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Effects of chromium nanoparticle dosage on growth, body composition, serum hormones and tissue chromium in Sprague-Dawley rats*

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Abstract: This 6-week study was conducted to evaluate the effects of seven different levels of dietary chromium (Cr) (0, 75, 150, 300, 450, 600, and 1200 ppb Cr) in the form of Cr nanoparticle (CrNano) on growth, body composition, serum hormones and tissue Cr in Sprague-Dawley (SD) rats. Seventy male SD rats (average initial body weight of (83.2±4.4) g) were randomly assigned to seven dietary treatments (n=10). At the end of the trial, body composition was assessed via dual energy X-ray absorptiometry (DEXA). All rats were then sacrificed to collect samples of blood, organs and tissues for determination of serum hormones and tissue Cr contents. The results indicated that lean body mass was significantly increased (P<0.05) due to the addition of 300 and 450 ppb Cr from CrNano. Supplementation of 150, 300, 450, and 600 ppb Cr decreased (P<0.05) percent body fat significantly. Average daily gain was increased (P<0.05) by addition of 75, 150, and 300 ppb Cr and feed efficiency was increased (P<0.05) by supplementation of 75, 300, and 450 ppb Cr. Addition of 300 and 450 ppb Cr decreased (P<0.05) the insulin level in serum greatly. Cr contents in liver and kidney were greatly increased (P<0.05) by the addition of Cr as CrNano in the dosage of from 150 ppb to 1200 ppb. In addition, Supplementation of 300, 450, and 600 ppb Cr significantly increased (P<0.05) Cr content in the hind leg muscle. These results suggest that supplemental CrNano has beneficial effects on growth performance and body composition, and increases tissue Cr concentration in selected muscles.

Key words: Rat, Chromium (Cr), Nanoparticle, Growth, Body composition, Hormone
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INTRODUCTION

Chromium (Cr) is a trace element found in the environment commonly in trivalent, Cr (III), and hexavalent, Cr (VI), forms (Bagchi *et al*., 2002). However, Cr (III) is the most stable form in the food supply and in vivo (Mertz, 1969). It functions as a cofactor for the hormone insulin and enhances the ability of insulin to regulate glucose, protein, and fat metabolism (Mossop, 1983; Mertz, 1993). Various trivalent chromate compounds have been used as nutritional supplements in humans and feed additives in domestic animals (Lukaski, 1999). Dietary Cr supplementation can alter body composition, but the ability of different forms of Cr may vary in doing this (Hasten *et al*., 1997a). It is suggested that the absorption and utilization of Cr may be dependent on its status in intestinal tract (Wang and Xu, 2004). Size, nature of the polymer, zeta potential and vehicle have been determined as critical factors influencing particle uptake (Delie, 1998). Nanoparticle, which is at least one dimension reduced to a nanometer size, exhibits new electrical, magnetic, mechanical, and biological properties (Gref *et al*., 1994). Therefore, the new phenomena and properties of nanoparticle may have unique potential application.

Chromium nanoparticle (CrNano) has shown beneficial effects on carcass characteristics, pork quality and individual skeletal muscle weight when it was supplemented to the diets of finishing pigs (Wang

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and Xu, 2004). However, no definite conclusion on the beneficial effect of CrNano on altering body composition could be drawn. Furthermore, the dosage of different forms of Cr plays a role in producing effects on animal bodies. In swine, Page *et al*.(1993) found that changes of body composition (increase in longissimus muscle area and percentage of muscling, and decrease in the 10th rib fat) took place at Cr levels of 100, 200, 400, and 800 μg Cr/kg feed (also expressed as parts per billion, ppb), but growth rate was decreased at the higher levels of 400 and 800 ppb. The dosages in animal studies which have observed increases in lean body mass of rats (Hasten *et al*., 1993) and pigs (Shelton *et al*., 2003) and decreases in body fat of pigs (Lindemann *et al*., 1993) and steers (Pollard *et al*., 2002) due to Cr supplementation ranged from 25 to 1 000 ppb. Furthermore, Lindemann *et al*.(1993) found linear increases in lean body mass with linear decreases in body fat in swine for chromium picolinate (CrPic) additions of 100, 200, and 1000 ppb Cr. Therefore, the purpose of the present study was to examine the effects of different levels of dietary Cr (0, 75, 150, 300, 450, 600, and 1200 ppb) in the form of CrNano on growth, body composition, serum hormones and tissue Cr in Sprague-Dawley (SD) rats.

