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# Effect of Different Levels of Chromium Methionine Supplementation on Growth Performance, Meat Oxidative Stability and Ruminal Metabolites of Male Goat Kids

Research Article

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## ABSTRACT

This study was performed to determine the effects of different levels of chromium methionine (Cr-Met) supplementation on growth performance, meat oxidative stability and ruminal metabolites in male kids. Thirty-two male Mahabadi goat kids, with an average initial body weight (BW) of 22 ± 2 kg at 4 months of age, were allocated in a completely randomized design with four treatments: control without Cr and three levels of Cr supplementation 0.5, 1 and 1.5 mg respectively as Cr-Met/animal/d. Diets were the same for the all groups with a ratio forage: concentrate of 30:70, except for the addition of Cr-Met. The animals were fed in two equal meals (at 08:00 and 16:00 h) and the leavings were collected before the morning meal. Animals were kept in individual pens for 100 days. Kids were weighed after 10 days of adaptation period and at 21 days intervals after feed restriction. Kids were slaughtered at the end of the trial and *Longissimus dorsi* muscle (LDM) samples were immediately stored at -20 ˚C. Thio Barbioturic Acid Reactive Substances (TBARS) methods were used in order to measure meat stability. Supplemental Cr-Met did not affect ($P>0.05$) the final BW, total BW gain, dry matter intake (DMI) and average daily gain (ADG). Concentrations of $NH_3$-N and total and individual volatile fatty acids (VFA) in the rumen were not affected by the Cr-Met ($P>0.05$). However, Cr-Met supplementation increased rumen pH and decreased ruminal protozoa count ($P<0.05$). TBARS values of the LDM were significantly increased as the storage time increased from 1 to 2 months ($P<0.05$). It was also found that increasing dietary chromium supplementation, especially at level of 1.5 mg Cr-Met, significantly decreased lipid oxidation and the TBARS value at 2 months of storage ($P<0.05$). These results indicate that supplementation of goat kid diet with Cr-Met had minimal effects on growth performance and rumen metabolites but improved oxidative stability of LDM during refrigerated storage kids.

KEY WORDS chromium-methionine, lipid oxidation, Mahabadi goat kid, performance, volatile fatty acids.

## INTRODUCTION

Chromium (Cr) has been implicated as an essential nutrient for humans and lab animals (Mertz, 1993). Chromium increases glucose tolerance by potentiating the action of insulin in clearing postprandial glucose from the blood (Mertz, 1993). This can lead to improved glucose utilization and increased growth efficiency. Organic ligands are required for Cr to be biologically available and active (Burton, 1995). However, organic Cr such as Cr picolinate (CrPic), Cr nicotinate (CrNic), amino acid-chelated Cr and high-Cr yeast have demonstrated good biological responses in domestic animals and were absorbed and utilized more effectively than inorganic Cr such as $CrCl_3$ (Chang and Mowat, 1992; Page *et al*. 1993; Mowat *et al*. 1993). The bioavailability of chromium methionine chelate is considered to be

higher than those of other organic chromium (Ohh and Lee, 2005). Chromium supplementation is probably the most controversial mineral supplementation within the livestock industry. Although some studies have demonstrated benefits after Cr supplementation (Moonsie-Shageer and Mowat, 1993; Page *et al*. 1993; Kegley *et al*. 1997; Mooney and Cromwell, 1997), other experiments sustain that the use of chromium does not present any effect (Arthington *et al*. 1997; Gentry *et al*. 1999; Mostafa-Tehrani *et al*. 2006). These conflicting results may be related to the presence of stress factors such as long journeys, exercise or alimentary restrictions (Chang and Mowat, 1992; Kegley *et al*. 1997). Lipid oxidation is one of the most important mechanisms of quality loss and directly affects the acceptability of meat and meat products by the consumers. Studies of chromium supplementation in goat kids are rare and no study has evaluated the effect of Cr on rumen microorganisms and lipid oxidation. Some heavy metals have been shown to be toxic, especially to simple life forms such as ruminal protozoa (Dallago *et al*. 2011). The equilibrium (chemical and biological) of rumen content must be maintained to allow for its normal physiology. This equilibrium is an important factor to be studied for improving livestock production systems. Farmers often ignore this fact and use Cr in mineral salt formulas without scientific support on the role of chromium in animal nutrition. This study was performed to determine the effects of diet supplementation with chromium methionine chelate on meat oxidative stability, growth performance and ruminal metabolites of Mahabadi goat kids.

