

Influence of dietary chromium yeast supplementation on apparent trace elements metabolism in growing camel (*Camelus dromedarius*) reared under hot summer conditions

Ibrahim A. Alhidary¹ · M. A. Alsofi¹ · K. A. Abdoun¹ · E. M. Samara¹ · A. B. Okab¹ · A. A. Al-Haidary¹

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Abstract This study aimed to evaluate the effect of dietary chromium (Cr) supplementation on the apparent metabolism of some trace elements in camel calves reared under hot summer conditions. The study was conducted on a total of 15 male camel calves (5–6 months old) reared under hot summer conditions for 12 weeks. The animals were housed individually under shelter and divided into three dietary treatment groups (diets supplemented with 0.0, 0.5, or 1.0 mg Cr/kg DM), five animals each. At the end of the study, a metabolic trial was conducted on all camels for the evaluation of trace elements metabolism. Cr excretion, absorption, and retention showed an increasing trend with the increasing level of dietary Cr supplementation. Dietary Cr supplementation at 0.5 mg Cr/kg DM to camel calves resulted in a significant ($P < 0.05$) increase in Cu and an increasing trend in Zn and Mn excretion via urine and feces. However, Fe retention increased significantly ($P < 0.05$) in camel calves fed on diet supplemented with Cr. Dietary Cr supplementation to camel calves resulted in an increasing trend of plasma Cr concentration, while plasma concentration of Cu and Zn tended to decrease and without any effect on plasma Fe concentration. The results of the present study suggests that care should be taken for the negative interaction of Cr with the utilization of other trace elements, in cases where Cr is supplemented to the diet as a feed additive to promote growth and immunity under hot climatic conditions.

Keywords Camel · Chromium · Heat stress · Trace elements

✉ Ibrahim A. Alhidary
ialhidary@ksu.edu.sa

¹ Department of Animal Production, College of Food and Agriculture Sciences, King Saud University, Riyadh 11451, Saudi Arabia

Introduction

Dromedary camel (*Camelus dromedarius*) is one of the highly valuable domestic animals in Saudi Arabia with a population of 1.4 million (Saudi General Authority for Statistics 2015). Camels can produce more milk and meat and sustain production for a longer time than any other species of farm animal under extreme temperatures, drought, and poor pasture (Raziq et al. 2008). Chromium (Cr) has been suggested to alleviate stress-associated effects (Kumar et al. 2017), where Cr is added to the diet of animals to improve animal productivity and endocrine profile as well as to enhance immune competence (Abdoun et al. 2015; Kumar et al. 2017; Xu et al. 2017). In domestic animals, Cr has been recognized as an essential trace mineral for its ability to enhance insulin activity (Vincent and Bennett 2007) and reduce stress-associated effects (Abdoun et al. 2015; Kumar et al. 2017). Chromium has received great attention due to its beneficial impact on the biological functions and health in animal and human (Khan et al. 2014).

The absorption and utilization of Cr may be dependent on its status in the intestinal tract (Wang and Xu 2004), where organic Cr has greater biological availability than inorganic Cr (Lukaski 1999). Inorganic Cr is characterized by a very low absorption rate ranging from 0.5 to 2%, whereas the absorption rate of organic Cr is far better than that of inorganic Cr and ranges from 10 to 25% (Gralak 2002). Ingested Cr is excreted primarily in the urine (Hooth 2009). Chromium requirement of animals has not been defined; however, it is obvious that stress and disease conditions could increase the requirement of Cr (Squires 2010). Increased urinary losses of zinc (Zn) and copper (Cu) have been reported with marked transit stress and fasting (Orr et al. 1990). However, Cr supplementation has been reported to protect against the stress-induced losses of several trace elements (Schrauzer et al.

1986). Iron-binding proteins are involved in chromium binding, transport, and storage (Feng et al. 2003). Mineral intake at higher doses might have antagonist effects on the retention of other minerals (Khan et al. 2014). The knowledge pertaining to this antagonism regarding Cr is still in its infant stage. Therefore, it was the intention of this study to evaluate the effects of dietary Cr supplementation on apparent trace elements metabolism in camel calves reared under hot summer conditions.