MATERIALS AND METHODS

Animals and dietary treatments

The animal welfare committee of Zhejiang Chinese Medical University, China, approved the protocol employed in this experiment. Seventy male SD rats with average initial body weight (BW) of (83.2±4.4) g were provided by Shanghai laboratory animal center, Chinese Academy of Sciences. The animals were randomly assigned to seven dietary treatments (n=10). The seven treatments used in the present study consisted of the following diets: (1) basal with no Cr supplementation; (2) basal+75 ppb Cr; (3) basal+150 ppb Cr; (4) basal+300 ppb Cr; (5) basal+450 ppb Cr; (6) basal+600 ppb Cr; (7) basal+1200 ppb Cr. Cr supplementation for all diets was in the form of CrNano. CrNano (average size 40~50 nm) provided by the Key Laboratory of Molecular Animal Nutrition, Ministry of Education, China. The basal diet consumed by all rats in this study was standard commercial rat pellets purchased from Zhejiang Academy of Medical Sciences, China. The composition of basal diet is shown in Table 1. The Cr content of ten random samples taken from different basal diet batches was found to be (200±12) ppb. The study was conducted for six weeks. The animals were kept in stainless steel housing with wire mesh flooring in a temperature (22~24 °C) and humidity (40%~60%) controlled environment with a 12-h light/dark cycle. All rats had free access to food and ultrapure water.

Table 1 Composition and nutritive value of basal diets

	Content (%)
Ingredients	
Corn meal	34.0
Soybean meal	25.0
Wheat starch	25.0
Wheat bran	5.0
Fish meal	5.0
Brewers dried yeast	1.0
Salt	1.0
Soybean oil	1.5
Mineral mixture[1]	1.5
Vitamin mixture[2]	1.0
Proximate composition	
Crude protein	21.4
Crude fat	4.2
Crude fibre	5.0
Crude ash	3.2
Calcium	1.2
Phosphorus	0.9
Gross energy (kJ/kg)	1676.2

[1] Contained per kg diet: Cu 10 mg, Zn 30 mg, Fe 120 mg, Mn 75 mg, Se 0.1 mg, I 0.5 mg; [2] Contained per kg diet: V_A 14000 IU, V_D 1500 IU, V_E 120 IU, V_K 5 mg, V_{B1} 13 mg, V_{B2} 12 mg, Niacin 60 mg, D-pantothenic acid 24 mg

Body composition evaluation

At the end of the feeding trial, body composition was determined by means of dual energy X-ray absorptiometry (DEXA) on the GE Lunar medical system. For this procedure, three rats from each dietary treatment were chosen to undergo DEXA analyses. Before the procedure, the animals were anaesthetized with a combination of ketamine (8.7 mg/100 g BW) and xylazine (1.3 mg/100 g BW). The rat whole body was then scanned to determine BW, lean body mass (LBM), body fat weight (BFW), and percent body fat (%BF).

Growth performance

Body weight and feed intake were recorded once a week over the 6-week period. Average daily gain (ADG) (g/d), daily feed intake (DFI) (g/d), and feed efficiency (FE) (g BW/100 g feed) were calculated from these records.

Serum hormones analyses

At the end of the 6-week treatment period, after an overnight fast, the rats were anaesthetized (ketamine, 8.7 mg/100 g BW, and xylazine, 1.3 mg/100 g BW) and weighed for final BW. The abdominal cavity was opened, and blood was drawn directly from the abdominal aorta. The blood samples were then centrifuged at 1000 r/min for 10 min. Following centrifugation, serum from each sample was collected and frozen at −70 °C until analysis. The concentrations of growth hormone (GH), insulin (INS), and cortisol (Corti) in serum were determined using commercial kits by a procedure of radioimmunoassay on a Scintillation Counter (Packard 8500, US). Kits of GH, INS, and Corti were purchased from Beijing North Institute of Biological Technology, China.

