011; Bicalho et al. 2014).

**Conclusions**

The results from the present study corroborate and extend the results from previous studies on the beneficial roles of multi trace minerals (Zn, Co, Se, Mg, Cu and I) on immune functions and antioxidant status of farm animals under natural grazing conditions. Indeed, supplementing a long-acting trace mineral rumen bolus has important implications for the camel industry, in term of providing the essential trace minerals needed in a long-term period and more reliable in preventing trace mineral deficiencies. Furthermore, several of the protective effects of multi-trace minerals on immune responses observed in this study have not been previously reported and warrant further investigation. Therefore, further studies are required to define the effects of trace mineral supplementation and to quantify interactions between these nutrients and the responses of growing camels. The level, source, and synergistic combinations of trace mineral supplementation should be considered when determining the most beneficial effect for productive performance and the health of camels raised on extensive grazing systems.
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