Biol Trace Elem Res (2009) 129:157–169
DOI 10.1007/s12011-008-8282-9

Chromium(III) Nanoparticles Affect Hormone and Immune Responses in Heat-Stressed Rats

Longying Zha · Jingwen Zeng · Suxia Sun · Hong Deng · Haiji Luo · Wanli Li

Received: 20 October 2008 / Accepted: 11 November 2008 /
Published online: 28 November 2008
© Humana Press Inc. 2008

Abstract This study was conducted to evaluate the effects of chromium nanoparticles (CrNano) on the hormone and immune responses of rats in heat stress condition. A total of 80 male Sprague–Dawley rats were randomly assigned to four dietary treatment groups (n=20). The first group was offered a basal diet as a control. The second, third, and fourth groups received basal diet supplemented with 150, 300, and 450 μg/kg Cr, respectively, in the form of CrNano. At the end of the 8-week trial, growth performance, food utilization, and sera concentrations of hormones, immunoglobulins, and alexins were determined. Lymphocyte proliferation activity, antibody response to injected sheep red blood cells (SRBCs), and phagocytosis of peritoneal macrophages were determined by ^{3}H-thymidine uptake method, plaque-forming cells (PFC) assay, and ingesting chicken red blood cells test, respectively. The results indicated that rats that received CrNano exhibited no changes in growth rate and food efficiency compared to the control group. However, dietary supplementation of 150, 300, and 450 μg/kg Cr from CrNano significantly decreased serum concentrations of insulin and cortisol, increased sera levels of insulin-like growth factor I and immunoglobulin G, and enhanced the lymphoproliferative response, anti-SRBC PFC response, and phagocytic activity of peritoneal macrophages. These results suggest that dietary supplementation of Cr as CrNano affects hormone and immune status in heat-stressed rats.

Keywords Chromium nanoparticles · Hormone · Immune · Rats · Stress

All authors listed have contributed to the work and have agreed to submit the manuscript to *Biological Trace Element Research*. The manuscript is original, has not been published before, and is not under consideration for publication elsewhere.

L. Zha (✉) · S. Sun · H. Deng · H. Luo · W. Li
Department of Nutrition and Food Hygiene, School of Public Health and Tropical Medicine,
Southern Medical University, No. 1838, Guangzhou Avenue North, Guangzhou 510515,
People's Republic of China
e-mail: lyzhasmu@126.com

J. Zeng
Department of Preventive Veterinary Medicine, College of Veterinary Medicine,
China Agricultural University, Beijing 100094, People's Republic of China

Humana Press

Introduction

Chromium (Cr), an essential nutrient for mammalian organisms, functions as a cofactor for insulin when in the trivalent oxidation state (Cr^{+3}). Therefore, Cr enhances the functions of insulin in cell metabolism [1]. Various trivalent chromate compounds have been used as nutritional supplements, weight-loss agents, and muscle-development agents in human and as feed additives in domestic animals [2–4]. However, responses of human or animals to Cr supplementation have been variable [5]. Moreover, it is unlikely for healthy individuals to develop Cr deficiency [6]. Thus, the use of Cr supplements is probably unnecessary for the general public. However, there are strong evidences indicating that dietary Cr addition is beneficial for people or animals under physiological, pathological, nutritional, or environmental stress [7–8]. The beneficial roles of Cr could be due to the fact that the storage of Cr in the body tends to be decreased or depleted under stress conditions [8–9]. Under stress conditions, dietary Cr supplementation has been found to promote immunomodulatory function for both innate and adaptive immune response [9]. For example, in stressed feeder calves, dietary Cr supplementation increased the level of total serum immunoglobulin, particularly IgM, and enhanced primary antibody response to human red blood cells in the model employing the injection of human red blood cells [10]. The enhanced immune responses following Cr supplementation under the conditions of stress occur in association with changed profiles of endocrine hormones (e.g., insulin and cortisol) [11].

Cr is absorbed primarily in the small intestine [12, 13]. Thus, the absorption and utilization of Cr should be dependent on its status in intestinal tracts. The factors affecting the intestinal absorption of particles include their size, nature of the polymer, zeta potential, and vehicle [14]. Particle, when its dimension is reduced to nanometer size, exhibits new electrical, magnetic, mechanical, and biological properties [15]. Previously, Cr nanoparticles (CrNano) with average particle size ranging from 40 to 70 nm was shown to have beneficial effects on growth performance, carcass characteristics, and pork quality in finishing pigs [16] and on body compositions in rats [6]. However, the effects of CrNano supplementation on hormone and immune responses under stress condition still remain unclear. Numerous studies have shown that nanoparticles are more inclined to be recognized by the immune system and ingested by immune cells such as macrophages, monocytes, and leukocytes [17]. Hence, the objective of the present study was to investigate the effects of supplementation of graded doses of Cr in the form of CrNano on the hormone and immune responses in heat-stressed Sprague–Dawley rats.

Materials and Methods

Experimental Materials and Design

The CrNano with average size ranging from 40 to 70 nm was obtained from the Key Laboratory of Molecular Animal Nutrition, Ministry of Education, China. Eighty male Sprague–Dawley rats with an average initial body weight (BW) of 60±3 g were purchased from Shanghai Laboratorial Animal Center, Chinese Academy of Science (Shanghai, China).

