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Effect of Chromium Dietary Supplementation on the Immune Response and Some Blood Biochemical Parameters of Transport-stressed Lambs

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ABSTRACT : Forty-eight Naemi lambs (avg. BW 31.7 kg) were transported by truck for a distance of 1,450 km from Al-Jouf to Riyadh, Saudi Arabia. On arrival day, the lambs were randomly allocated to four groups receiving diets supplemented with 0.0, 0.3, 0.6 and 0.9 ppm organic chromium (Cr). Each group consisted of four separately housed replicates of three lambs each. The animals were fed *ad libitum* on a grower diet for 84 days. Blood samples were obtained shortly before transportation, upon arrival and at weekly intervals thereafter from all lambs for analysis of plasma and serum. Plasma glucose and serum cortisol, total protein, albumin, urea-N and total cholesterol concentrations were determined. A cursory clinical examination of the lambs, along with rectal temperature, was undertaken at different intervals during the experiment. The lambs were inoculated each with 2 ml i.v. chicken red blood cells (CRBC) on days 0, 21, and 42. Serum total, IgG and IgM antibody titers were determined at weekly intervals post-immunization. An *in vivo* intradermal hypersensitivity test was carried out on 6 lambs from each group on days 10 and 70. Transportation of the lambs resulted in a significant ($p<0.001$) elevation of serum cortisol, total protein and albumin levels, as well as increased plasma glucose concentration, with corresponding decrease in total cholesterol, while blood urea-N remained largely unchanged. These constituents returned to normal levels during subsequent weeks, with no significant differences in their concentrations being observed between the Cr-supplemented groups and controls. Rise in rectal temperature after transportation was reduced to a greater extent ($p<0.05$) in Cr-supplemented versus control lambs. Total, IgG and IgM antibody titers against CRBC rose significantly ($p<0.05$) during immunizations in all groups, with significantly and linearly higher ($p<0.05$) total and IgG titers in Cr-supplemented *versus* control lambs. By contrast, no significant effect due to Cr supplementation was recorded in IgG titers, which increased equally in Cr-fed and control groups. Skin thickness in response to intradermal inoculation of phytohaemagglutinin (PHA) was also significantly ($p<0.01$) increased as a result of Cr supplementation. These results indicate that dietary Cr supplementation might be useful during stress especially for enhancing immune responses in transport-stressed lambs. (**Key Words :** High Chromium Yeast, Immune Response, Some Blood Parameters, Lambs)

INTRODUCTION

Transportation over long distances is a significant cause of stress in animals. This effect is exacerbated by denying the animals feed and water during transit. Under these circumstances, animals usually manifest depression and stop eating, resulting in reduced microbial fermentation in the rumen and decreased alimentary canal motility. Malabsorption might consequently ensue and the animals' performance might be severely jeopardized. Loss of adipose tissue might be observed, even if the animals are consuming some food, due to inadequate nutrient intake to meet body requirements for maintenance and growth. This, coupled with metabolic dysfunction, leads to increased susceptibility to diseases (Cole et al., 1982; Hutcheson and Cole, 1986; Hutcheson, 1990).

Previous studies have shown that the use of antibiotics, vitamins and minerals as dietary supplements increases feed intake and improves performance and immunological functions, thereby ameliorating the effects of stress in man and animals (Nockels, 1990; Anderson, 1994). Similar effects have been attributed to chromium supplementation which was shown to enhance carbohydrate, fat, and protein metabolism during stress, probably through potential action of insulin (Anderson, 1987; Mertz, 1993). In addition to its role in metabolic processes, Cr has been shown to affect nuclear protein (Weser and Koolman, 1969) and RNA synthesis (Okada et al., 1989) and improve immunological responses (Chang et al., 1994) especially when organic forms of the metal are supplemented to stressed animals

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Table 1. Basal diet (control, 0 ppm Cr supplementation) composition and analysis

Ingredients	Content (%, as DM)
Alfalfa hay	27.0
Yellow corn	37.3
Barley	23.5
Soybean meal	9.2
Sodium bicarbonate	1.0
Ground limestone	0.9
Dicalcium phosphate	0.5
Salt	0.4
Mineral & vitamin premix[1]	0.2
Cr premix[2]	variable[3]
Chemical analysis[4]	
DM (%)	89.0
OM (%)	92.9
CP (%)	14.71
GE (Mcal/kg)	4.41
ME (Mcal/kg, tabulated)	2.74
DE (Mcal/kg)	3.20
CF (%)	8.48
NDF (%)	50.8
ADF (%)	16.3
Ca (%)	1.00
P (%)	0.41
Concentrate:roughage ratio	73:27

[1] Mineral and vitamin premix, contain per kilogram: CO, 300 mg; Cu, 20,000 mg; I, 700 mg; Fe, 10,000 mg; Mg, 150,000 mg; Mn, 40,000 mg; Se, 150 mg; Zn, 50,000 mg; and vit. A, 5,000,000 IU; vit. D, 500,000 IU; vit. E, 10,000 IU.

