



Efficacy of Cr (III) Supplementation on Growth, Carcass Composition, Blood Metabolites, and Endocrine Parameters in Finishing Pigs*

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ABSTRACT : The study was conducted to evaluate the effects of trivalent chromium from different sources on growth, carcass composition, and serum parameters in finishing pigs. Ninety-six crossbred pigs with an initial average body weight of 65.57 ± 1.05 kg were blocked by body weight and randomly assigned to four treatments with three replicates. Pigs were offered one of four diets including a control diet or the control diet supplemented with 200 $\mu\text{g}/\text{kg}$ chromium from either chromium chloride (CrCl_3), chromium picolinate (CrPic) or chromium nanocomposite (CrNano) for 40 days. After completion of the feeding trial, eight pigs from each treatment were selected to collect blood samples, and slaughtered to measure carcass composition. The results showed that supplemental chromium had no significant effect on growth performance, while CrNano increased carcass lean proportion and loin *Longissimus* muscle area ($p < 0.05$), and decreased carcass fat proportion and 10th rib backfat depth ($p < 0.05$). CrPic supplementation also resulted in lower fat proportion and larger *Longissimus* muscle area ($p < 0.05$). The addition of Cr from CrNano or CrPic decreased serum glucose ($p < 0.05$) and increased concentrations of total protein and free fat acid in serum ($p < 0.05$). Serum urea nitrogen, triglyceride and cholesterol were decreased ($p < 0.05$), and serum high density lipoprotein and lipase activity were increased ($p < 0.05$) with the supplementation of CrNano. Serum insulin was decreased ($p < 0.05$) by supplemental Cr from CrNano or CrPic, and serum insulin-like growth factor I was increased significantly in the CrNano treated group. These results suggest that chromium nanocomposite has higher efficacy on carcass composition in pigs compared to the traditional chromium sources. (**Key Words :** Chromium, Pigs, Nanocomposite, Carcass Composition, Serum Parameters)

INTRODUCTION

Trivalent chromium (Cr (III)) is a component of glucose tolerance factor (GTF) and is vital in carbohydrate, fat, and protein metabolism presumably by potentiating the action of insulin (Anderson, 1987; Mertz, 1993). Various trivalent chromate compounds have been used as nutritional supplements, weight-loss agents, and muscle-development agents in humans and as feed additives in domestic animals (Lindemann et al., 1995; Vincent, 2004). It is generally

accepted that organic sources of Cr have a higher bioavailability than inorganic sources (NRC, 1997). Interest has focused on the potential use of the organic chromium complex, chromium picolinate (CrPic), to increase carcass leanness. Positive responses were reported by Page et al. (1993), Lindemann et al. (1995), Bolemann et al. (1995), and Mooney and Cromwell (1995). However, others reported no responses in carcass leanness to supplemental Cr in this form (Harris et al., 1995; Ward et al., 1995; Mooney and Cromwell, 1996).

Size, nature of the polymer, zeta potential and vehicle can be critical factors influencing particles uptake (Florence, 1998). It is postulated that the absorption and utilization of Cr is dependent on its status in the gastrointestinal tract. New and emerging technologies such as nanotechnology have the potential to advance the science of animal nutrition and to improve growth, health and other productive index (Ross et al., 2004). The efficiency of 100 nm size particle was 15- to 250- fold higher than larger size microparticle in intestinal tissues, and the size dependency of uptake was

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confirmed in Caco-2 cell lines (Desai et al., 1996, 1997). In previous work at our laboratory, chromium nanocomposite (CrNano) was shown to produce beneficial effects on carcass characteristics, pork quality and individual skeletal muscle weight, with approximate 2-3 fold higher tissue chromium deposition in selected muscle and organs compared to the control group, which implicated higher absorptivity and bioavailability (Wang and Xu, 2004). Further studies in finishing pigs and rats showed that dietary supplementation of Cr as CrNano affected blood metabolites, alters the levels of some endocrine parameters (Wang et al., 2007; Zha et al., 2007). CrNano exhibited considerably higher absorption efficiency than both CrPic and chromium chloride (CrCl₃) (Zha et al., 2007).

Hence, the objective of the present investigation was to assess the efficacy of three different sources of chromium as CrCl₃, CrPic and CrNano on growth, carcass composition, blood metabolites and endocrine parameters in pigs.

MATERIALS AND METHODS

CrCl₃, CrPic and CrNano

The CrCl₃ (CrCl₃·6H₂O, 99%) and CrPic (Chromium Picolinate, 98%) were purchased from Sangon Co. Ltd (Shanghai, China). CrNano (nanocomposite of CrCl₃, size ranges from 40-70 nm) was provided by the Key Laboratory of Molecular Animal Nutrition, Ministry of Education, China.

