Journal of Animal and Veterinary Advances 7 (5): 564-569, 2008
ISSN: 1680-5593
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Effects of Chromium Picolinate on Weight Gain, Selected Blood Metabolites, Leptin and Immunity in Calves

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Abstract: This study was performed to investigate the effects of chromium picolinate (CrPic) on weight gain, some blood variables including leptin and to evaluate whether there is any relationship between leptin and immune response in chromium supplemented non-stressed calves. Twenty-four. Holstein calves evenly divided into 3 groups. The age of the calves ranged from 6 to 8 weeks and their initial weight were 76.17±8.56 kg. Calves received orally either 0, 200 or 400 μg Cr/calf/day for 12 weeks in the form of CrPic. Calves in all groups were offered a commercial starter and grower diets *ad libitum* up to 2.5 kg and alfalfa hay and water were provided *ad libitum*. Animals were vaccinated with inactivated IBR-marker vaccine (Bayovac-Bayer) at 6 and 9th weeks of the experiment. The weight of the animals was recorded and weight gain was calculated. Blood samples were collected on 21 at days of both vaccinations for determination of primary and secondary antibody responses. Sera were analysed for serum total protein, albumin, glucose, iron, chromium and leptin. Supplementation of 200 and 400 μg Cr/calf/day slightly increased weight gain and significantly enhanced the primary and secondary antibody responses against IBR-marker vaccine in the calves without effecting blood leptin and other serum variables. Supplementation of 400 μg Cr/calf/day had no further improvement in immune response, therefore a daily supplementation of 200 μg chromium may be recommended to enhance the efficacy of vaccination in calves in the field conditions.

Key words: Calves, chromium, immunity, leptin, blood metabolites, weight gain

INTRODUCTION

Trivalent chromium (Cr^{+3}), which has been considered as an essential trace element for human and animals, involves in carbohydrate, lipid and protein metabolisms by enhancing the insulin action (Mertz, 1993; McDowell, 1992; BANR, 2001). Several previous studies investigating the effects of various chromium sources on growth performance in calves revealed inconsistent results. In some studies, different form and levels of chromium increased average daily weight gain, especially in the early phase of growing period (Chang and Mowat, 1992; Moonsie-Shageer and Mowat, 1993; Kegley *et al*., 1997a, b; Depew *et al*., 1998). On the other hand, in some other studies, supplemental chromium had no effect on daily weight gain in ruminants (Bunting *et al*., 1994; Kegley and Spears, 1995; Kegley *et al*., 1995, 1996, 2000; Arthington *et al*., 1997; Besong *et al*., 2001).

Inconsistent results were also reported concerning the effects of a range of chromium supplements including chromium nicotinate, chromium chloride, chelated chromium, high chromium yeast and chromium picolinate on immunity in response to vaccination or foreign proteins in various animal species (Chang and Mowat, 1992; Moonsie-Shageer and Mowat, 1993; Kegley and Spears, 1995; Kegley *et al*., 1996; van Heugten and Spears, 1997; Arthington *et al*., 1997; Uyanik *et al*., 2002).

Leptin, an ob gene product, is mainly produced and secreted by the adipose tissue (Nelson and Cox, 2000). It is also present within both primary and secondary lymphoid organs and has a significant metabolic and immunomodulatory role. Leptin's functional receptor

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(ObRb) is expressed in the hypothalamus where it regulates energy homeostasis and neuroendocrine function and in all cell types of innate and adaptive immunity (Matarese *et al*., 2005). It has been reported that leptin plays an important role in T lymphocyte response, and it can serve as a neuroendocrine signal between body fat and immunity regulating humoural immune response (Fantuzzi and Faggioni, 2000; Busso *et al*., 2002; Demas and Sakaria, 2005).

Lymphocytes, including T and B cells, express insulin receptor on their surface. When they are activated by antigen or mitogen, their receptor number and affinity are increased. It was suggested that chromium has a direct effect on lymphocytes proliferation thus may affect immune response by interactions of Cr and insulin action (Chang *et al*., 1996). In previous studies, the reducing effects of chromium compounds on body fat have been shown in animals (Page *et al*., 1993; Lien *et al*., 1999; Uyanik, 2001) whereas in some other studies same effect was not found (Wenk *et al*., 1995; Mooney and Cromwell, 1997). In recent years it has been shown that chromium decreases blood leptin, which is mainly secreted from the fat tissue (Sun *et al*., 2000; Bennett *et al*., 2006; İnanç *et al*., 2006). From these findings, it can be hypothesized that a relationship may exist between immunity, leptin and chromium. However, to the authors' knowledge, no research has evaluated this possible relationship in non-stressed chromium supplemented calves.