[2] High Cr-yeast (1,000 mg of Cr/kg of Cr-yeast; Alltech, Lexington, KY).

[3] Supplemental Cr diets (0.3, 0.6 and 0.9 ppm Cr) were obtained by replacing the exact amounts of Cr premix with a similar amount of corn in the basal (control) diet.

[4] Laboratory analysis as DM basis.

such as feeder calves (Chang and Mowat, 1992; Mowat et. al., 1993) and dairy cows (Burton et al., 1993).

The present study was undertaken to investigate the effect of organic Cr supplementation from a high-Cr yeast source on the immune response and some blood biochemical parameters of transport-stressed lambs.

MATERIALS AND METHODS

Experimental animals and design

Forty-eight, 4-6 month old *Naemi* lambs (mean body wt. 31.7 kg) were used. The animals were procured from a farm in Al-Jouf, northern Saudi Arabia, and transported together by truck over a distance of 1,450 km from that city to Riyadh in the central region of the Kingdom. All lambs were born and reared on the same farm and all were clinically normal upon purchase. A review of their health records revealed none with a previous history of infectious or metabolic disease. Prior to transportation, the lambs were deprived of feed and water for 16 h; shortly before loading, blood samples were collected from the lambs and their rectal temperatures were recorded. The journey lasted ~30 h, during which the animals remained without food or water. The lambs were randomly allocated to four groups each consisting of four separately housed replicates of 3 lambs each. One group received no Cr supplementation (0.0 Cr) and served as a control group. The remaining three groups received supplementary Cr at the rate of 0.3, 0.6 and 0.9 mg per kg dry matter, respectively, from a high-Cr yeast source (Alltech, Lexington, KY, USA). The experiment lasted for 84 days during which the lambs were fed *ad libitum* on a grower diet formulated according to NRC (1985) recommendations (Table 1), while water was freely available. The experimental procedures used in the study were consonant with animal ethics guidelines approved by the Agricultural Research Council of King Saud University.

Blood sampling and biochemical analysis

Blood samples were collected by jugular veinpuncture from the lambs shortly before transportation, upon arrival (day 0) and at weekly intervals thereafter, using 10 ml vacuotainer tubes with and without anticoagulant (EDTA) for collection of plasma and serum, respectively. The animals were fasted for 16 h before sampling. Plasma was separated shortly after collection by centrifugation of non-coagulated blood samples, while serum was separated from clotted samples that were allowed to stand overnight at room temperature. Both serum and plasma were stored at -80°C until analyzed. Plasma glucose, serum total protein (TP), albumin, urea-N and total cholesterol were determined spectrophotometrically, at weekly intervals in all lambs using commercial reagent kits (Randox Laboratories, UK). Serum cortisol level was determined before and after transportation and at weekly intervals up to 70 days of the experiment, in 8 animals per group, using radio-immunoassay procedure (DRG Instruments, GMBH, Germany).

Body temperature

Rectal temperature was recorded on days 0, 2, 5, 7, 14 and 21 using a digital thermometer.

Cellular-mediated response

On days 10 and 70 of the experiment, six lambs from each group were inoculated intradermally with 0.15 mg phytohemagglutinin (PHA) per lamb dissolved in 100 µl sterile phosphate buffer saline (PBS) into a shaven area of the skin of the right shoulder. An equal quantity of phosphate buffered saline (PBS) was inoculated into the left shoulder for comparison. Skin fold thickness was measured before and at 2, 4, 6, 8, 24, 48 and 72 h after inoculation using a skin caliper.