Animals and experimental design

The protocol of this study was approved by the Institution Animal Care and Use Committee at Zhejiang University and was conducted in accordance with the National Institutes of Health guidelines for the care and use of experimental animals. The feeding trial was carried out in Guangzhou Breeding Farm. A total of 96 crossbred pigs (Duroc×Landrace×Yorkshire) were selected and fed with control diet for 7 days pre-test. After that, those pigs with an average body weight of 65.57±1.05 kg were grouped into four blocks by initial body weight and randomly assigned to one of the following dietary treatment: control and control diet supplemented with 200 µg/kg chromium from either CrCl₃, CrPic or CrNano, with three replicate pens per treatment and 8 pigs per pen with balanced gender and ancestry. All experimental treatments used the same maize-soybean meal basal diet formulated met or exceeded NRC (1998) recommendations for nutrients except digestible energy (Table 1).

The pigs were penned in 3.25×5.25-m pens with concrete floors, with a nipple drinker and a stainless steel feeder to allow pigs *ad libitum* access to feed and water. The duration of the feeding trial was 40 days. Preceding the

Table 1. Ingredient inclusion and chemical composition of basal diet, as-fed basis

Ingredients (g/kg)	
Maize	645.0
Soybean meal	215.0
Rapeseed meal	40.0
Wheat bran	60.0
Limestone	13.0
Calcium phosphate	15.0
Salt	3.0
Mineral premix ^a	7.0
Vitamin premix ^b	2.0
Chemical composition(analyzed, g/kg) ^c	
Digestible energy (MJ/kg)	13.4
Crude protein (%)	171.0
Calcium (%)	7.6
Phosphorus (%)	6.2
Lysine (%)	10.5
Methionine (%)	4.5

^a Contained per kg of diet: Cu, 10 mg from CuSO₄·5H₂O; Zn, 100 mg from ZnSO₄·7H₂O; Fe, 140 mg from FeSO₄·H₂O; Mn, 40 mg from MnSO₄·5H₂O; Se, 0.1 mg from Na₂SeO₃·5H₂O; I, 0.3 mg from KI.

^b Contained per kg of diet: vitamin A, 6,000 IU; vitamin D₃, 700 IU; vitamin E, 88 IU; vitamin K, 4.4 mg; riboflavin, 8.8 mg; D-pantothenic acid 24.2 mg; niacin, 33 mg; choline chloride 330 mg; vitamin B₁₂, 22 µg; D-biotin, 300 µg; folic acid, 2.5 mg.

^c All data were analyzed values except digestible energy which was calculated using swine NRC (1998) values.

study, the pigs were kept in the same house and were offered no chromium supplementation diet for *ad libitum* consumption. Average daily gain (ADG), average daily feed intake (ADFI) and feed:gain ratio were collected for all pigs throughout the experimented period.

Blood sampling

On day 40 of the experiment, 8 pigs from each treatment were selected on the basis of closer body weight, and blood samples were taken by anterior vena cava puncture after 12 h fasting. The samples were then centrifuged at 1,500×g at 4°C for 15 min. Serum from each sample was collected and stored at -20°C until needed for analysis.

Carcass evaluation

After blood sampling, the selected 32 pigs were transported to meat factory and slaughtered by exsanguination after electrical stunning. At slaughter, the head, hair and viscera were removed from carcass, hot carcass (for determination of dressing proportion) was collected, and the right and left halves of the carcasses were separated. Measurements of backfat depth and longissimus muscle area were made from left carcass tracings taken at

Table 2. Effects of different sources of Cr supplementation on growth performance and feed utilization in finishing pigs after 40 days of administration

Item	Control	Cr (200 µg/kg)			SEM
		CrCl ₃	CrPic	CrNano	
Initial weight (kg)	65.25	65.20	66.83	66.27	0.498
Final weight (kg)	89.80	89.67	91.93	92.37	1.518
ADG (g/d)	614	612	628	653	33
ADFI (kg/d)	2.13	2.18	2.22	2.16	0.042
Feed:gain	3.47	3.56	3.54	3.31	0.173

Values are presented as means; n = 3 per treatment with eight pigs per pen contributing to a pen mean; SEM means standard error of the mean.

the 10th rib. The left half of the carcass was dissected by separating bone, muscle, fat and skin. Each component was weighed respectively. Carcass dressing proportion was calculated by the following formula: hot carcass weight divided by final live weight × 100. Proportion of lean or fat was calculated by the following formula: lean or fat weight divided by hot carcass weight × 100.