Materials and methods

Study design

This study was conducted on 15 Al-Mejaheem male camel calves with an average body weight of 90.0 ± 5 kg and 5–6 month old. The study was executed during the hot summer months of Saudi Arabia (June to September) at the Animal Research Station affiliated to the Department of Animal Production, King Saud University, Riyadh, Saudi Arabia. Calves were housed individually in a shaded pen (3×3 m²) provided with a plastic water bucket and feed trough. Animals were offered alfalfa hay and basal concentrate diet for 4 weeks as an adaptation period prior to the commencement of the experiment. On arrival and during the adaptation period, camel calves were ear-tagged and treated with prophylaxis doses of antibiotics, anthelmintic, and multivitamins. At the beginning of the experiment, camel calves were assigned randomly into three equal groups, five animals each, and fed ad libitum on either basal commercial diet without Cr supplementation, commercial diet supplemented with 0.5 mg Cr/kg DM, or commercial diet supplemented with 1.0 mg Cr/kg diet DM for 12 weeks. All experimental diets were provided by ARASCO (WAFI, ARASCO, Riyadh, Saudi Arabia) and formulated to meet the nutritional requirements of growing camels (Table 1). Where, Cr was added to the experimental diets in the form of Cr yeast (0.1% Cr). At the termination of the first phase of the experiment (12 weeks), all camel calves were transferred to metabolic cages for a 12-day period (4 days adaptation period, followed by 8 days sample collection period) for conduction of the second phase of the experiment (metabolic trial). The camel calves in all experimental groups were exposed to the same care and management procedures throughout the study.

Data collection and analysis

Ambient temperature (T_a , °C) and relative humidity (RH%) were recorded throughout the study period at 30-min intervals using data logger (HOBO Pro Series, ONSET, Bourne, MA, USA). Temperature–humidity index (THI) was calculated according to the equation used by Marai et al. (2001):

Table 1 Nutrient composition of the basal diet used in the experiment

Items	Content
Nutrient composition, DM basis	
Dry matter (%)	91.04
Crude protein (%)	12.40
Crude fiber (%)	11.98
Ether extract (%)	2.61
Neutral detergent fiber (%)	41.95
Acid detergent fiber (%)	26.10
Ash (%)	9.09
Metabolizable energy (MJ/kg)	11.95
Calcium (%)	1.23
Phosphorus (%)	0.58
Sodium chloride (%)	0.76
Chromium (mg/kg)	5.00
Copper (mg/kg)	22.00
Iron (mg/kg)	140.71
Manganese (mg/kg)	136.00
Zinc (mg/kg)	137.00

$$\text{THI} = 0.8 \text{ dry bulb temperature (dbT)}$$

$$+ \% \text{ relative humidity (RH)} \times (\text{dbT} - 14.4) + 46.4$$

During the metabolic trial, feed, offered, and refused and the masses of feces and urine voided were measured daily for each individual animal. Urine sample were separated from feces using a separator attached to the floor of the metabolism cage, and 100 ml of hydrochloric acid solution were added daily to urine containers for preventing nitrogen volatilization. Representative samples were collected (5% of feed offered and refused, 10% of urine, and 20% of feces) from each animal, then pooled and frozen at -20 °C for the determination of trace elements (Cr, Zn, Cu, Fe, and Mn) concentrations. The collected samples were then dried for 24 h at 100 °C using an oven, then ground through a 1-mm screen. Dry matter content was determined by drying samples in an oven at 100 °C for 24 h. Prior to the determination of trace elements concentrations in the collected samples (feed, feces, urine), the samples were subjected to microwave digestion (Multiwave 3000, Microwave Digestion System, Anton Paar GmbH). Thereafter, inductively coupled plasma mass spectrometry (Perkin Elmer ICP-OES 7000DV) was used to determine Cr, Cu, Fe, Mn, and Zn concentrations in the collected samples.

Blood samples (7 ml) were collected from all camel calves before feeding by jugular venepuncture on day 1, 28, 56, and 84 of the first phase of the experiment using a 10-ml syringe (BD Franklin Lakes, NJ, USA). Plasma was obtained after centrifugation of whole blood at 3000 rpm for 15 min at 4 °C. Duplicate samples of plasma were then frozen at

– 20 °C for further analysis. Plasma samples were subjected to microwave digestion (Multiwave 3000, Microwave Digestion System, Anton Paar GmbH) prior to the analysis of Cr, Zn, Cu, and Fe concentrations using inductively coupled plasma mass spectrometry (Perkin Elmer ICP-OES 7000DV).