Humeral antibody response

Each lamb was immunized intravenously on day 0 and

Table 2. Effect of transportation on some metabolic and hormonal parameters in lamb serum[1]

Item	Before shipping	After shipping	SEM[2]	P^3
Glucose (mmol/L)	3.67	4.38	0.066	0.001
Total protein (gm/L)	68.99	72.97	0.464	0.001
Albumin (gm/L)	33.39	36.51	0.063	0.001
Urea N (mmol/L)	2.66	2.80	0.046	0.120
Total cholesterol (mg/dl)	73.98	58.03	1.340	0.001
Cortisol (ng/ml)	59.38	124.12	8.240	0.001

[1] Values represent least squares means (n = 36 lambs). [2] Pooled standard error of means. [3] Probability.

re-immunized on days 21 and 42 with 2 ml suspension of 20% chicken red blood cells (CRBC) in PBS. Serum total antibody and IgG titers were determined prior to immunization and at weekly intervals thereafter using hemagglutination (Witlin, 1966) and 2-mercaptoethanol (Solomon and Delhanty, 1966) methods, respectively, while IgM titers were calculated as the difference between total and IgG antibody titers.

Statistical analysis

Data were analyzed using a general linear procedure (GLM) in SAS (1998) according to the following model:

$$Y_{ijk} = \mu + C_i + T_j + CT_{ij} + e_{ijk}$$

Where Y_{ijk} = k^{th} observation of the j^{th} Cr level and i^{th} time post-injection;

μ = common mean;

C_i = effect of the i^{th} Cr level,

T_j = effect of the j^{th} time post injection;

CTij = interaction of the i^{th} Cr level with j^{th} time post injection;

e_{ijk} = residual error

Least squares means were used to compare treatment means, and were used in the tables and figures with pooled standard error of means.

RESULTS AND DISCUSSION

All lambs maintained satisfactory health throughout the duration of the experiment. The mean rectal temperature of the lambs upon arrival was 39.7°C, an average increase ($p<0.001$) of 0.4°C from pre-transportation temperature. During the following three weeks, rectal temperatures decreased by about 0.7°C in chromium-fed and 0.4°C in control-fed lambs, with largest ($p<0.05$) reduction being recorded in lambs receiving 0.6 ppm Cr supplementation *versus* controls. These results concur with the observations of Moonsie-Shageer and Mowat (1993) and Wright et al. (1994) that Cr supplementation might help reduce stress-induced pyrexia in animals.

Transportation of the lambs resulted in a marked increase ($p<0.001$) of serum cortisol level, with corresponding reduction in total cholesterol and elevation of total protein and albumin, while blood urea-N was only slightly and non-significantly elevated (Table 2). A significant increase ($p<0.001$) in plasma glucose concentration was also observed following transportation. These results agree with Fordham et al. (1989) and Kent and Ewbank (1986) who reported more than 66% and 5 fold increases in blood cortisol of lambs and calves, respectively, following road transportation for several hours. These changes were to be expected since exposure to stress factors has long been known to stimulate increased secretion of corticosteroid hormones (Seyle, 1956), which in turn, contribute to elevated glucose, total protein and urea-N levels in blood (Hutcheson et al., 1984; Cole et al., 1986; Kent and Ewbank, 1986; 1988; Bennett et al., 1989; Nockels, 1994). According to Galean et al. (1981) increased glucose concentration in transport-stressed calves could be attributed to increased gluconeogenesis during stress.

Weekly determination of these blood biochemical constituents continued over the following 12 weeks of the experiment, during which their values fluctuated within normal levels (data not shown). Statistical analysis of the results showed that Cr-supplementation had no significant effect on plasma glucose concentration, thus confirming the results of other investigators that no significant change in blood glucose was associated with Cr supplementation in other species of animals (Mowat et al., 1993; Chang et al., 1995; Lindemann et al., 1995; Kegly et al., 1997b; Ward et al., 1997; Gentry et al., 1999). In human beings, however, Cr supplementation has been associated with reduction in blood glucose in type II diabetics (Vinson and Bose, 1984; Thiel, 1996) while neither blood glucose nor cholesterol values were affected in subjects with non-insulin dependent diabetes (Anderson, 1997). Similarly, total protein, albumin, total cholesterol, urea-N and cortisol values recorded in the present study were comparable in all Cr supplemented lambs and the controls. To our knowledge, this is the first study on the effect of Cr supplementation on these blood constituents, in transport-stressed sheep.