All rats were randomly allotted to four dietary treatment groups of 20. The first group was offered a basal diet (composition is shown in Table 1) as a control. The second, third, and fourth groups received basal diet supplemented with 150, 300, and 450 μg/kg Cr,

Table 1 Composition of the Basal Diets

Ingredients	g/kg
Cornstarch	389.488
Casein	200.000
Dextrose	150.000
Sucrose	100.000
Soybean oil	60.000
Cellulose	50.000
Mineral mixture[a]	35.000
Vitamin mixture[b]	10.000
L-cysteine	3.000
Choline	2.500
T-butylhydroquinone	0.012

The basal diets were formulated to meet the American Institute of Nutrition (AIN-93G) recommendations for growing rats. The Cr content of basal diets was found to be 150±14 μg/kg as determined by atomic absorption spectrometry (AA6510, Schimadzu, Japan)

[a] Contained per kilogram diet: 35 mg iron, 5,000 mg calcium, 1,570 mg phosphate, 3,600 mg potassium, 300 mg sulfur, 1,020 mg sodium, 1,570 mg chlorine, 507 mg magnesium, 30 mg zinc, 50 mg manganese, 10 mg copper, 0.2 mg iodine, 0.15 mg selenium

[b] Contained per kilogram diet: 30 mg niacin, 16 mg pantothenic acid, 7 mg vitamin B_6, 6 mg thiamine, 6 mg riboflavin, 2 mg folic acid, 0.75 mg vitamin K, 0.2 mg D-biotin, 0.025 mg vitamin B_{12}, 4,000 IU vitamin A, 300 IU vitamin D_3, 75 IU vitamin E

respectively, in the form of CrNano. The rats were raised in stainless steel cage with five each. The diets and ultrapure water were provided ad libitum. The study was conducted for 8 weeks, and during the experiment, the house's temperature was measured four times a day (0600, 1200, 1800, and 2400). The mean value of daily temperature in the house was 30± 3°C. The environmental humidity is 40–60% and a 12-h light/dark cycle was maintained. BW and food intake were recorded once a week over the 8-week period. Average daily gain (ADG), daily food intake (DFI), and food efficiency (FE) were calculated from these records. The protocol employed in this experiment was approved by Zhejiang University Animal Care and Use Committee.

Blood and Tissue Sampling

At the end of the feeding trial, ten rats from each dietary treatment group were chosen to be killed by anesthetization (ketamine, 8.7 mg/100 g BW, and xylazine, 1.3 mg/100 g BW) following an overnight fast and weighed for final BW. The abdominal cavity was opened, and blood was drawn directly from the abdominal aorta. The blood sample from each rat was divided into two portions. One portion of blood sample was used to prepare for serum by centrifugation at 1,000 rpm for 10 min and the other was heparinized to further isolate peripheral blood lymphocytes (PBL) for lymphocyte proliferation assay. The PBL was prepared by using lymphocyte separation medium (Sigma, St. Louis, MO, USA), then counted and assessed for viability by trypan blue dye exclusion, and adjusted at 2×10^6 cells/mL in RPMI-1640 medium containing 20% fetal bovine serum (FBS; GIBCO, Grand Island, NY, USA).

The thymus was removed and weighed. The spleen was aseptically removed, weighed, and crushed in a homogenizer with 5 mL RPMI-1640 medium (GIBCO, Grand Island, NY, USA). The homogenate was centrifuged at 1,000 rpm for 5 min at 4 °C, the supernatant

discarded, and the pellet suspended in 5 mL sterile distilled water under slight agitation for 25 s to lyse the erythrocytes. The spleen cells were then diluted in 10 mL RPMI-1640 medium and centrifuged again. The resulting pellet of lymphocytes was washed again in 2 mL RPMI-1640 medium and filtered through a gauze (80 μm) to eliminate impurities. The recovered cells were diluted in RPMI-1640 medium containing 20% FBS, counted, and assessed for viability by trypan blue dye exclusion. The spleen cells concentration was adjusted at 2×10^6 cells/mL for subsequent test.

Determination of Serum Hormone, Immunoglobulin, and Alexin

The concentrations of insulin (INS), growth hormone (GH), insulin-like growth factor I (IGF-I), thyrotropic-stimulating hormone (TSH), free triiodothyronine (FT_3), free thyroxine (FT_4), and cortisol in serum were determined using commercially available ^{125}I RIA kits (Beijing North Institute of Biological Technology, China) by the procedure of radioimmunoassay on a scintillation counter (Packard 8500, USA).

The levels of immunoglobulin A (IgA), IgG, IgM, alexin 3 (C_3), and alexin 4 (C_4) in serum were determined by special commercial kits (Cicheng Biochemical Reagent, Ningbo, China) with the recommended procedures.