The objective of this study was to determine the effects of chromium picolinate on weight gain, some blood variables including leptin and immune response as well as to evaluate the possible interaction between chromium, leptin and immune response in calves.

MATERIALS AND METHODS

Animals and experimental design: Twenty-four Holstein calves, which were born on a commercial dairy farm and reared according to the routine practice in the field, were used. Calves were ear-tagged and divided into three groups (3 bull calves and 5 heifer calves in each group) according to their weight and birth dates. The age of the calves ranged from 6-8 weeks and their initial weight were 76.17±8.56 kg (mean±SD).

Calves in one group was kept as control without supplemental chromium, calves in the remaining two groups received orally either 200 or 400 μg Cr/calf/day for 12 weeks in the form of CrPic in gelatine capsules as a bolus. A commercial starter calf diet was provided for the first month of the study and thereafter a grower calf diet was provided (Table 1) *ad libitum* up to 2.5 kg for each animal and alfalfa hay and water were provided *ad libitum* throughout the experiment. Calves were individually housed in separated pens of 2.5 m^2 of floor space for the first month of the experiment. Then all animals were kept together in a half-open stall allowing 15 m^2 of floor space for per calf for the remaining period of the experiment.

Table 1: Ingredients and chemical composition of diets fed to calves

Ingredients	Starter diet (%)	Grower diet (%)
Corn	39.25	35.00
Wheat	19.00	7.00
Soy meal	14.00	12.00
Fulfat soy	8.00	2.00
Barley	5.00	16.00
White beet meal	5.00	9.00
Sunflower meal	5.00	14.25
Lime	3.00	3.00
Dicalcium phosphate	1.00	1.00
Salt	0.50	0.50
Vitamin and mineral premix	0.25	0.25
Chemical composition by calculation		
Dry matter (DM)	90.10	90.00
Crude protein	18.20	17.30
Crude fiber	4.60	6.90
Crude ash	7.20	7.60
Calcium	1.50	1.50
Phosphorus	0.60	0.60
ME (MJ/kg)	11.72	11.09

Vitamin and mineral premix (provided per kg): Vit. A, 15 000 000 IU;Vit D, 3 000 000 IU;Vit E, 30 000 mg, Niacin, 125 000 mg; Mn, 50 000 mg; Fe, 50 000 mg; Zn 50 000 mg; Cu, 10 000 mg; I, 800 mg; Co, 150 mg; Se 150 mg

Bovine herpesvirus 1 (BHV1), a pathogenic agent causing Infectious Bovine Rhinotracheitis (IBR) and Infectious Pustular Vulvovaginitis (IPV) infections in cattle, results in important economical loses (Ackermann *et al*., 1990). Therefore, vaccination of animals with inactivated IBR vaccine against BHV1, which causes latent infection (Ackermann and Wyler, 1984), is a routine practice in the field conditions. To determine the effect of chromium on immune response, animals in all groups were vaccinated with inactivated IBR-marker vaccine (Bayovac, Bayer AG, Leverkusen) at 6 and 9th weeks of the experiment for primary and secondary antibody responses.

Data and sample collection and analysis: At the beginning of the study and thereafter 2 weekly intervals, the weight of the animals was recorded and weight gain was calculated. Blood samples were collected from V. jugularis on days 21 after both vaccinations. Sera were separated by centrifugation at 1500 g for 10 min. after one hour incubation at room temperature and stored at 20°C until the determination of primary and secondary antibody responses.

At the end of the study, sera were analysed for serum total protein, albumin, glucose (Biolabo, France) and iron (Futura, Italy) with commercially available kits by a

Shimadzu UV/VIS 1208 model spectrophotometer. Serum globulin levels were calculated by subtracting albumin values from total protein values. Serum leptin levels were determined with a multi-species. Radioimmunoassay kit (Linco, Research Inc. USA). Sera were analyzed for chromium by an atomic absorption spectrophotometer (Varian AA 880) equipped with a graphite furnace (GTA-110). The sera were mixed with 0.1% of triton-X (1:1) and then chromium levels were measured.

Bovine Herpes Virus1 (BHV1) specific antibodies were detected by a micro-neutralization technique by the method of Frey and Liess (1971). Madin Darby Bovine Kidney (MDBK) cells that were cultured with Dulbecco's Modified Minimal Essential Medium (DMEM) (Biochrom, T-041-10, Germany) containing 10% calf serum was used for microneutralization test. Neutralisation was performed with BHV1 Cooper strain. Two sets of twofold serial dilutions of heat-inactivated (at 56°C for 30 min) sera were made with PBS (pH 7.4) for testing serum neutralization 50 (SN_{50}).