Repeated immunization of the lambs with CRBCs elicited significant cell-mediated and humoral antibody responses in all groups. These responses were more pronounced in lambs receiving dietary Cr supplementation in comparison to the controls. The average skin fold thickness induced by intradermal inoculation of PHA on

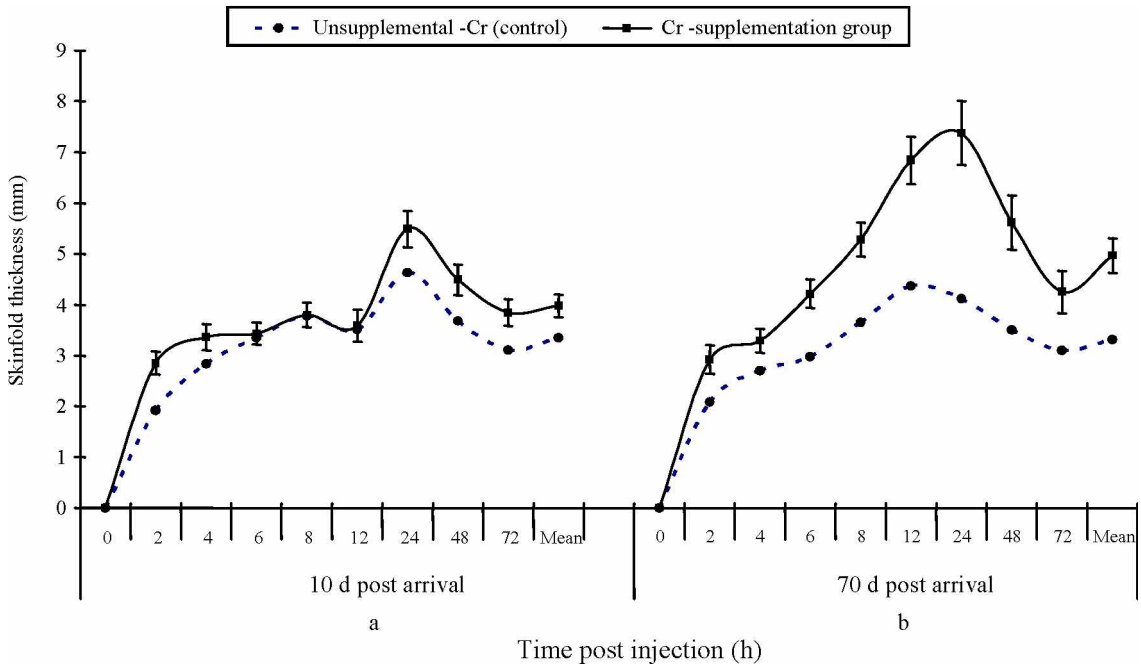

Figure 1. Effect of supplemental chromium level as high Cr- yeast on skinfold thickness (mm) of lambs at 10 and 70 days post arrival, values are least squares means (n = 6 observations for control and 18 observations for Cr treatment groups).