Lymphocyte Proliferation

The mitogen-stimulated T and B cell proliferations were performed in vitro with isolated PBL and splenocytes. The concentrations of 5 μg/mL of concanavalin A (ConA; Sigma, MO, USA) for T cell proliferation and 10 μg/mL of lipopolysaccharides (LPS; Sigma, MO, USA) for B cell proliferation were used. Triplicate samples of splenocytes or PBL with or without mitogen were pipetted into individual wells of a 96-well microtiter plate and incubated for 72 h in a humidified atmosphere of 95% air and 5% CO_2 at 37°C. Four hours before harvesting, 50 μL medium containing 1 μCi ^3H-thymidine was added to the cultures. The ^3H-thymidine uptake by lymphocytes was measured by a liquid scintillation counter (Packard 8500, USA), and mean count per minute was determined for the triplicate samples. Mean count per minute was used to calculate the stimulation indices (SI) as follows: count per minute of stimulated cell cultures/count per minute of unstimulated cell cultures.

Anti-SRBC PFC Assay

Four days before the end of the feeding trial, five rats from each dietary treatment group were immunized via injection of the lateral tail vein with 0.4 mL of 1.25×10^8 sheep red blood cells (SRBCs) per milliliter sterile saline. At the end of the experiment, the rats were killed and the spleens were removed aseptically. Spleen cell suspensions were prepared by methods as described above. Aliquots of spleen cells were added to glass test tubes held at 47°C in a constant temperature water bath. Earle's balanced salt solution (GIBCO; containing 5% agar and 0.05% diethyl aminoethyl dextran), SRBCs, and guinea pig complement (GIBCO) were also added into the tube and mixed by vortexing. The content in each test tube was then poured into a 100-mm Costar Petri dish and covered with a $45\times$ 50-mm glass cover slip. The Petri dishes were incubated for 3 h at 5% CO_2 and 37°C, after which the number of plaques were counted. Results were then expressed as plaque-forming cells (PFC) per 10^6 cells. Each sample was assayed in triplicate.

Phagocytosis of Peritoneal Macrophages

Five rats from each dietary treatment group were selected to perform assay of phagocytosis of peritoneal macrophages. On the last day of the feeding trial, 0.5 mL of 1×10^7 chicken red blood cells (CRBC) was intraperitoneally injected into each rat, and the rats were euthanized 1 h later. The fluid of the abdominal cavity was collected to make a smear for each rat. The smear were incubated at 37°C for 30 min in a wet box, fixed with 95% ethanol, and then stained by Wright dye. The number of macrophage ingesting CRBC out of a total of at least 100 cells was calculated by direct visual enumeration using a light microscope. The phagocytic index (PI) was calculated using the following formula: PI= number of total ingested CRBC/number of macrophage ingesting CRBC.

Statistical Analysis

Data are presented as the mean±standard deviation (SD). Statistical differences among the means of the various groups were evaluated by analysis of variance using generalized linear model procedures and SAS software (SAS Institute, 1989). A probability of $P<0.05$ was considered significant.

Results

Growth Performance and Food Utilization

The effects of CrNano supplementation on the growth performance and food utilization of the heat-stressed rats were summarized in Table 2. No differences for growth performance and food efficiency were observed between the control group and the groups with supplementation of 150, 300, and 450 μg/kg Cr from CrNano.

Serum Hormone, Immunoglobulin, and Alexin

The effects of CrNano on the levels of hormones and immunoglobulins in the animal serum were determined. Dietary supplementation of 150, 300, and 450 μg/kg Cr from CrNano significantly decreased serum concentrations of insulin and cortisol and increased serum

Table 2 Growth Performance and Food Utilization of Heat-Stressed Male Sprague–Dawley Rats Fed CrNano for 8 Weeks

Parameters	Cr (μg/kg) as CrNano			
	Control	150	300	450
Initial BW (g)	59.0±3.2	60.6±2.6	60.5±2.4	59.9±2.9
Final BW (g)	358.0±6.8	363.2±5.9	364.8±9.4	366.7±7.6
ADG (g/day)	5.3±0.1	5.4±0.1	5.4±0.2	5.5±0.1
DFI (g/day)	25.7±1.7	25.3±2.3	24.5±2.9	24.7±1.4
FE (g weight gain/100 g food)	20.9±1.4	21.6±2.3	22.5±3.0	22.3±1.3

Data are presented as the means±SD, $n=20$ per treatment

IGF-I level in heat-stressed rats (Table 3). However, concentrations of GH, TSH, FT_3, and FT_4 in serum were not significantly affected.

Sera IgG levels in heat-stressed rats were significantly increased by dietary addition of 150, 300, and 450 μg/kg Cr in the form of CrNano (Fig. 1). However, supplementation of Cr from CrNano did not produce significant effects on serum concentrations of IgA, IgM, C_3, and C_4 (Fig. 1).

Weights of Thymus and Spleen

As shown in Table 4, no significant effect of supplemental Cr from CrNano on the weights of the thymus and spleen of heat-stressed rats was observed.

Lymphocyte Proliferation

The mitogen-stimulated T and B lymphocyte response to ConA and LPS, respectively, of both PBL and spleen cells, harvested from dietary-treated (150, 300, and 450 μg/kg Cr from CrNano) and control rats is shown in Fig. 2. With ConA and LPS, the mean lymphoproliferative response was significantly elevated in dietary Cr-treated rats. In addition, the ConA-stimulated response of spleen lymphocytes was elevated in a Cr dose-dependent manner.