Statistical analysis: Data were analysed by SPSS 13.0 version for Windows. One-way analysis of variance (ANOVA) was used for the differences between groups. When the F values were significant, Duncan's Multiple Range Test was performed. All data were expressed as means±SEMs unless otherwise stated. Differences were considered as significant at $p<0.05$.

RESULTS

Chromium supplementation increased the overall weight gain of calves by 10.07 and 17.68% for 200 and 400 μg chromium levels respectively without reaching any significance (Table 2). As indicated in another part of this study, which was submitted previously, the serum chromium levels increased slightly but not significantly reaching 2.16±0.86 and 3.21±0.9 mg mL^{-1} in 200 and 400 μg Cr supplemented groups respectively compare to the control's level of 1.23±0.36 mg mL^{-1}. No effects of both level of chromium supplementation were found on serum iron, total protein, albumin, globulin, glucose and leptin levels (Table 3).

Compare to control, 200 and 400 μg Cr supplementation increased primary and secondary antibody responses against inactivated IBR marker vaccine ($p<0.001$). There was no significant difference between 200 and 400 μg Cr supplemented groups. Primary and secondary antibody responses in all groups did not differ from each other (Fig. 1 and 2).

Table 2: Effects of chromium picolinate on weight gain in calves

	Chromium picolinate (μg/calf/day)			
Parameters	Control n: 8	200 n: 8	400 n: 8	p
Initial body weight (kg)	75.00±2.27	80.75±2.18	72.75±3.91	p>0.05
Final body weight (kg)	122.32±5.79	132.82±2.63	128.49±4.01	p>0.05
Total weight gain (kg)	47.33±4.58	52.08±2.07	55.74±2.78	p>0.05
Daily weight gain	0.526±0.051	0.579±0.023	0.619±0.031	p>0.05

Table 3: Effects of chromium picolinate on serum blood chemistry in calves

	Chromium picolinate (μg/calf/day)			
Parameters	Control n: 8	200 n: 8	400 n: 8	p
Total protein (g dL^{-1})	5.81±0.43	6.31±0.70	5.80±0.51	p>0.05
Albumin (g dL^{-1})	3.67±0.50	3.39±0.30	3.12±0.36	p>0.05
Globulin (g dL^{-1})	2.13±0.41	2.92±0.64	2.68±0.58	p>0.05
Glucose (mg dL^{-1})	55.20±5.32	55.88±2.49	53.24±4.11	p>0.05
Leptin (ng mL^{-1})	2.28±0.21	2.09±0.23	2.20±0.20	p>0.05
Iron (μol dL^{-1})	16.91±1.18	17.14±1.16	17.94±1.31	p>0.05

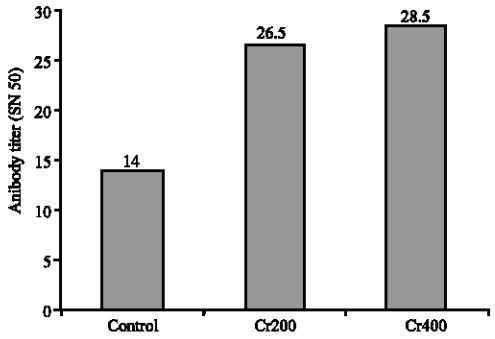

Fig. 1: Effect of chromium picolinate on the primary antibody response against inactivated IBR marker vaccine

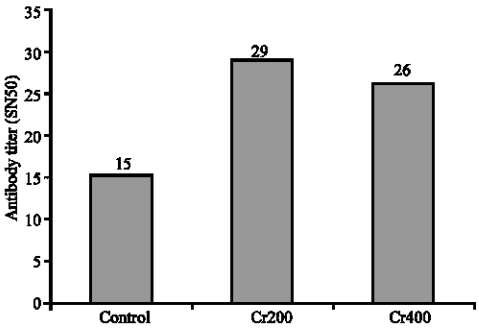

Fig. 2: Effect of chromium picolinate on the secondary antibody response against inactivated IBR marker vaccine

DISCUSSION

Some previous studies have shown that chromium supplementation from different sources had no effect on

weight gain (Bunting *et al*., 1994, Kegley and Spears, 1995; Kegley *et al*., 1996, 2000; Arhington *et al*., 1997; Besong *et al*., 2001).

On the other hand, Moonsie-Shageer and Mowat (1993) reported a 27% increase in average daily gain for Holstein calves fed a diet supplemented with 0.2 or 0.1 mg kg^{-1} of chromium as yeast. Kegley *et al*. (1997a) reported that 0.4 mg kg^{-1} of supplemental chromium-nicotinic acid complex or Cr Cl_3 increased the average daily gain from d 28 to 42, but not over the entire 63 day performance phase in calves. In the present study, although statistically not significant, daily supplementation of calves with 200 and especially 400 μg Cr in the form of CrPic tended to improve weight gain as in the study of Depew *et al*. (1998) who found a minor effect of chromium tripicolinate at a level of 1 ppm on growth performance.