Tissue weight determination

The hind leg muscle was excised, connective tissue was carefully trimmed away, and the muscle wet weight was determined. The heart, liver, kidney, spleen, and testicle were collected with excess fat and veins carefully trimmed away and the weights of these tissues were also determined.

Tissue chromium analyses

Tissue Cr content was determined by the method of Anderson *et al*.(1996) with atomic absorption spectrometry (AA6510, SHIMADZU, Japan).

Statistical analyses

Data were analyzed by one-way ANOVA using Statistical Analysis System (SAS Version 8.0). Data were presented as means. A significant level of $P<0.05$ was used to determine differences.

RESULTS

The LBM of the rats was significantly increased ($P<0.05$) due to the addition of 300 and 450 ppb Cr from CrNano in the diet (Table 2). In comparison to basal diet, no effect on BFW was detected due to the supplementation of 75 to 1200 ppb Cr from CrNano in the diet. However, dietary supplementation of 150, 300, 450, and 600 ppb Cr from CrNano significantly decreased %BF ($P<0.05$).

As seen in Table 3, addition of 75 and 150 ppb Cr from CrNano produced higher ($P<0.05$) final body weight (FBW) than the basal diet. ADG was increased ($P<0.05$) when 75, 150, and 300 ppb Cr from CrNano was added. However, DFI was unaffected ($P>0.05$) by the supplementation of different doses of Cr as CrNano. In addition, FE was significantly increased ($P<0.05$) when 75, 300, and 450 ppb Cr from CrNano was supplemented to the diets of the rats.

Supplementation of 300 and 450 ppb Cr from CrNano decreased ($P<0.05$) the insulin level in serum (Table 4). However, addition of any dosage of Cr from CrNano did not change the GH levels in serum ($P>0.05$). Addition of 1200 ppb Cr from CrNano produced higher ($P<0.05$) serum cortisol level relative to supplementation of 0, 75, 150, 300, 450, and 600 ppb Cr from CrNano.

In comparison to the basal diet, supplementation of 75 to 1200 ppb Cr from CrNano produced no effect ($P>0.05$) on weights of heart, liver, and kidney (Table 5). However, addition of 450 ppb Cr from CrNano decreased ($P<0.05$) the weight of spleen. Testicle weight was decreased ($P<0.05$) by supplementation of 75, 300, 450, 600 and 1200 ppb Cr. Addition of 75 and 150 ppb Cr increased ($P<0.05$) the weight of hind leg muscle.

Cr contents in heart, spleen, and testicle were not affected ($P>0.05$) by the addition of different doses of Cr from CrNano (Table 6). Cr contents in liver and kidney were both greatly increased ($P<0.05$) by the dietary addition of 150, 300, 450, 600, and 1200 ppb Cr from CrNano (Table 6). Furthermore, dietary supplementation of 300, 450, and 600 ppb Cr from CrNano increased ($P<0.05$) Cr content in the hind leg muscle.

DISCUSSION

It was reported that Cr plays a role in the regulation of lean body mass (LBM), percent body fat, and weight reduction (Evans, 1989; Hasten *et al*., 1992).

Table 2 Body composition of SD rats fed with different dosages of chromium in the form of CrNano[1]

	Cr (ppb)							*SEM*[2]
	0	75	150	300	450	600	1200	
BW (g)	292.0	326.3	316.7	322.7	326.0	315.3	316.7	11.99
LBM (g)	221.7^{b}	255.7^{ab}	254.0^{ab}	262.0^{a}	270.7^{a}	254.3^{ab}	245.7^{ab}	11.72
BFW (g)	63.3^{ab}	63.0^{ab}	52.3^{b}	53.0^{ab}	52.3^{b}	52.0^{b}	64.7^{a}	3.99
%BF	22.2^{a}	19.7^{abc}	16.6^{bc}	16.8^{bc}	16.2^{c}	16.8^{bc}	20.5^{ab}	1.34

[1]Data are presented as means, n=3 per treatment. Means in a row with different letters differ significantly (P<0.05); [2]Standard error of the mean