Analysis of blood samples

The concentrations of serum glucose (GLU), total protein (TP), urea nitrogen (SUN), triglyceride (TG), high density lipoprotein (HDL), cholesterol (CHL), and free fat acid (FFA) were determined by corresponding commercial kits (Cicheng Biochemical Reagent Co., Ningbo, China) with the recommended procedures. The enzymatic activities of lipase (LIP), glutamic-oxaloacetic transaminase (GOT), and glutamic-pyruvic transaminase (GPT) were also determined by commercial kits (Jiancheng Biochemical Reagent Co., Nanjing, China).

The concentration of insulin (INS) was analyzed using a commercially available ¹²⁵I RIA kit (Beijing North Institute of Biological Technology, Beijing, China). The assay used human INS and antibodies against human INS as the standard. Minimum detectability of INS was 0.1 µIU/ml, and the intraassay CV was 10%.

Serum insulin-like growth factor I (IGF-I) was analyzed using a commercially available ¹²⁵I RIA kit (INCSTAR Co., Stillwater, MN, USA). In the assay, recombinant human IGF-I and mouse antiIGF-I monoclonal antibody were used as the standard. Recovery rate ranged from 92.3 to 110.0%.

The intraassay CV was 10%, and the minimum detectable concentration of IGF-I was 0.1 ng/ml.

Statistical analysis

The experimental data were analyzed using GLM procedures of SAS (SAS Inst. Inc., Cary, NC, 1989). Each pen was considered as an experimental unit for average daily gain (ADG), average daily feed intake (ADFI), and feed: gain ratio; individual animals served as the experimental unit for other measurements. The alpha level used for determination of significance was 0.05.

RESULTS

Growth performance and feed utilization

The effects of dietary Cr supplementation on growth performance and feed utilization are presented in Table 2. There were no significant difference on ADG, feed gain ratio and feed intake between treatments.

Carcass composition

The effects of supplemental Cr on carcass composition are presented in Table 3. There is no significant effect of Cr on dressing proportion. Compared to the control group, dietary CrNano supplementation increased lean proportion and longissimus muscle area by 10.55% (p<0.05) and 20.22% (p<0.05), and reduced fat proportion and 10th rib backfat thickness by 32.54% (p<0.05) and 24.32% (p<0.05), respectively. Compared to the control group, Longissimus muscle area of pigs fed with supplemental CrPic diet were

Table 3. Effects of different sources of Cr supplementation on carcass composition in finishing pigs after 40 days of administration

Item	Control	Cr (200 µg/kg)			SEM
		CrCl ₃	CrPic	CrNano	
Dressing proportion (%)	73.16	74.09	75.45	74.24	1.591
Lean proportion (%)	54.49 ^a	56.39 ^a	55.42 ^a	60.24 ^b	0.754
Fat proportion (%)	21.54 ^a	18.47 ^b	16.24 ^{bc}	14.53 ^c	0.873
10 th rib backfat (cm)	1.85 ^a	1.80 ^a	1.66 ^{ab}	1.40 ^b	0.134
Longissimus muscle area (cm ²)	46.84 ^a	44.31 ^a	54.94 ^b	56.31 ^b	2.182

Values are presented as means; n = 8 animals per treatment; SEM means standard error of the mean.

Means in a row with different letters differ significantly (p<0.05).

Table 4. The effects of different sources of Cr supplementation on serum metabolites in pigs measured after 40 days of administration

Item	Control	Cr (200 µg/kg)			SEM
		CrCl ₃	CrPic	CrNano	
GLU (mmol/L)	4.86 ^a	4.69 ^a	3.09 ^b	3.30 ^b	0.417
TP (g/L)	42.50 ^a	44.27 ^a	53.98 ^b	62.44 ^c	2.683
SUN (mmol/L)	10.65 ^a	8.79 ^{ab}	8.87 ^{ab}	7.52 ^b	0.926
TG (mmol/L)	0.70 ^a	0.58 ^{ab}	0.52 ^{ab}	0.44 ^b	0.094
CHL (mmol/L)	2.86 ^a	2.56 ^{ab}	2.39 ^{ab}	1.97 ^b	0.263
HDL (mmol/L)	0.50 ^a	0.51 ^a	1.03 ^{ab}	1.35 ^b	0.284
FFA (µmol/L)	145.39 ^a	182.09 ^a	258.07 ^b	333.95 ^c	13.497
LIP (U/L)	59.55 ^a	62.06 ^a	68.10 ^{ab}	93.51 ^b	9.572
GOT (IU/L)	53.45	33.78	30.83	30.17	13.361
GPT (IU/L)	145.71	117.46	116.79	111.41	15.830

Values are presented as means; n = 8 animals per treatment; SEM means standard error of the mean.