## MATERIALS AND METHODS

This study was performed at the Experimental Farm of Agriculture and Natural Resource College, University of Tehran, Karaj, Iran. Thirty-two male Mahabadi goat kids (4 months of age) were allocated by stratified randomization on the basis of body weight (22±2 kg on average) into four equal groups. Kids were individually penned and measurements were made on each kid. Kids were allowed *ad libitum* access to water and feed was offered twice daily at approximately 08:00 and 16:00 h for 100 days. The forage:concentrate ratio was gradually adjusted to 30:70 in totally mixed ration (TMR) form. This took place over the 10 day adaptation period, and then kids were randomly assigned to one of four dietary treatments (n=8 per group) consisting of supplementation with 0, 0.5, 1.0 or 1.5 mg of Cr in the form of Cr-Met [10% Chromium and 90% Methionine (wt/wt); Micro Plex 1000, Zinpro, Inc., Eden Prairie, MN] once daily, mixed with 50 g of ground barley for 90 days. The basal diet (Table 1) was formulated for maximum growth to meet the requirements recommended by NRC (1985). The range of Cr levels was selected based on previous studies with sheep (Haldar *et al*. 2009). Samples of the diet and the leavings were collected weekly in polyethylene sachets and pooled at monthly intervals for analysis of dry matter (DM), organic matter (OM), ether extract (EE), crude protein (CP), ADF and NDF (AOAC, 1990). The goats were weighed at 1, 21, 42, 63 and 90 days of trial after an overnight deprivation of feed. Feed conversion ratio (FCR) was calculated according to: FCR= (DM; kg) / (ADG; kg/d).

Ruminal contents were sampled at 22, 43, 64 and 85$^{th}$ days of the experimental phase to quantify the ruminal protozoa, volatile fatty acids (VFA) and $NH_3$-N concentrations and pH. On these days, three hours after the morning feed, approximately 20 mL of ruminal content was sampled by an esophagus probe coupled with a collecting pump. Ruminal pH was measured immediately after sampling using a pH meter (HI 8314 membrane pH meter, Hanna Instruments, Villafranca, Italy) and the samples were prepared for protozoa counting following the procedure described previously by Dehority (1984). At the end of experiment, ruminal content samples were drawn at 0 and 3 h after morning feeding. The rumen fluid was stabilized by adding 1 ml of sulfuric acid per 50 ml and frozen at -20 ˚C until VFA analysis by using gas chromatography (0.25×0.32, 0.3 µm i.d. fused silica capillary, model no. CP-9002 Vulcanusweg 259 a.m., Chrompack, Delft, the Netherlands) as outlined by Kowsar *et al*. (2008). A subsample of 5 mL was combined with 1 mL of HCl 0.2 N for determination of NH3-N concentration by following the Weatherburn (1967) technique.

At the end of trial, kids were weighed and slaughtered following 16-h fasting. The *Longissimus dorsi* muscles (LDM) were immediately stored at -20 ˚C to facilitate assessment of the effect of Cr on lipid oxidation. The extent of lipid peroxidation after refrigerated storage (1 and 2 months) was assessed by measuring Thio Barbioturic Acid Reactive Substances (TBARS) using the method described by Esterbauer and Cheeseman (1990).