Table 3 Effects of CrNano dosage on growth and feed utilization in SD rats[1]

	Cr (ppb)							*SEM*[2]
	0	75	150	300	450	600	1200	
IBW[3] (g)	84.7	83.5	83.2	81.8	83.5	83.8	82.0	1.45
FBW (g)	341.0^{b}	377.8^{a}	372.2^{a}	368.6^{ab}	358.7^{ab}	365.8^{ab}	363.4^{ab}	10.51
ADG (g/d)	7.1^{b}	8.2^{a}	8.0^{a}	8.0^{a}	7.6^{ab}	7.8^{ab}	7.8^{ab}	0.29
DFI (g/d)	27.9	29.2	30.3	25.1	28.9	29.8	29.9	1.95
FE (g BW/100 g feed)	24.3^{c}	28.7^{b}	26.7^{bc}	32.3^{a}	27.4^{b}	26.8^{bc}	26.5^{bc}	0.94

[1]Data are presented as means, n=10 per treatment. Means in a row with different letters differ significantly (P<0.05); [2]Standard error of the mean; [3]IBW: Initial body weight

Table 4 Effects of CrNano dosage on serum hormones in SD rats[1]

	Cr (ppb)							*SEM*[2]
	0	75	150	300	450	600	1200	
INS (μIU/ml)	47.66^{a}	38.75^{abc}	28.41^{abc}	26.03^{c}	28.22^{bc}	45.99^{ab}	34.09^{abc}	6.666
Corti (ng/ml)	3.86^{b}	3.91^{b}	4.35^{b}	4.27^{b}	5.07^{b}	5.08^{b}	8.75^{a}	1.226
GH (ng/ml)	1.37	1.25	1.19	1.16	1.21	1.19	1.33	0.091

[1]Data are presented as means, n=10 per treatment. Means in a row with different letters differ significantly (P<0.05); [2]Standard error of the mean

Table 5 Weight of tissues expressed as percentage of body weight in SD rats fed with different dosages of chromium in the form of CrNano[1]

	Cr (ppb)							*SEM*[2]
	0	75	150	300	450	600	1200	
Heart	0.33^{ab}	0.32^{ab}	0.37^{a}	0.33^{ab}	0.30^{b}	0.33^{ab}	0.34^{ab}	0.024
Liver	2.75	2.87	3.12	2.93	2.64	2.86	2.93	0.183
Kidney	0.75	0.85	0.80	0.75	0.74	0.78	0.72	0.058
Spleen	0.22^{ab}	0.22^{ab}	0.22^{ab}	0.18^{bc}	0.16^{c}	0.18^{bc}	0.23^{a}	0.015
Testicle	1.05^{a}	0.87^{b}	0.94^{ab}	0.88^{b}	0.88^{b}	0.82^{b}	0.84^{b}	0.044
Hind leg muscle	8.79^{b}	10.46^{a}	10.54^{a}	9.91^{ab}	10.07^{ab}	10.26^{ab}	9.95^{ab}	0.502

[1]Data are presented as means, n=10 per treatment. Means in a row with different letters differ significantly (P<0.05); [2]Standard error of the mean

Table 6 Chromium content in selected tissues of SD rats fed different dosages of chromium in the form of CrNano[1]

	Cr (ppb)							*SEM*[2]
	0	75	150	300	450	600	1200	
Heart (ng/g)	1.13	1.74	1.91	1.92	2.32	2.11	2.54	0.528
Liver (ng/g)	16.13^{b}	23.96^{ab}	29.93^{a}	31.60^{a}	32.05^{a}	29.83^{a}	32.88^{a}	4.280
Kidney (ng/g)	49.08^{b}	58.95^{ab}	69.55^{a}	75.47^{a}	76.82^{a}	76.74^{a}	85.92^{a}	5.585
Spleen (ng/g)	1.16	1.46	0.95	1.83	1.15	1.31	1.66	0.325
Testicle (ng/g)	0.77	1.28	0.76	0.89	0.98	0.92	1.22	0.263
Hind leg muscle (ng/g)	11.22^{c}	11.57^{c}	15.06^{c}	20.61^{ab}	23.63^{a}	22.44^{a}	15.60^{bc}	1.785