Means in a row with different letters differ significantly ($p < 0.05$).

increased by 17.29% ($p < 0.05$), with decreased fat proportion by 24.61% ($p < 0.05$).

Blood metabolites

Effects of Cr supplementation on serum metabolites are presented in Table 4. Compared to the control group, the supplementation of 200 µg/kg Cr from CrNano decreased the serum concentrations of GLU (32.10%, $p < 0.05$), SUN (29.39%, $p < 0.05$), TG (37.14%, $p < 0.05$), CHL (31.12%, $p < 0.05$), and increased the serum contents of TP (46.98%, $p < 0.05$), HDL (170.00%, $p < 0.05$), FFA (129.62%, $p < 0.05$) and activity of LIP (57.03%, $p < 0.05$). Compared to the control group, supplemental Cr from CrPic also resulted in decreased serum GLU (36.42%, $p < 0.05$), and increased serum TP (27.01%, $p < 0.05$) and FFA (77.50%, $p < 0.05$). The activities of GOT and GPT were not significantly affected by Cr supplementation.

Insulin and IGF-I levels

Serum INS was decreased by 39.55% ($p < 0.05$), and 37.58% ($p < 0.05$) respectively with the supplementation of Cr from CrNano or CrPic (Table 4). Serum IGF-I level was elevated by 87.94% ($p < 0.05$) with the supplementation of CrNano.

DISCUSSION

The research with Cr supplementation to animal diets

has been primarily conducted with CrPic, chromium nicotinate (CrNic), CrCl₃ or chromium propionate (CrProp), with widely disparate results among studies. Chromium nanocomposite, a new form of Cr with 40-70 nm size, was produced on the basis of nanotechnology. Nanocomposite was investigated in various biomedical applications (Sahoo and Labhasetwar, 2003) and proved to exhibit a high rate of absorption in the gastrointestinal tract (Desai et al., 1996; Hussain et al., 2001). Because of its small size, Nanocomposite can penetrate through small capillaries and be taken up by cells, which allow efficient accumulation at the target sites (Sahoo and Labhasetwar, 2003). The efficiency of uptake of 100 nm size particles by the intestinal tissues was 15-250 fold higher compared to larger size microparticles (Desai et al., 1996), and the significantly greater uptake of small diameter microparticles and size dependency of uptake were confirmed in Caco-2 cell lines (Desai et al., 1997). In our previous studies, CrNano was shown to produce striking effects on carcass characteristics and individual skeletal muscle weight in pigs, with approximate 2-3 fold higher tissue chromium deposition in selected muscle and organs compared to the control group (Wang and Xu, 2004). Further study showed that absorption of CrNano in small intestine was mainly via transcellular pathway, while CrPic and CrCl₃ were mainly via paracellular pathway, and so that CrNano exhibited considerably higher absorption efficiency than both CrPic and CrCl₃ (Zha et al., 2007).

Table 5. The effects of different source of Cr supplementation on levels of serum insulin and IGF-I measured after 40 days of administration in finishing pigs

Item	Control	Cr (200 µg/kg)			SEM
		CrCl ₃	CrPic	CrNano	
INS (µIU/ml)	16.31 ^a	14.18 ^a	10.18 ^b	9.89 ^b	1.322
IGF-I (ng/ml)	107.61 ^a	118.64 ^a	150.97 ^{ab}	202.24 ^b	27.940

Values are presented as means; n = 8 animals per treatment; SEM means standard error of the mean.

Means in a row with different letters differ significantly ($p < 0.05$).

The effect of CrNano on growth performance reported here is partly in consistent with our previous study in finishing pigs, in which no change in growth rate but an improvement in feed efficiency was observed with the supplementation of CrNano (Wang and Xu, 2004). In this study, there was 6.35% and 4.61% improvement in daily gain and feed efficiency respectively from CrNano supplementation, though no significant difference. Supplementation of bioavailable source of Cr is not uniformly efficacious on growth. Improvements in growth rate of swine as a results of supplementing diets with 200 to 500 µg Cr/kg as CrPic or 500 µg to 5 mg Cr/kg as CrCl₃ were reported in 11 of 31 studies, and feed efficiency was improved by Cr supplementation of diets in 8 of 31 studies (NRC, 1997). Therefore, no definite conclusion on the effect of Cr on growth performance can be drawn from those studies at the moment, and the current result of CrNano on growth also remains to be confirmed further.