TBARS concentrations were expressed as mg malonaldehyde/kg meat. Data were analyzed by completely randomized design using the General Linear Model (GLM) procedure of the statistical analysis software package SAS, (2002). Least-square means were computed and tested for differences by the Tukey's test. Differences of least-square means were considered to be significant at $P<0.05$, and that of ($P<0.1$) was described as a trend.

## RESULTS AND DISCUSSION

The final BW, total BW gain, DMI and ADG were not affected by supplemental Cr ($P > 0.05$; Table 2).

**Table 1** Ingredients and chemical composition of diet

| Nutrient | % of DM | Chemical components | | Macro mineral and micro mineral | |
|---|---|---|---|---|---|
| Alfalfa hay | 16.49 | DM (%) | 80.78 | Calcium (%) | 0.89 |
| Corn silage | 8.32 | | | Phosphorus (%) | 0.48 |
| Ground barley grain | 50.65 | NEL (Mcal/kg) | 2.41 | Magnesium (%) | 0.27 |
| Soybean meal | 2.21 | | | Sulfur (%) | 0.28 |
| Canola meal | 4.55 | CP (%) | 13.5 | Zn (mg/kg DM) | 219 |
| Wheat bran | 9.09 | | | Fe (mg/kg DM) | 368 |
| Wheat straw | 5.19 | NDF (%) | 36.6 | I (mg/kg DM) | 3 |
| Carbonate Calcium | 1.3 | | | Mn (mg/kg DM) | 216 |
| Sodium bicarbonate | 0.78 | Ether extract (%) | 2.6 | Cu (mg/kg DM) | 54 |
| Salt | 0.52 | | | Co (mg/kg DM) | 1 |
| Mineral and vitamins supplement[1] | 0.91 | Ash (% ) | 9 | Cr (mg/kg DM) | 0.83 |

[1] Containing: vitamin A: 250000 IU/kg; vitamin D: 50000 IU/kg; vitamin E: 1500 IU/kg; Manganese: 2.25 g/kg; Calcium: 120 g/kg; Zinc: 7.7 g/kg; Phosphorus: 20 g/kg; Magnesium: 20.5 g/kg; Sodium: 186 g/kg; Iron: 1.25 g/kg; Sulfur: 3 g/kg; Copper: 1.25 g/kg; Cobalt: 14 mg/kg; Iodine: 56 mg/kg and Selenium: 10 mg/kg.

**Table 2** Effect of chromium methionine (Cr-Met) on final body weight (BW), total BW gain, ADG, DMI and FCR in Mahabadi goat kids[1]

| Measurement | Treatments* | | | | SEM | P-value |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | | |
| Final BW (kg) | 32.19 | 31.32 | 32.62 | 32.95 | 1.64 | 0.91 |
| Total BW gain (kg) | 10.75 | 9.83 | 11.38 | 12.33 | 0.72 | 0.11 |
| ADG (kg/d) | 0.12 | 0.11 | 0.13 | 0.14 | 0.01 | 0.11 |
| DMI (kg/d) | 1.00 | 1.00 | 1.02 | 1.06 | 0.04 | 0.64 |
| FCR | 8.64 | 9.54 | 8.15 | 7.77 | 0.47 | 0.09 |

* 1: 0 mg Cr/animal/d; 2: 0.5 Cr/animal/d; 3: 1 Cr/animal/d and 4: 1.5 mg Cr/animal/d; Cr provided as chromium methionine (CrMet).
[1] Values are least-square means.
SEM: standard error of the mean and n=8.
BW: body weight; ADG: average daily gain; DMI: dry matter intake and FCR: feed conversion ratio.

Although not significant (P=0.09), there was tendency for improvement in FCR from the level of 1 mg Cr/animal/d. These findings are consistent with earlier research in goats (Haldar *et al.* 2006), pigs (Amoikon *et al.* 1995; Lindemann *et al.* 1995), lambs (Fornea *et al.* 1994; Gentry *et al.* 1999; Samsell and Spears 1989; Kitchalong *et al.* 1995; Mostafa-Tehrani *et al.* 2006; Dallago *et al.* 2011), calves (Bunting *et al.* 1994; Mathison and Engstrom, 1995), beef steers (Chang and Mowat, 1992) and dairy calves (Bunting *et al.* 1994).