Anti-SRBC PFC Response

The antibody response of spleen cells isolated from dietary Cr-treated rats to SRBC was significantly higher than that of those isolated from control rats (Fig. 3). In addition, treatment of heat-stressed rats with Cr from CrNano appeared to improve the PFC response in a dose-dependent manner.

Phagocytosis Ability

The phagocytic activities of macrophages isolated from both control and dietary Cr-treated rats were assessed (Fig. 4). The uptake capacity of peripheral phagocytes was increased by CrNano treatment. Although the PI was increased significantly by treatment of 150, 300,

Table 3 Hormone Levels in Sera of Heat-Stressed Male Sprague–Dawley Rats Fed CrNano for 8 Weeks

Parameters	Cr (μg/kg) as CrNano			
	Control	150	300	450
Insulin (μIU/mL)	45.32±5.68a	27.31±2.98b	26.34±1.96b	26.31±2.21b
GH (ng/mL)	1.12±0.08	1.09±0.11	1.14±0.16	1.11±0.06
IGF-I (ng/mL)	1.94±0.12a	2.96±0.16b	3.33±0.21b	3.31±0.32b
TSH (μIU/mL)	0.10±0.02	0.07±0.02	0.15±0.05	0.14±0.02
FT_3 (fmol/mL)	2.66±0.25	2.70±0.20	2.59±0.27	2.66±0.26
FT_4 (fmol/mL)	2.41±0.17	2.36±0.23	2.29±0.30	2.21±0.20
Cortisol (ng/mL)	5.07±1.93a	4.35±1.02b	4.47±1.33b	4.16±0.91b

Data are presented as the means±SD, $n=10$ per treatment. Means in a row with different letters differ significantly ($P<0.05$)

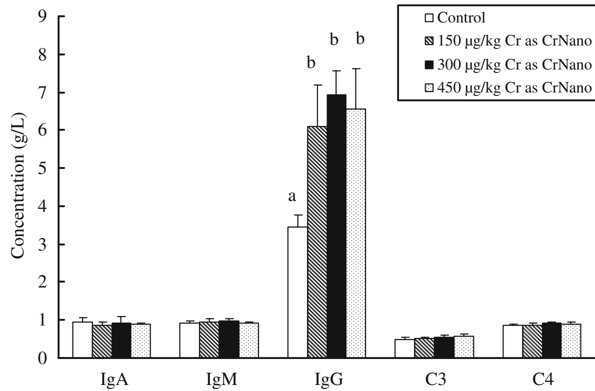

Fig. 1 Serum contents of immunoglobulin and alexin in heat-stressed male Sprague–Dawley rats fed CrNano for 8 weeks. Data are presented as the means±SD, n=10 per treatment. Different superscript letters connote statistically significant differences (P<0.05)

and 450 μg/kg Cr as CrNano, PI with treatment of 450 μg/kg Cr was slightly lower than that with treatment of 300 μg/kg Cr.

Discussion

Two major types of trivalent Cr compounds, i.e., the organic and inorganic Cr compounds, have been used as nutritional supplements in human and feed additives in animals. Although the positive effects of dietary supplementation of these Cr compounds have varied among reports, it is generally believed that inorganic Cr salts are not as biologically active as organic forms of Cr [5]. The underlying reason could be the superior absorptivity of organically complexed Cr [5, 7]. It is known that nanoparticles, for their small size effects, are shown to exhibit greater absorption rate both in vivo and in vitro in comparison to macroparticles [18–19]. The beneficial effects of CrNano on growth performance, carcass characteristics, and pork quality in finishing pigs [16] and on body compositions in rats [6] have attributed to its superior absorptivity [20]. High ambient temperature is considered to be a physiological stress which promotes the loss of body Cr store [8–9]. Therefore, the supplementation of Cr with high absorptivity might contribute to alleviate the negative effects of unfavorable conditions on animal growth.

In the present study, no change in growth rate and food efficiency was observed in the heat-stressed rats with dietary treatment of CrNano. The effect of CrNano on growth performance in rats reported in this study is inconsistent with our previous 6-week study under no heat stress condition in which both average daily gain and food efficiency were improved by the supplementation of 300 μg/kg Cr from CrNano [6]. In fact,

Table 4 Weights of the Thymus and Spleen of Heat-Stressed Male Sprague–Dawley Rats Fed CrNano for 8 Weeks

	Cr (μg/kg) as CrNano			
	Control	150	300	450
Thymus (g/kg)	1.64±0.13	1.74±0.12	1.52±0.19	1.83±0.18
Spleen (g/kg)	2.22±0.14	2.24±0.17	1.94±0.13	2.14±0.14