The role of Cr in regulation of body composition is still controversial. CrPic is the most widely used organic chromium complex to increase lean body mass or carcass leanness. Lindemann (1999) reviewed the reported carcass effects from those trial comparisons and found a mean increase in longissimus muscle area of 2.7 cm² (about 7%; with 13 numerically positive responses out of 15 comparisons) and a mean reduction in 10th rib backfat depth of 3.3 mm (about 13%; with 13 positive responses out of 15 comparisons). The lack of a consistent response may be related to Cr levels of the diets, Cr status of subjects, and amino acid levels of diet (White et al., 1993; Lindemann et al., 1995). In the present study, supplementation of 200 µg Cr/kg diet from both CrPic and CrNano increased longissimus muscle area and decreased carcass fat proportion, and CrNano addition also significantly increased lean proportion and decreased 10th rib backfat. These results are in line with the striking effect on carcass composition of finishing pigs in our previous study (Wang and Xu, 2004), which implicates that CrNano may be a more bioavailable source of Cr and potentially be used as an effective carcass-modifier for animals.

The reduction in SUN concentration reported here is consistent with the results of our previous study, and is likely associated with the effects of CrNano on protein deposition as indicated by the increased lean proportion and longissimus muscle area, and reduction in 10th rib backfat thickness. However, most of the literatures indicate that Cr from CrPic, CrNic, CrCl₃ and CrProp do not affect plasma or serum urea N concentrations (Page et al., 1993; Ward et al., 1997; Matthews et al., 2001). The striking effect of supplemental Cr from the form of CrNano on an increase in leanness and longissimus area, and decreases in fat proportion and 10th rib backfat thickness in the present study would be involved in an increased utilization of

nitrogen, thereby, resulting in a decrease in the circulating concentrations of urea nitrogen. The concentration of TP was increased in pigs fed CrNano or CrPic in this study, which is in agreement with our previous report of CrNano, and not in agreement with report of CrPic in about 20 kg BW growing pigs from Amoikon et al. (1995).

The reduced serum cholesterol level exhibited by pigs offered the diet supplemented with CrNano is in line with our previous study. The results of both experiments suggest a role of Cr in cholesterol (fatty acid) metabolism, which is further supported by our results for HDL. Similar differences between experiments exist for the effects of supplemental Cr on TG levels. In the present experiment, TG was reduced by CrNano which is in line with our previous study. Serum TG response to CrPic reported here is in agreement with the results of Page et al. (1993) but different from those reported by Min et al. (1997). Nevertheless, Min et al. (1997) reported that the effect of CrPic on TG was affected by the weight of the pig and differences in weight and possibly even breed may explain some of the differences between experiments. Serum free fat acid was increased with the supplementation of CrNano, which is consistent with the results of increased lipase level in serum. The apparent effects of CrNano on energy/fat metabolism may simply be reflective of the positive effects on protein metabolism and/or may independent of these. The actual causes remain to be elucidated.

The decreases in concentrations of glucose and insulin in serum in present study are in accordance with our previous study of CrNano and with Page et al. (1993), Amoikon et al. (1995) and Lindemann et al. (1995), who reported a reduction of plasma insulin in pigs fed Cr from CrPic. It is reported that CrPic is able to increase the rate of insulin internalization and uptake of glucose into rat skeletal muscle cells (Evans and Bowman, 1992). Thus, an increase in insulin internalization would be in accordance with the observed reduction in circulating concentrations of insulin and glucose (Amoikon et al., 1995).

The CrNano induced changes in GLU and insulin are in line with the effects on SUN and the results for the longissimus muscle and backfat. Overall the results of this and our previous experiment suggest that supplementation of the diets offered pigs from approximately 65 to 100 kg with CrNano affects protein and energy metabolism. The elevated IGF-I level measured in pigs offered the supplemented diets may have played a role in the protein and energy metabolism changes indicated by the results since IGF-I mediates the effects of GH on protein metabolism and the uptake of glucose by muscle tissue.

IMPLICATIONS

The results of present study suggest that chromium

nanocomposite has higher efficacy on growth and carcass composition in pigs, which demonstrated significant effect on blood metabolites and some endocrine parameters, and indicated obvious effect on fat or lipid catabolism. The mechanisms underlying these effects and their scientific and commercial implications, and long-term effects are yet to be established and require further research.

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