[1]Chromium content value is per gram of wet weight tissue samples. Data are presented as means, n=10 per treatment. Means in a row with different letters differ significantly (P<0.05); [2]Standard error of the mean

But the point of view is still highly controversial (Anderson, 1998). In swine, dietary addition of Cr was reported to increase carcass leanness and decrease carcass fatness (NRC, 1998). Page *et al*.(1993) reported that longissimus muscle area and percentage of muscling were increased and 10th rib fat was decreased by chromium picolinate (CrPic) added to the diets of growing-finishing pigs. However, inconsistent or no effect on carcass traits with supplementation of CrPic and chromium chloride ($CrCl_3$) was also reported (Evock-Clover *et al*., 1993; Mooney and Cromwell, 1997). In human, there were also reports of increased muscle mass and decreased body fatness due to Cr supplementation (Evans, 1989; Bulbulian *et al*., 1996; Kaats *et al*., 1996). However, reports from Clancy *et al*.(1994) and Campbell *et al*.(1999) did not support an effect of supplemental Cr on altering body composition. The dosages in studies on rats (Hasten *et al*., 1993), pigs (Shelton *et al*., 2003) and steers (Pollard *et al*., 2002), which have observable changes in body composition due to Cr supplementation ranged from 25 to 1000 ppb. In the present study, LBM was increased by the addition of 300 and 450 ppb Cr as CrNano and %BF was decreased by the addition of 150, 300, 450, and 600 ppb Cr as CrNano. This further substantiated our previous results in finishing pigs (Wang and Xu, 2004). Whether CrNano is a source of Cr with higher bioavailability remains to be further investigated.

Previous studies reported loss of BW due to supplemental Cr (Grant *et al*., 1997). However, in the present study addition of 75 and 150 ppb Cr from CrNano increased BW and addition of 75, 150, and 300 ppb Cr increased ADG in SD rats. These were in agreement with Page *et al*.(1993) who first reported increases in growth rate and ADG by the addition of 50 and 200 ppb Cr as CrPic to the diets of pigs. However, other studies have reported no changes in body weight and growth rate in pigs fed 250 to 500 ppb Cr from CrPic (Lindemann *et al*., 1995), rats fed 25 to 1500 ppb Cr from CrPic (Hasten *et al*., 1997b), and lambs fed 200 to 1000 ppb Cr from Cr nicotinate (Mostafa-Tehrani *et al*., 2006). The reason that low levels (less than 300 ppb) of Cr as CrNano increase BW and ADG in rats and high levels of Cr do not is not well understood. Therefore, further studies should be conducted to make substantiation. Hasten *et al*.(1997b) reported that a level of 1500 ppb Cr as CrPic appeared to produce an inhibition on appetite and feed intake in SD rats (Hasten *et al*., 1997b). Page *et al*.(1993) also reported that feed intake in pigs decreased with the higher dosages of 400 and 800 ppb Cr as CrPic, but not with the lower dosages of 100 and 200 ppb Cr as CrPic. However, DFI in SD rats was not affected by the supplementation of Cr as CrNano in the dosage range from 75 to 1200 ppb in this study. In addition, there did not appear to be a tendency for DFI to be decreased with the increase of Cr dosages either. FE was significantly increased by the addition of 75, 300, and 450 ppb Cr as CrNano. This agrees with Lindemann *et al*.(1995) who reported that supplemental Cr as CrPic improved FE in growing-finishing pigs. However, Amoikon *et al*.(1995) and Boleman *et al*.(1995) both reported that CrPic had no effect on FE in pigs. Sahin *et al*.(2001) also found that addition of 200 and 400 ppb Cr from $CrCl_3$ had no effect on FE in growing rabbits. Furthermore, in a study with growing beef steers, supplementation of 100 to 400 ppb Cr as Cr yeast had no effect on FE (Swanson *et al*., 2000). These results suggest that the effects of different forms of Cr on growth performance vary greatly.