Data are presented as the means±SD, n=10 per treatment

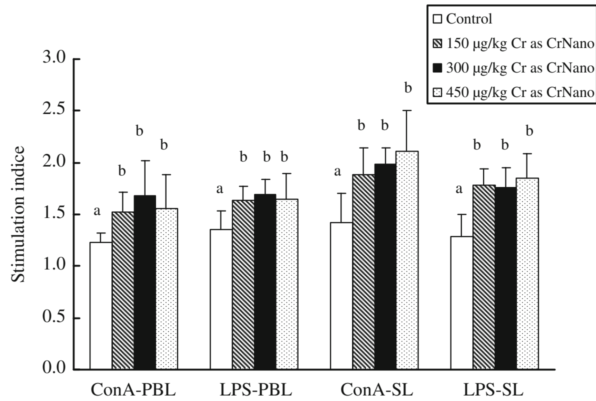

Fig. 2 Stimulation indices with T or B cell mitogen stimulation of peripheral blood and spleen lymphocytes in heat-stressed male Sprague–Dawley rats fed CrNano for 8 weeks. Data are presented as the means±SD, n=10 per treatment. Different superscript letters connote statistically significant differences (P<0.05). *ConA-PBL* ConA-stimulated PBL, *LPS-PBL* LPS-stimulated PBL, *ConA-SL* ConA-stimulated spleen lymphocyte, *LPS-SL* LPS-stimulated spleen lymphocyte

supplementation of bioavailable source of Cr is not uniformly efficacious on growth performance. Hasten et al. [21] observed no change in growth rate in Sprague–Dawley rats fed basal diets supplemented with Cr chloride, Cr picolinate, and Cr nicotinate. Results from Van de Ligt et al. [22] and Lien et al. [23] showed that Cr (in the form of Cr tripicolinate and propionate, respectively) supplementation produced no effect on growth rate and feed efficiency in pigs during the postweaning period. However, Barajas et al. [24] reported that supplementation of Cr methionine improved average daily gain and feed/gain ratio of transport-stressed bull calves. Therefore, no definite conclusion on the effect of Cr supplementation on growth performance can be drawn from these existing studies. In addition, the effect of CrNano supplementation on growth performance in physiological and heat-stressed conditions remains to be further investigated.

Cr is usually claimed to be associated with the function of insulin [1]. In the present study, dietary addition of 150, 300, and 450 μg/kg Cr from CrNano significantly decreased the serum insulin levels in heat-stressed rats. This is in agreement with our previous results of CrNano in finishing pigs [25] and rats [6] experiencing no external stresses. Under no externally applied stress conditions, Cr picolinate supplementation was found to decrease sera insulin levels in pigs [2, 26], but not in rats [21, 27]. The decrease of peripheral insulin levels may be due to the enhanced binding of insulin to its receptors on targeted tissues such as muscle and fat since Cr is thought to facilitate the interactions between insulin and

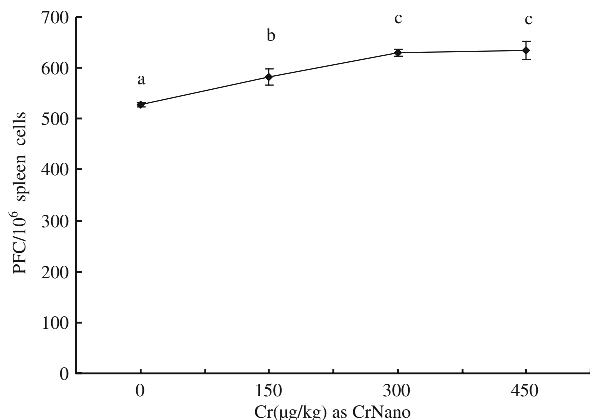

Fig. 3 Anti-SRBC response in heat-stressed male Sprague–Dawley rats fed CrNano for 8 weeks, as determined by the PFC assay. Data are presented as the means±SD, n=5 per treatment. Different superscript letters connote statistically significant differences (P<0.05)

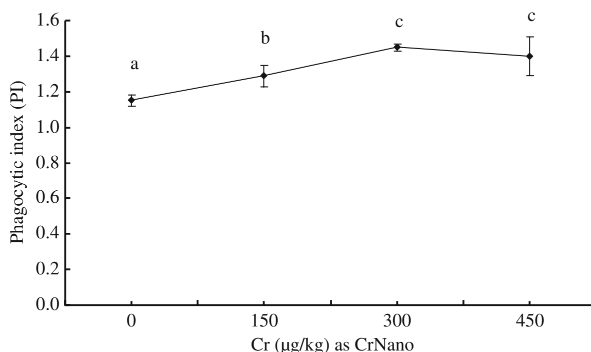

Fig. 4 PI of CRBC by peritoneal macrophages from heat-stressed male Sprague–Dawley rats fed CrNano for 8 weeks. Data are presented as means±SD, $n=5$ per treatment. Different superscript letters connote statistically significant differences ($P<0.05$)

insulin receptors and promote insulin internalization into cells [1]. Insulin causes cellular uptake and use of glucose after binding to its receptor. Once acquired by cells, glucose is used as an energy source which, along with the anabolic actions of GH and IGF-I, drives protein synthesis and proper maintenance and functioning of all the body's organs [9]. The elevated serum IGF-I level measured in heat-stressed rats offered the diets with supplemental CrNano may have played a role in the protein and energy metabolism. The lack of any significant effect of CrNano on serum GH in heat-stressed rats, however, suggests that the effect on IGF-I may have been independent of GH though a single blood sample is not a sensitive enough technique to assess treatment effects on GH given the pulsatile nature of GH release in most mammals [25, 28].