Statistical analysis

All data obtained from the study were analyzed using repeated measures and the Proc Mixed model (SAS Institute Inc., Cary, NC, USA). Dietary treatments (levels of Cr) were included in the model as main effects. Data were presented as least square means \pm SE and differences were considered significant at $P < 0.05$. All recorded and calculated variable were subjected to analysis of variance (ANOVA) in a completely randomized design (CRD) by following a statistical package using SAS, statistical computer package program. Duncan's multiple range test (DMRT) was used to compare treatment means.

Results

Meteorological measurements

The maximum and minimum T_a (°C), relative humidity (%), and THI throughout the experimental period (July to September) are presented in Table 2. The maximum values of THI recorded during the experimental period indicated that camel calves were exposed to heat stress during certain periods of the study.

Table 2 Mean ambient temperature (T_a), relative humidity (RH), and temperature–humidity index (THI) during the experimental period

Month	Mean	Maximum	Minimum
July			
T_a (°C)	36.0 \pm 5.8	45.3	20.9
RH (%)	11.0 \pm 5.3	31.3	3.9
THI	74.0 \pm 6.5	85.4	56.8
August			
T_a (°C)	35.0 \pm 5.8	45.8	22.8
RH (%)	12.5 \pm 5.9	35.2	5.3
THI	73.0 \pm 6.4	85.6	59.1
September			
T_a (°C)	31.9 \pm 5.8	44.4	17.9
RH (%)	15.3 \pm 7.7	43.2	4.5
THI	70.7 \pm 6.4	84.0	57.6

T_a and RH were recorded every 30 min for 84 days through July and August, and hourly means were calculated. THI was calculated as $(0.8 \times T_a) + [(RH/100) \times (T_a - 14.4)] + 46.4$

Trace elements metabolism

Dietary Cr supplementation showed variable effects on the trace elements intake, excretion, and retention in camel calves exposed to moderate heat stress (Table 3). Fecal and urinary excretion of Cr showed numerical increase with the increasing level of dietary Cr. This was reflected on a numerical increase in Cr absorption and a significant ($P < 0.05$) increase in Cr retention with the increasing level of Cr supplementation.

Copper intake of the growing camel calves showed a numerical decrease with the increasing level of Cr supplementation, while the excretion of Cu via urine and feces was significantly ($P < 0.05$) increased at 0.5 mg Cr/kg DM. This was reflected in a significant reduction in the percentage of Cu retained in camel calves supplemented with Cr (Table 4).

Iron intake in camel calves supplemented with Cr did not show significant variations. Nevertheless, dietary supplementation at 0.5 mg Cr/kg DM resulted in a numerical increase in Fe intake (Table 4) whereas Fe voided in feces and urine was numerically reduced. This resulted in a significant ($P < 0.05$) increase in Fe retention and the percentage of Fe retained in camel calves supplemented with Cr. It is worth to mention that dietary Cr supplementation resulted in more than twofold increase in Fe retention.

Manganese intake and retention in camel calves was not affected significantly by Cr supplementation to the diets. However, Mn excretion via urine and feces tended to increase ($P = 0.08$) in camel calves fed on diet supplement at 0.5 mg Cr/kg DM (Table 4). Consequently, this was reflected on a significant ($P < 0.05$) decrease in the percentage of Mn retained in camel calves fed on supplemented diet at 0.5 mg Cr/kg DM.

Zinc intake and retention was not altered significantly by Cr supplementation to the diet of camel calves (Table 4). However, Zn voided in feces and urine showed a significant ($P < 0.05$) increase in camel calves fed on diet supplement at 0.5 mg Cr/kg DM. This resulted in a significant ($P < 0.05$) reduction in the percentage of Zn retained in camel calves fed on 0.5 mg Cr/kg DM supplemented diet.

Plasma trace element concentration

Dietary chromium supplementation showed a numerical increase in plasma chromium concentration of camel calves (Table 5). However, Cr supplementation to the diet of camel calves showed a numerical decrease in plasma concentration of Cu and Zn without affecting Fe concentration.