In contrast to this, Moonsie-Shageer and Mowat (1993) reported improvement in ADG and DMI in calves supplemented with high-Cr yeast in a corn-silage diet.

Haldar *et al.* (2009) also showed improved FCR and ADG in castrated male black Bengal goats supplemented with inorganic Cr, whereas Boleman *et al.* (1995) reported reduced ADG and DMI in pigs fed with Cr tripicolinate from the growing to the finishing phase. Lindemann *et al.* (1995) reported an improved feed efficiency in pigs fed grower diets supplemented with Cr picolinate. In ruminants, positive performance responses to Cr appear to depend on the presence of stressors such as stress due to transit (Chang and Mowat, 1992; Moonsie-Shageer and Mowat, 1993). When serum cortisol concentration is high more glucose is used by the cells.

This causes an increase in the use of chromium resulting in increase in chromium excretion by depleting body chromium.

This lost chromium may be replaced by chromium supplementation or the entrance of glucose into the cell may be impaired by the lack of chromium if the animal is not supplemented (Dallago *et al.* 2011). Alternatively, when a stress factor is not associated, the demand for chromium by the body does not increase and the chromium supplementation is not necessary. Dry matter intake has direct consequences on animal performance. In this study, all animals presented similar nutrient intake, showing no differences between treatments. As no differences were observed in DMI, no differences were expected for performance traits. The reason for lacking effects on performance in this study may be because of adequacy of Cr status or its source in the basal diet.

TBARs values of the LDM significantly increased as the storage time increased from 1 to 2 months ($P<0.05$). It was also found that increasing dietary chromium supplementation, especially 1500 ppb Cr, significantly decreased lipid oxidation and TBARs value for 2 months storage ($P<0.05$). On the first month of storage, dietary Cr supplement did not significantly decrease the lipid oxidation of LDM (Table 3). Observed that Cr supplementation significantly decreased lipid oxidation and the TBAR values of thigh and breast muscles of broilers during refrigerated storage.

Chromium is a component of glucose tolerance factor (GTF) and is important in carbohydrate, fat and protein metabolisms presumably by potentiating the action of insulin (Mertz, 1993).

It has been well recognized that insulin metabolism influences lipid peroxidation (Gallaher *et al*. 1993).

**Table 3** Effects of chromium supplementation on the lipid oxidation of *longissimus dorsi* muscle (LDM; mg malonaldehyde/kg meat) following different refrigerated storage period[1]

| Period of storage | Treatments* | | | | SEM | P-value |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | | |
| First month | 1.77 | 1.43 | 1.15 | 1.11 | 0.19 | 0.08 |
| Second month | 2.04[a] | 1.95[a] | 1.69[ab] | 1.50[b] | 0.10 | 0.01 |

* 1: 0 mg Cr/animal/d; 2: 0.5 Cr/animal/d; 3: 1 Cr/animal/d and 4: 1.5 mg Cr/animal/d; Cr provided as chromium methionine (CrMet).
[1] Values are least-square means.
SEM: standard error of the mean and n=8.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Chromium (insulin cofactor) is, therefore, postulated to function as an antioxidant (Preuss *et al.* 1997). According to antioxidant theory (Klasing, 1993), when the concentrations of antioxidant vitamins (vitamin C and vitamin E) decrease, lipid peroxidation increases in plasma and tissues leading to damage of cell membranes. Anderson *et al.* (2001) reported the potential beneficial antioxidant effects of the individual and combined supplementation of Cr and Zn for six months in Tunisian adult subjects with type 2 diabetes mellitus. Sahin *et al.* (2003) also reported that supplementation of chromium and vitamin C resulted in an increase in serum concentrations of vitamin C and vitamin E and an decrease in malonaldehyde concentration in serum. Preuss, *et al*. (1997) reported decreased hepatic TBARS formation upon supplementation of chromium picolinate and nicotinate in rats.