In the present study, serum insulin levels were greatly decreased by the addition of CrNano. This corresponds with Evans and Bowman (1992) who demonstrated that CrPic can increase the rate of insulin internalization. Thus, an increase in insulin internalization would be in accordance with the observed reduction in circulating concentrations of insulin.

It was shown that stressors can increase plasma cortisol (Arave *et al*., 1988; Hashizume *et al*., 1994). The increased serum cortisol by addition of 1200 ppb Cr from CrNano in the present study suggests that high level of Cr may be a stressor to rat. There is no change in serum GH levels due to supplemental Cr as CrNano, which was inconsistent with Evock-Clover *et al*.(1993) who reported an increase of plasma GH in pigs by dietary addition of CrPic.

In this experiment, addition of Cr as CrNano in the dosage range of from 75 to 1200 ppb had no effect on weights of heart, liver, and kidney. This was partly in agreement with previous reports. Page *et al*.(1993) reported no differences in heart weights in pigs fed CrPic. However, it was reported that dietary CrPic could increase heart weights of lambs (Kitchalong *et*

al., 1993) and rats (Hasten *et al*., 1993). Weights of liver and kidney were also observed to be decreased in pigs fed CrPic (Boleman *et al*., 1995). The decrease in weight of spleen by addition of 450 ppb Cr from CrNano observed in this study indicated that supplemental Cr might have effect on immune system of rat. Previous studies reported that supplemental Cr had effect on reproductive qualities. Hagen *et al*.(2000) reported that supplementation of 200 Cr from CrPic could increase a little size. Lindemann *et al*.(2004) found Cr supplementation resulted in increased litter size in a dose-related manner up to 600 ppb Cr from CrPic. In this study, decreases in testicle weights by addition of 75, 300, 450, 600, and 1200 ppb Cr from CrNano suggested that supplemental Cr might have effect on reproductive system of male rats. There is little information about Cr supplementation on individual muscle weight. The increased weight of hind leg muscle by addition of 75 and 150 ppb Cr from CrNano was consistent with the observed increase in LBM.

Previous studies indicated that supplemental Cr had effects on Cr content in tissues, but the results varied (Chang and Mowat, 1992; Anderson *et al*., 1996). In this study, Cr contents in liver and kidney were significantly increased with the addition of Cr as CrNano in the dosage of from 150 ppb to 1200 ppb. This is consistent with Anderson *et al*.(1996), who reported that there was an approximate doubling of kidney Cr concentration and roughly 50% increased liver Cr concentration with the supplementation of 300 ppb Cr as Cr picoliante in the diets of pigs. Their results from rats (Anderson *et al*., 1996) showed that increase in tissue Cr following Cr supplementation were greatest for the kidney, followed by the liver, with considerably smaller changes in the heart and skeletal muscle tissues. Thus, the biggest difference from previous reports observed in this study was that supplemental Cr from CrNano produced greatly higher Cr concentration in muscles. This agreed with our previous results in pigs (Wang and Xu, 2004). The enhanced Cr absorption in vivo by CrNano might improve the efficacy of insulin as shown by its accelerated internalization. Internalization of insulin into muscle cells might increase protein synthesis which ultimately increased the LBM as seen in this study. In addition, a potentiation of insulin by CrNano might also decrease the rate of fat deposition which contributed to the observed decrease in %BF in SD rats (Mooney and Cromwell, 1997). It was suggested that CrNano may have higher bioavailability, which may be partly because of the unique absorption method of nanoparticle in intestinal tract and its novel distribution pathway in vivo. Uptake of nanoparticles has been shown to occur transcellularly through normal enterocytes and Peyer's patches via M-cells (Hussain *et al*., 2001; Jevprasesphant *et al*., 2004). Therefore, further researches should concentrate on the absorption mechanism of CrNano.

CONCLUSION

From the results of this study it could be concluded that supplemental Cr in the form of CrNano has beneficial effects on growth performance and body composition, and increases tissue Cr concentration in selected muscles. The effectiveness differed between the dosages supplemented. Taking into account all the traits determined, it is recommended that 300 ppb Cr as CrNano may be the optimum dosage for supplementation to diets of rats.

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