Discussion

Climatic conditions

The maximum T_a and THI values prevailed during the current study were 45.89 °C and 85.65, respectively, while the

Table 3 Effects of Cr supplementation on Cr utilization in growing camels under hot conditions

Items	Supplemental Cr mg/kg DM			SEM	P value
	0.0	0.5	1.0		
Fecal Cr excretion (mg/day)	14.0	17.0	16.4	1.3	0.26
Urinary Cr excretion (mg/day)	0.05	0.06	0.11	0.03	0.37
Cr retention (mg/day)	4.0 ^b	5.5 ^{ab}	8.2 ^a	1.1	0.05
Cr retention (%)	22.6	24.5	32.4	3.6	0.17
Cr absorption (%)	22.8	24.8	32.9	3.8	0.15

Values are for growing camels ($n = 5$ per treatment) fed on diets supplemented with either 0.0, 0.5, or 1.0 mg of Cr/kg DM for 84 days. Fecal and urinary samples were collected for 4 days in the digestion trial

Superscript letters within a row are means without a common superscript difference ($P < 0.05$)

average relative humidity was $12.85 \pm 0.29\%$. THI has been utilized for more than four decades to assess heat stress in farm animals using the integrative effect of T_a and relative humidity as a one-dimensional approach (Marai et al. 2001). Temperature–humidity index values of 70 or less are considered comfortable, 75–78 stressful, and values greater than 78% cause extreme distress and animals are unable to maintain thermoregulatory mechanisms or normal body temperature (Silanikove 2000). Therefore, camel calves used in this study were considered to be exposed to heat stress (max THI = 85.65).

Table 4 Effects of Cr supplementation on trace mineral (Cu, Fe, Mn, and Zn) utilizations in growing camels under hot conditions

Items	Supplemental Cr mg/kg DM			SEM	P value
	0.0	0.5	1.0		
Cu utilization					
Total intake (mg/day)	80.3	83.0	75.0	5.3	0.57
Total excretion (mg/day)	45.5 ^b	60.4 ^a	47.9 ^b	3.9	0.04
Retention (mg/day)	34.8	22.6	27.1	4.8	0.10
Retention (%)	43.4 ^a	27.3 ^b	35.0 ^{ab}	6.5	0.02
Fe utilization					
Total intake (mg/day)	486.5	520.2	475.7	31.1	0.59
Total excretion (mg/day)	441.8	415.0	381.1	34.9	0.49
Retention (mg/day)	45.7 ^b	105.8 ^a	96.6 ^a	24.7	0.03
Retention (%)	9.4 ^b	20.9 ^a	20.9 ^a	3.4	0.05
Mn utilization					
Total intake (mg/day)	492.1	502.4	459.4	32.7	0.63
Total excretion (mg/day)	24.8	34.2	25.0	2.99	0.08
Retention (mg/day)	467.2	468.2	434.4	30.52	0.86
Retention (%)	95.0 ^a	93.1 ^b	94.5 ^a	0.42	0.03
Zn utilization					
Total intake (mg/day)	495.3	505.7	462.4	42.88	0.63
Total excretion (mg/day)	25.7 ^b	41.1 ^a	27.9 ^b	3.96	0.04
Retention (mg/day)	469.9	464.6	434.6	30.78	0.69
Retention (%)	94.7 ^a	91.8 ^b	94.0 ^a	0.66	0.03

Values are for growing camels ($n = 5$ per treatment) fed on diets supplemented with either 0.0, 0.5, or 1.0 mg of Cr/kg DM for 84 days. Fecal and urinary samples were collected for 4 days in the digestion trial

Superscript letters within a row are means without a common superscript difference ($P < 0.05$)

Trace elements metabolism and plasma concentration

In the present study, Cr retention in camel calves exposed to heat stress increased with the increasing level of dietary chromium. This indicates that dietary Cr level up to 1.0 mg Cr/kg DM could be retained in animal tissues without any signs of saturation. This is consistent with the previous reports on buffalo calves (Kumar et al. 2013) and broiler chickens (Sirirak et al. 2012).

Chromium supplementation to the diet of camel calves resulted in a significant increase of Fe retention. Iron is known

Table 5 Effects of Cr supplementation on plasma concentrations ($\mu\text{g}/100\text{ ml}$) of trace minerals in growing camels under hot conditions

Items	Supplemental Cr mg/kg DM			SEM	P value
	0.0	0.5	1.0		
Chromium	7	9	9	1	0.17
Copper	57	50	47	13	0.56
Iron	230	220	240	14	0.66
Zink	190	180	170	28	0.92

Values are for growing camels ($n = 5$ per treatment) fed on diets supplemented with either 0.0, 0.5, or 1.0 mg of Cr/kg DM for 84 days. Blood samples were collected on days 1, 28, 56, and 84

The result of plasma Mn concentration was not presented, because the method used in this study failed to detect Mn concentration in the collected blood samples