Cortisol is generally regarded as a calorigenic agent in many mammalian species and acts antagonistically to insulin because it prevents entry of glucose into peripheral tissues (e.g., muscle and fat) to spare it for tissues of higher demand (e.g., brain and liver) [9, 11]. Cold and heat exposure, isolation, strenuous exercise, disease, and numerous other forms of emotional, physical, metabolic, and environmental stresses increase plasma cortisol. Supplemental Cr has been demonstrated to reduce serum cortisol in pigs [25], guinea pigs [29], lambs [30], horses [31], calves [32], and humans [33]. In vitro, high concentration of Cr from Cr picolinate can directly inhibit cortisol secretion in agonist-stimulated adrenocortical cell line [34]. The present result is in line with these reports, indicating that Cr from CrNano may also have an "antistress" effect by lowering plasma cortisol. However, there are also a number of reports indicating no effect of Cr supplementation on serum cortisol levels [35–37]. The disparity of response may be related to the level and type of stress experienced by the animals. Furthermore, the ultimate target of dietary Cr supplementation may be the dynamic balance of cortisol, insulin, and probably, other endocrine modulatory effects [11].

Less data is available about the effects of Cr on thyroid hormone which increases metabolic rate causing increased oxygen consumption, heat production, and metabolism of fats, proteins, and carbohydrates in the liver, kidney, heart, and muscle [9]. In this study, no significant effect of supplemental Cr from CrNano on TSH, T_3, and T_4 were observed in heat-stressed rats, indicating a lack of role of CrNano in the metabolisms of thyroid hormones.

Dietary supplementation of 150, 300, and 450 μg/kg Cr from CrNano has not affected the relative weights of the thymus and spleen. In addition, no macroscopic physical damage was observed during the experiment. This indicates that the dosage of CrNano used in this study may have no negative toxic effect on rats.

The documented effects of dietary Cr supplementation on immune response in mammals have been inconsistent. The increased serum IgG level in heat-stressed rats in the present

study is in line with our previous result in finishing pigs experiencing no external stress [25], and with Chang and Mowat [10] and Moonsie-Shageer and Mowat [37] who demonstrated that total IgG was increased in calves supplemented with high-Cr yeast under transport stress. These studies also indicated that total IgM was elevated by supplemental Cr. However, in this study, serum total IgM was unaffected by supplementation of any dosage of Cr from CrNano. Kegley et al. [38] observed no change in total IgM but a decline in total IgG in transport-stressed calves fed diets supplemented with Cr nicotinate. The effect of supplemental Cr on serum total immunoglobulin may be associated with the experimental period. Van de Ligt et al. [22] have studied the effect of Cr tripicolinate supplementation of diet on porcine immune response during the postweaning period and found that total IgG was not affected by Cr on day 0 or on day 7, but on day 28, total IgG had risen and was affected by Cr, and total IgM followed a similar pattern.

The effect of Cr on lymphocytes has been investigated in several studies. Lymphocyte blastogenesis in response to phytohemagglutinin (PHA) in stressed calves receiving supplemental Cr nicotinate [35] and to ConA in morbid calves receiving supplemental chelated Cr [39] was found to be increased. Burton et al. [40] reported that supplementation of chelated Cr in diets of dairy cows resulted in an increase in lymphocyte blastogenesis of 57% during late gestation and parturition and of 31% at the time of peak milk yield. In this study, the lymphoproliferative response of PBL and splenocytes to ConA and LPS were significantly elevated in heat-stressed rats offered diets supplemented with Cr from CrNano, indicating that CrNano may have an immunostimulatory effect. However, Van de Ligt et al. [22] reported that no difference was detected in ear-swelling in response to the PHA mitogen in weanling pigs. Van Heugten and Spears [41] indicated that Cr chloride, Cr nicotinate, and Cr picolinate supplementation in weanling pigs enhanced lymphocyte blastogenesis in response to pokeweed mitogen, but not in response to PHA. Thus, the varied effects of Cr on lymphocyte may be associated with the differences of animals, mitogens, and stress levels used among reports.

The PFC assay allows the evaluation of immunomodulating compounds and presents the appropriate conditions to emphasize changes in immune functions [42]. In this study, dietary supplementation of Cr from CrNano significantly increased antibody production in response to SRBCs. This is in accordance with previous reports of increased antibody production in response to human red blood cells and ovalbumin in stressed calves with supplementation of high-Cr yeast [37, 43]. In contrast to these results, antibody production in response to ovalbumin when measured 14 days following immunization was decreased by dietary supplementation of Cr nicotinate to weanling pigs [41]. However, dietary addition with Cr chloride or Cr picolinate did not affect antibody response to either ovalbumin or SRBCs in weanling pigs [41]. These results indicated that different forms of Cr may have varied in their immunomodulatory roles. In addition, the immunomodulatory role of Cr seems to be antigen-dependent.