The averages values for pH, concentration of $NH_3$-N and volatile fatty acids (VFA) at 0 and 3 h after feeding, in the ruminal liquor of goat kids fed with the experimental diets, revealed that dietary supplementation of CrMet had no effect on the concentration of total VFA (m*M*), acetate, propionate, butyrate, valerate and isovalerate (P>0.05), but significantly increased rumen pH (P<0.05). Besong *et al.* (2001) observed that supplementation with Cr had no effect on molar proportions of ruminal VFA in Holstein steers that were fed with diet supplemented with 0.8 mg/kg of Cr as chromium picolinate, However, results of *in vitro* studies indicated that the molar proportion of propionate decreased, whereas butyrate and isobutyrate increased linearly with increasing Cr content at 12 h of incubation, also molar proportion of valerate alone increased linearly with increasing Cr content at 24 h of incubation. Our results are consistent with those of Rikhari *et al.* (2010) who added 0.5 and 1 mg/kg of Cr as CrP to the diet of fistulated male cattle and observed no difference in ammonia nitrogen concentrations and TVFA.

The mean concentration of ruminal protozoa for the treatments revealed a negative relationship between Cr supplementation and ruminal protozoa population (Figure; P<0.01).

Dallago *et al*. (2011) showed that Cr as chromium picolinate decreased protozoa in the rumen of sheep.

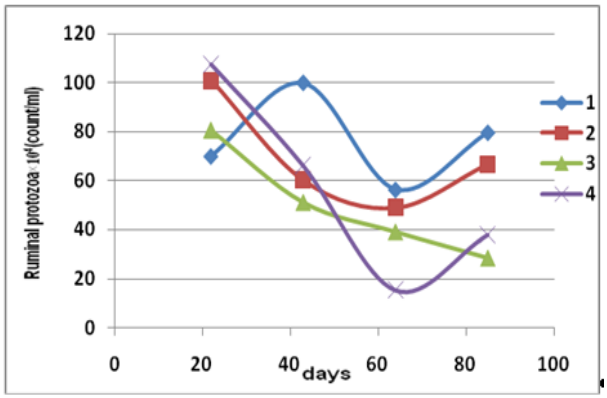


**Figure 1** Relationship between Cr supplementation and ruminal protozoa count
* 1: 0 mg Cr/animal/d; 2: 0.5 Cr/animal/d; 3: 1 Cr/animal/d and 4: 1.5 mg Cr/animal/d; Cr provided as chromium methionine (CrMet)

For the ruminal protozoa population, it should be taken in to account that Cr is a heavy metal chelate and, therefore, has toxic potential, which can induce damage to DNA, cause interference with essential metabolic functions or produce reactive metabolites (Hodgson *et al*. 2004). These, in turn, may increase oxidative stress to ruminal protozoa, causing their death or impairing their reproduction. When manipulating ruminal microbes, care must be taken with the maintenance of ruminal equilibrium.

The rumen is a complex ecologic niche, where the survival of certain organisms depends greatly on the presence of others and their auto-regulation is important for ruminants (Mackie, 1996).

## CONCLUSION

The results of this study indicate that supplementation with chromium methionine may be beneficial in the oxidative stability of meat in growing goat kids even in a non-stressed management regime without changing their performance.

## REFERENCES

Amoikon E., Fernandez J., Southern L., Thompson Jr.D., Ward T. and Olcott B. (1995). Effect of chromium tripicolinate on growth, glucose tolerance, insulin sensitivity, plasma metabolites, and growth hormone in pigs. *J. Anim. Sci.* **73,** 1123-1130.

Anderson R.A., Roussel A.M., Zouari N., Mahjoub S., Matheau J.M. and Kerkeni A. (2001). Potential antioxidant effects of zinc and chromium supplementation in people with type 2 diabetes mellitus. *J. Am. Coll. Nutr.* **20,** 212-218.