Superscript letters within a row are means without a common superscript difference ($P < 0.05$)

to be present in the diet in its trivalent ferric form (Fe^{+3}) and should be reduced to its divalent ferrous form (Fe^{+2}) to be biologically available for absorption in the duodenum. There are numerous dietary components capable of reducing Fe^{3+} to Fe^{2+} including ascorbic acid and amino acids such as cysteine and histidine (Sharp and Srari 2007). Chromium is postulated to function as antioxidant (Kumar et al. 2017). Therefore, it could be capable of reducing Fe^{+3} to Fe^{+2} and hence enhancing Fe absorption. This clearly explains the mechanism deriving the observed increase in Fe retention in camel calves supplemented with Cr. The results obtained in the present study confirm the previous reports on the positive influence of dietary Cr on iron retention in goats (Halder et al. 2009).

Dietary Cr supplementation increased the fecal and urinary excretion and consequently reduced the retention of Cu, Zn, and Mn in camel calves. This could be attributed to the competition of Cr with these trace elements for the intracellular-binding protein (metallothionein) during the intestinal absorption (Feng et al. 2003). In addition, this could also be due to the possible competition between Cr and the other trace elements for the binding sites on transferrin (Pechova and Pavlata 2007). Surprisingly, the effect of dietary Cr supplementation on the excretion and retention of trace elements was not linear. This non-linear dose-independent effect of dietary Cr could partially be attributed to the observed decline in trace element intake with the increasing dose of dietary Cr. However, the processes that control and regulate the absorption of trace elements in feeds consumed in mixed diets are not fully understood and needs to be investigated. In contrast to the results of the present study, Kumar et al. (2013) reported that dietary Cr supplementation did not change the apparent absorption and retention of Cu, Zn, and Fe in summer-exposed buffalo calves.

Dietary Cr supplementation at 0.5 mg Cr/kg DM resulted in a numerical increase in plasma Cr level of camel calves.

However, further increase of dietary Cr to 1.0 mg Cr/kg DM did not result in further increase in the level of plasma Cr. In consistent with these results, blood Cr concentration changed only in case of excessive intake in grazing cattle (Sahin et al. 1996). Furthermore, little dose-independent changes in plasma Cr concentration were observed in pre-pubertal dairy heifers (Biswas et al. 2006).

Findings of the present study indicated that supplementation of Cr yeast to the diet of camel calves did not alter plasma concentration of Cu, Zn, and Fe. This indicates that the homeostatic control mechanisms of the plasma levels of these trace minerals could compensate for the reported reduction or enhancement of absorption and retention of these trace minerals caused by Cr supplementation to the diet of camel calves. In consistent to the results of the present study, Kumar et al. (2013) did not report any significant effect of dietary Cr supplementation on plasma concentration of Cu, Fe, and Zn in buffalo calves reared under heat stress conditions. Furthermore, it has been reported that dietary Cr supplementation influenced plasma Cr levels without affecting plasma concentration of Cu, Zn, and Fe in buffaloes (Zade et al. 2014). However, in contrast to the results presented herein, Ghazi et al. (2012) there were reported increase in serum Zn and Fe levels and reduced serum Cu concentrations in heat-stressed broilers.

It is worth to mention that plasma Cu concentration reported in the present study is a little bit lower than the normal range (60–120 $\mu\text{g}/100\text{ ml}$) reported in camels (Faye and Bengoumi 1997). With an average value of 57 $\mu\text{g}/100\text{ ml}$, the copper status of the control camel calves studied herein was at the deficiency limit, whereas plasma Zn concentration reported in the present study was quite higher than the normal level (30–50 $\mu\text{g}/100\text{ ml}$) reported in camel (Faye et al. 2008). The higher plasma Zn concentration obtained in this study could be attributed to the use of recently weaned camel calves in this study (4–5 months), where highest values of plasma Zn concentrations were observed by some authors on non-weaning camel calves due to the milk feeding which provided sufficient zinc in the diet (Faye et al. 2008). However, plasma Fe concentration reported in the current study is corresponding to the values reported for male camels (Faye et al. 2008).

Conclusions

Dietary Cr supplementation to promote growth performance and immune competence of camel calves reared under hot climatic conditions resulted in negative interactions with Cu, Zn, and Mn utilization. Therefore under such conditions, care should be taken for the metabolism and utilization of these trace elements.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Statement of ethical approval The animal experiment was conducted according to the ethics regulations of research on living creatures approved by the ethics committee at King Saud University.

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