Phagocytes acts as regulator and effector cells in the immune system and phagocytosis represents the indispensable step of the immunological defense system [44]. Lee et al. [45] have shown a dose-dependent effect of Cr picolinate on phagocytosis of *Escherichia coli* by incubation of pulmonary alveolar macrophages in medium in the presence or absence of insulin. Our results showed that dietary supplementation of 150, 300, and 450 μg/kg Cr from CrNano enhanced the phagocytosis of peritoneal macrophages, indicating immunostimulatory effects. However, 450 μg/kg Cr from CrNano showed a slightly lower PI than 300 μg/kg Cr from CrNano. It suggested that high dosage of CrNano may have immunosuppressive effect on macrophages, which should await further verification.

The apparent effect of CrNano on immune status in the heat-stressed rats in this study may be explained by the notion that nanoparticles are inclined to be recognized by the immune system in animals and humans [17]. Evidence has shown that nanoparticles can be absorbed via gastrointestinal lymphatics and enhance the duration of immune response and concurrently stimulate humoral, cellular, and mucosal immune responses [46].

Conclusion

The present endocrine and immune parameter results suggest that dietary chromium supplementation in the form of chromium nanoparticles affects hormone and immune status in heat-stressed rats. Although the underlying molecular and cellular mechanism is not clear, our study indicates that the use of chromium nanoparticles might promote the healthiness of animals under stress conditions.

References

1. Vincent JB (2001) The biochemistry of chromium. J Nutr 130:715–718
2. Page TG, Southern TL, Ward TL, Thompson DL (1993) Effect of chromium picolinate on growth and serum and carcass traits of growing-finishing pigs. J Anim Sci 22:349–352
3. Anderson RA (1998) Effects of chromium on body composition and weight loss. Nutr Rev 56:266–270
4. Vincent JB (2004) Recent advances in the nutritional biochemistry of trivalent chromium. Proc Nutr Soc 63:41–47
5. Lukaski HC (1999) Chromium as a supplement. Annu Rev Nutr 19:279–302
6. Zha LY, Wang MQ, Xu ZR, Gu LY (2007) Efficacy of chromium (III) supplementation on growth, body composition, serum parameters, and tissue chromium in rats. Biol Trace Elem Res 119:42–50
7. Vincent JB (2004) Recent developments in the biochemistry of chromium (III). Biol Trace Elem Res 99:1–16
8. Al-Saiady MY, Al-Shaikh MA, Al-Mufarrej SI, Al-Showeimi TA, Mogawer HH, Dirrar A (2004) Effect of chelated chromium supplementation on lactation performance and blood parameters of Holstein cows under heat stress. Anim Feed Sci Technol 117:223–233
9. Burton JL (1995) Supplemental chromium: its benefits to the bovine immune system. Anim Feed Sci Technol 53:117–133
10. Chang X, Mowat DN (1992) Supplemental chromium for stressed and growing feeder calves. J Anim Sci 70:559–565
11. Borgs P, Mallard BA (1998) Immune-endocrine interactions in agricultural species: chromium and its effect on health and performance. Domest Anim Endocrinol 15:431–438
12. Chen NSC, Tsai A, Dyer IA (1973) Effect of chelating agents on chromium absorption in rats. J Nutr 103:1182–1186
13. Beitz DC (1997) Absorption, transport, and content in animal tissues. In: Committee on Animal Nutrition Board on Agriculture National Research Council (ed) The role of chromium in animal nutrition. National Academy Press, Washington, DC, pp 7–9
14. Delie F (1998) Evaluation of nano- and microparticle uptake by the gastrointestinal tract. Adv Drug Deliv Rev 34:221–233
15. Gref R, Minamitake YM, Peracchia T, Trubetskoy V, Torchilin V, Langer R (1994) Biodegradable long-circulating polymeric nanospheres. Science 263(5153):1600–1603
16. Wang MQ, Xu ZR (2004) Effect of chromium nanoparticle on growth performance, carcass characteristics, pork quality and tissue chromium in finishing pigs. Asian Australas J Anim Sci 17:1118–1122
17. Dobrovolskaia MA, Aggarwal P, Hall JB, McNeil SE (2008) Preclinical studies to understand nanoparticle interaction with the immune system and its potential effects on nanoparticle biodistribution. Mol Pharmacol 5(4):487–495
18. Desai MP, Labhasetwar V, Amidon GL, Levy RJ (1996) Gastrointestinal uptake of biodegradable microparticles: effect of particle size. Pharm Res 13:1838–1845