AOAC. (1990). Official Methods of Analysis. Vol. I. 15th Ed. Association of Official Analytical Chemists, Arlington, VA.

Arthington J., Corah L., Minton J., Elsasser T. and Blecha F. (1997). Supplemental dietary chromium does not influence ACTH, cortisol, or immune responses in young calves inoculated with bovine herpesvirus-1. *J. Anim. Sci.* **75,** 217-223.

Besong S., Jackson J., Trammell D. and Akay V. (2001). Influence of supplemental chromium on concentrations of liver triglyceride, blood metabolites and rumen vfa profile in steers fed a moderately high fat diet. *J. Dairy Sci.* **84,** 1679-1685.

Boleman S.L., Boleman S.J., Bidner T.D., Southern L.L., Ward T.L., Pontif J.E. and Pike M.M. (1995). Effect of chromium picolinate on growth, body composition, and tissue accretion in pigs. *J. Anim. Sci.* **73,** 2033-2042.

Bunting L., Fernandez J., Thompson Jr.D. and Southern L. (1994). Influence of chromium picolinate on glucose usage and metabolic criteria in growing holstein calves. *J. Anim. Sci.* **72**, 1591-1599.

Burton J.L. (1995). Supplemental chromium: Its benefits to the bovine immune system. *Anim. Feed Sci. Technol.* **53,** 117-133.

Chang X. and Mowat D. (1992). Supplemental chromium for stressed and growing feeder calves. *J. Anim. Sci.* **70,** 559-565.

Dallago B., McManus C., Caldeira D., Lopes A., Paim T., Franco E., Borges B., Teles P., Correa P. and Louvandini H. (2011). Performance and ruminal protozoa in lambs with chromium supplementation. *Vet. Sci. Res.* **90,** 253-256.

Dehority B.A. (1984). Evaluation of subsampling and fixation procedures used for counting rumen protozoa. *Appl. Environ. Microbiol.* **48,** 182-185.

Esterbauer H. and Cheeseman K.H. (1990). Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. *Methods. Enzymol*. **186,** 407-421.

Fornea R., Bunting L., Fernandez J., Depew C. and Southern L. (1994). Chromium picolinate has minimal effects on glucose usage of lambs in either normal or diabetic states. *J. Anim. Sci.* **72,** 257-262.

Gallaher D.D., Csallany A.S., Shoeman D.W. and Olson J.M. (1993). Diabetes increases excretion of urinary malondehyde conjugates in rats. *Lipids.* **28,** 663-666.

Gentry L.R., Fernandez J.M., Ward T.L., White T.W., Southern L.L., Bidner T.D., Thompson D.L., Jr., Horohov D.W., Chapa A.M. and Sahlu T. (1999). Dietary protein and chromium tripicolinate in Suffolk wether lambs: effects on production characteristics, metabolic and hormonal responses, and immune status. *J. Anim. Sci.* **77,** 1284-1294.

Haldar S., Ghosh T., Pakhira M. and De K. (2006). Effects of incremental dietary chromium (cr3+) on growth, hormone concentrations and glucose clearance in growing goats (*Capra hircus*). *J. Agri .Sci.* **144,** 269-280.

Haldar S., Mondal S., Samanta S. and Ghosh T. (2009). Effects of dietary chromium supplementation on glucose tolerance and primary antibody response against peste des petits ruminants in dwarf bengal goats (*Capra hircus*). *Animal*. **3,** 209-217.

Hodgson E., Cope W.G. and Leidy R.B. (2004). Classes of toxicants: use classes. Pp. 49-74 in Textbook of Modern Toxicology. E.A. Hodgson Ed. 3th Ed. Wiley-Interscience, New Jersey.

Kegley E., Spears J. and Eisemann J. (1997). Performance and glucose metabolism in calves fed a chromium-nicotinic acid complex or chromium chloride. *J. Dairy Sci.* **80**, 1744-1750.