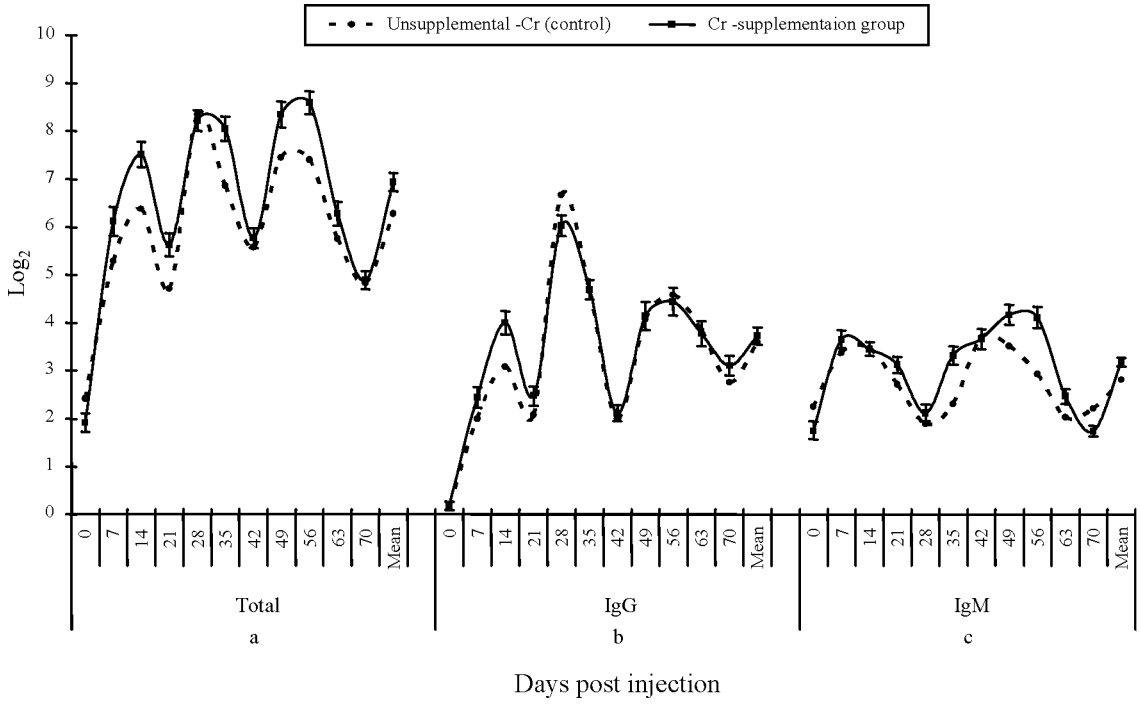

Figure 2. Effect of supplemental chromium level as high Cr- yeast on Log_2 antibody titer (total, IgG and IgM) of lambs, values are least squares means (n = 12 observations for control and 36 observations for Cr treatment groups).

day 10 of the experiment was nearly 20% ($p<0.01$) greater in Cr-supplemented *versus* control lambs (Figure 1a). Re-inoculation of PHA on day 70 of the experiment induced an even greater skin reaction, with more than 50% increase in Cr-supplemented *versus* control groups (Figure 1b) and there was a significant linear increase in that response with increasing dietary Cr-supplementation ($p<0.01$).

Weekly and overall anti-CRBC antibody titers were also significantly enhanced by Cr-supplementation (Figure 2a, b). The overall log_2 means of total and IgM antibody titers in Cr-supplemented lambs were 6.92 and 3.18, with corresponding means of 6.27 and 2.81, respectively, in the

controls and there was a significant linear increase ($p<0.01$) in that response with increasing Cr supplementation compared to controls. On the other hand, mean IgG titers were closely similar in Cr-supplemented and control groups (3.72 and 3.86, respectively) except on day 14 post-immunization when IgG titers in Cr fed groups significantly ($p<0.05$) surpassed the controls (Figure 2c). There are no previous studies on the effect of Cr supplementation on immune responses of lambs. However, studies in beef and dairy cattle (Chang and Mowat, 1992; Moonsee-Shageer and Mowat, 1993) as well as pigs (Van Heugten and Spears, 1997) have shown that Cr supplementation enhanced humoral antibody response to inoculation with foreign RBCs. It was also reported that Cr dietary supplementation at 0.4 mg/kg significantly increased humoral antibody response to triple immunization with IBR-BVD-PI vaccines in calves by nearly 20% (Kegley et al., 1997a). Cr-supplementation was also shown to induce a higher Concanavalin-A stimulated blastogenic response of peripheral blood mononuclear cells in production-stressed cows as compared to controls (Burton et al., 1993).

Several studies indicate that normal dietary intake of Cr in humans and animals may be suboptimal (reviewed by Anderson, 1997) and that further Cr deficiency may be induced by stress factors (Stoeker, 1999). Therefore, the supplementation of appropriate amounts of Cr can be beneficial in reducing some of the effects of stress. As shown in the present and several previous studies, one of the benefits of Cr supplementation during stress seems to be augmentation of immune system functioning. The exact mechanism by which that action is brought about is not yet known. Primarily, Cr is an essential trace element that potentiates the action of insulin and hence influences protein, lipid and carbohydrate metabolism (Nielson, 1995) including the synthesis of nuclear proteins and nucleic acid (Weser and Koolman, 1969; Okada et al., 1989). These functions contribute to improved performance and health of stressed animals, and could be associated with some immuno-endocrine interactions that might be coordinated through the production of regulatory cytokines (Borgs and Mallard, 1998).

CONCLUSIONS

The present study suggests that Cr dietary supplementation is beneficial in reducing the effect of stress and increasing resistance to diseases in lambs. Both humoral and cell-mediated immune responses of transport-stressed lambs are significantly enhanced in Cr-supplemented versus un-supplemented animals, and no untoward clinical or blood biochemical effects are found that may be attributed to the Cr supplementation. These findings will hopefully stimulate further investigations into the role of Cr as an anti-stress factor in sheep and the mechanisms by which it potentiates their immune responses. The daily Cr requirement of sheep and the optimal supplemental dose of Cr during stress should also be determined, while larger-scale studies should be undertaken to ascertain the role of Cr supplementation in non-stressed lambs as compared to those subjected to different forms of stress in the field and under experimental conditions.

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