19. Desai MP, Labhasetwar V, Walter E, Levy RJ, Amidon GL (1997) The mechanism of uptake of biodegradable microparticles in Caco-2 cells is size dependent. Pharm Res 14:1568–1573
20. Zha LY, Xu ZR, Wang MQ, Gu LY (2008) Chromium nanoparticle exhibits higher absorption efficiency than chromium picolinate and chromium chloride in Caco-2 cell monolayers. J Anim Physiol Anim Nutr 92:131–140
21. Hasten DL, Hegsted M, Keenan MJ, Morris GS (1996) Effects of various forms of dietary chromium on growth and body composition in the rat. Nutr Res 17:283–294
22. Van de Ligt JLG, Lindemann MD, Harmon RJ, Monegue HJ, Cromwell GL (2002) Effect of chromium tripicolinate supplementation on porcine immune response during the postweaning period. J Anim Sci 80:449–455
23. Lien TF, Yang KH, Lin KJ (2005) Effects of chromium propionate supplementation on growth performance, serum traits and immune response in weaned pigs. Asian Australas J Anim Sci 18:403–408
24. Barajas R, Cervantes BJ, Virgilio RJ, Almeida L, Romo JM, Calderon JC (2005) Influence of chromium methionine supplementation on growth performance of medium stressed bull-calves during the receiving period in the feedlot. Proc West Sect Am Soc Anim Sci 56:430–432
25. Wang MQ, Xu ZR, Zha LY, Lindemann MD (2007) Effects of chromium nanocomposite supplementation on blood metabolites, endocrine parameters and immune traits in finishing pigs. Anim Feed Sci Technol 139:69–80
26. Amoikon EK, Fernandez JM, Southern LL, Thompson Jr DL, Ward TL, Olcott BM (1995) Effects of chromium tripicolinate on growth, glucose tolerance, insulin sensitivity, plasma metabolites, and growth hormone in pigs. J Anim Sci 73:1123–1130
27. Hasten DL, Hegsted M, Keenan MJ, Morris GS (1997) Dosage effects of chromium picolinate on growth and body composition in the rat. Nutr Res 17:1175–1186
28. Barb CR, Kraeling RR, Rampacek GB (2002) Metabolic regulation of the neuroendocrine axis in pigs. Reprod Suppl 59:203–217
29. Seaborn CD, Cheng N, Adeleye B, Owens F, Stoecker BJ (1994) Chromium and chronic ascorbic acid depletion effects on tissue ascorbate, manganese and C14 retention from C14-ascorbate in guinea pigs. Biol Trace Elem Res 41:279–294
30. Ward TL, Gentry LR, Fernandez JM, Horohov DW, Thompson Jr DL, Chapa AM, Southern LL (1995) Dietary protein and chromium tripicolinate in Suffolk lambs: II. Hematological and immunological measurements, and metabolic responses to feeding, glucose and epinephrine. J Anim Sci 73(suppl 1):265
31. Pagan JD, Jackson SG, Duren SE (1995) The effect of chromium supplementation on metabolic response to exercise in thoroughbred horses. In: Lyons P, Jacques KA (eds) Biotechnology in the feed industry: proceedings of Altech's 11th annual symposium. Nottingham University Press, Nottingham, pp 249–256
32. Kegley EB, Spears JW, Brown Jr TT (1996) Immune response and disease resistance of calves fed chromium nicotinic acid complex or chromium chloride. J Dairy Sci 79:1278–1283
33. McCarty MF (1993) Homologous physiological effects of phenformin and chromium picolinate. Med Hypotheses 41:316–324
34. Kim BG, Adams BA, Jackson BA, Lindemann MD (2006) Effect of chromium (III) picolinate on cortisol and DEHAs secretion in H295R human adrenocortical cells. FASEB J 20:142.2, (abstract)
35. Kegley EB, Spears JW (1995) Immune response, glucose metabolism, and performance of stressed feeder calves fed inorganic or organic chromium. J Anim Sci 73:2721–2726
36. Pollard GV, Richardson CR, Karnezos TP (2002) Effects of supplemental organic chromium on growth, feed efficiency and carcass characteristics of feedlot steers. Anim Feed Sci Technol 98:121–128
37. Moonsie-Shageer S, Mowat DN (1993) Effect of level of supplemental chromium on performance, serum constituents, and immune status of stressed feeder calves. J Anim Sci 71:232–238
38. Kegley EB, Spears JW, Brown Jr TT (1997) Effect of shipping and chromium supplementation on performance, immune response, and disease resistance of steers. J Anim Sci 75:1956–1964
39. Chang X, Mallard BA, Mowat DN (1994) Proliferation of peripheral blood lymphocytes of feeder calves in response to chromium. Nutr Res 14:851–864
40. Burton JL, Mallard BA, Mowat DN (1993) Effects of supplemental chromium on immune responses of periparturient and early lactation dairy cows. J Anim Sci 71:1532–1539
41. Van Heugten E, Spears JW (1997) Immune response and growth of stressed weanling pigs fed diets supplemented with organic or inorganic forms of chromium. J Anim Sci 75:409–416
42. Mimouni J, Ramsteiner A, Fontanges R (1995) In vivo study of the immunomodulating activity of OM-85 using the plaque-forming cells technique (PFC) in mice. Int J Immunopharmacol 17(9):787–792
43. Chang X, Mallard BA, Mowat DN, Gallo GF (1996) Effect of supplemental chromium on antibody responses of newly arrived feeder calves to vaccines and ovalbumin. Can J Vet Res 60:140–144

44. Zhang WY, Wang Y, Hou YY (2004) Effects of Chinese medicinal fungus water extract on tumor metastasis and some parameters of immune function. Int Immunopharmacol 4:461–468
45. Lee DN, Yen HT, Shen TF, Chen BJ (2000) Chromium-induced glucose uptake, superoxide anion production, and phagocytosis in cultured pulmonary alveolar macrophages of weanling pigs. Biol Trace Elem Res 77:53–64
46. He Q, Mitchell AR, Johnson SL, Wagner-Bartak C, Morcol T, Bell SJD (2000) Calcium phosphate nanoparticle adjuvant. Clin Diagn Lab Immunol 11:899–903