Kitchalong L., Fernandez J., Bunting L., Southern L. and Bidner T. (1995). Influence of chromium tripicolinate on glucose metabolism and nutrient partitioning in growing lambs. *J. Anim. Sci.* **73,** 2694-2705.

Klasing K.C. (1993) Comparative Avian Nutrition, Cab International. Univ. Michigan.

Kowsar R., Ghorbani G., Alikhani M., Khorvash M. and Nikkhah A. (2008). Corn silage partially replacing short alfalfa hay to optimize forage use in total mixed rations for lactating cows. *J. Dairy Sci.* **91,** 4755-4764.

Lindemann M.D., Wood C.M., Harper A.F., Kornegay E.T. and Anderson R.A. (1995). Dietary chromium picolinate additions improve gain:Feed and carcass characteristics in growing-finishing pigs and increase litter size in reproducing sows. *J. Anim. Sci.* **73,** 457-465.

Mackie R.I. (1996). Gut environment and evolution of mutualistic fermentative digestion. *Gastroint. Microbiol.* **1,** 13-35.

Mathison G. and Engstrom D. (1995). Chromium and protein supplements for growing-finishing beef steers fed barley-based diets. *Canadian J. Anim. Sci*. **75,** 549-558.

Mertz W. (1993). Chromium in human nutrition: a review. *J. Nutr*. **123,** 626-633.

Mooney K. and Cromwell G. (1997). Efficacy of chromium picolinate and chromium chloride as potential carcass modifiers in swine. *J. Anim. Sci.* **75,** 2661-2671.

Moonsie-Shageer S. and Mowat D.N. (1993). Effect of level of supplemental chromium on performance, serum constituents, and immune status of stressed feeder calves. *J. Anim. Sci.* **71,** 232-238.

Mostafa-Tehrani A., Ghorbani G., Zare-Shahneh A. and Mirhadi S. (2006). Non-carcass components and wholesale cuts of iranian fat-tailed lambs fed chromium nicotinate or chromium chloride. *Small Rumin. Res.* **63,** 12-19.

Mowat D., Chang X. and Yang W. (1993). Chelated chromium for stressed feeder calves. *Canadian J. Anim. Sci.* **73,** 49-55.

NRC. (1985). Nutrient Requirements of Sheep, 6th Ed. Natl. Acad. Sci, Washington, DC.

Ohh S.J. and Lee J.Y. (2005). Dietary chromium-methionine chelate supplementation and animal performance. *Asian-Australasian J. Anim. Sci.* **18,** 898-907.

Page T., Southern L., Ward T. and Thompson Jr.D. (1993). Effect of chromium picolinate on growth and serum and carcass traits of growing-finishing pigs. *J. Anim. Sci.* **71,** 656-662.

Preuss H., Grojec P., Lieberman S. and Anderson R. (1997). Effects of different chromium compounds on blood pressure and lipid peroxidation in spontaneously hypertensive rats. *Clin. Nephrol.* **47,** 325-331.

Rikhari K., Tiwari D. and Kumar A. (2010). Effect of dietary supplemental chromium on nutrient utilization, rumen metabolites and enzyme activities in fistulated crossbred male cattle. *Indian J. Anim. Sci.* **80,** 785-893.

Sahin K., Sahin N. and Kucuk O. (2003). Effects of chromium, and ascorbic acid supplementation on growth, carcass traits,

serum metabolites, and antioxidant status of broiler chickens reared at a high ambient temperature (32 ˚C). *Nutr. Res*. **23,** 225-238.

Samsell L.J. and Spears J.W. (1989). Chromium supplementation effects on blood constituents in lambs fed high or low fiber diets. *Nutr. Res*. **9,** 889-899.

SAS Institute. (1996). SAS®/STAT Software, Release 6.11. SAS Institute, Inc., Cary, NC.

Weatherburn M. (1967). Phenol-hypochlorite reaction for determination of ammonia. *Anal. Chem*. **39,** 971-974.