In the present study, chromium had no effects on serum concentrations of total protein, albumin, globulin (Moonsie-Shageer and Mowat, 1993) and glucose (Moonsie-Shageer and Mowat, 1993; Kegley *et al*., 1997a; Bryan *et al*., 2004) as indicated previously. Functional ruminants derive little glucose from intestinal absorption and the role of insulin in glucose homeostasis differs from non-ruminants (Depew *et al*., 1998; Stahlhut, 2004) from milk-fed ruminants. The lack of the effect of chromium on glucose may be due to the possible insulin resistance with the aging of the calves. The results of this study concerning the growth performance and metabolism confirmed the results of some previous studies but did not confirm some other studies. The variable results obtained in various studies may be due to differences in chromium status of calves, the amount of stress to which the calves had been exposed, the amount and bioavailability of chromium in the diet, the bioavailability of the supplemental chromium source or duration of supplementation.

Since, the chromium losses occur in stressed animals (Anderson *et al*., 1982; Anderson *et al*., 1991), supplemental chromium seems to improve immune status mostly in stressed animals (Chang and Mowat, 1992; Moonsie-Shageer and Mowat, 1993; Kegley and Spears, 1995). On the other hand, similar effect of chromium supplementation was not found (Arhington *et al*., 1997; Kegley *et al*., 1997b). Burton *et al*. (1994) found an improvement in the primary antibody response against IBR vaccine by supplemental chromium. Similarly, in the present study, the 200 and 400 μg Cr supplementation increased primary and secondary antibody responses against inactivated IBR marker vaccine in the calves, which were not exposed to extreme conditions, may show that chromium supplementation in non-stressed animals is also effective.

In recent years, it has been shown that leptin plays an important role in T lymphocyte response (Fantuzzi and Faggioni, 2000; Busso *et al*., 2002) and it can serve as a neuroendocrine signal between body fat and immunity regulating humoural immune response (Demas and Sakaria, 2005). Lymphocytes, including T and B cells, express insulin receptor on their surface. When the cells are activated by an antigen or mitogen, their receptor number and affinity are increased (Chang *et al*., 1996). The serum leptin level is considered as an indicator of fat mass in the body, because leptin production and release rises with the number and size of adipocytes (Nelson and Cox, 2000). Since, the chromium reduced fat deposition in several animal species (Page *et al*., 1993; Lien *et al*., 1999; Uyanik, 2001) and lowered blood leptin in human and laboratory animals (Sun *et al*., 2000; Bennett *et al*., 2006; İnanç *et al*., 2006), existence of a possible interaction can be expected between leptin and immunity. However, in the present study, none of the chromium level influenced the serum leptin concentration of the calves as in the study of Woodworth *et al*. (2004) who investigated the effect of chromium on plasma leptin level of gestating sows. Lack of any difference in leptin level may show that chromium has no effect on fat deposition in the present study as indicated by Wenk *et al*. (1995) and Mooney and Cromwell (1997). No difference in leptin level may be due to the low fat tissue of the animals because they were in growing phase. Low or high leptin levels lead to alterations in immune response whereas the normal leptin level maintains optimal immune response (Matarese *et al*., 2005). Improvement in immune response without any changes in leptin levels by chromium supplementation suggests that chromium may enhance immune response trough other possible mechanisms. Cortisol, the most important glucocorticoid, is immunosuppressive by inhibiting the production and actions of cytokines and antibodies, lymphocyte function and leucocyte population (Moonsie-Shageer and Mowat, 1993). Although, in this study the cortisol level was not determined, the improvement in immune response by chromium supplementation may result from either reduced cortisol level (Chang and Mowat, 1992; Moonsie-Shageer and Mowat, 1993; Kegley *et al*., 1996; Uyanik *et al*., 2002) or trough other mediators released by cells of the immune system (Moonsie-Shageer and Mowat, 1993) or trough the effects of chromium on the metabolisms of other elements which are incorporated in the structure of specific enzymes regulating immunoglobulin production (Chang and Mowat, 1992).

CONCLUSION

The results of the present study have shown that chromium supplementation in the form of chromium

picolinate has minor effect on growth performance. Daily supplementation of 200 and 400 μg chromium enhanced the immune response of the conventionally managed non-stressed calves without effecting blood leptin. Lack of the significant difference between 200 and 400 μg of chromium supplementation showed that 200 μg chromium may be sufficient to stimulate immune response in calves. However, further well-controlled investigations are warranted to highlight the interrelationship between chromium supplementation, leptin and immune response.

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