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Effect of Small Peptide Chelate Chromium on Growth Performance, Organ Development and Serum Traits in Spargue-Dawley Rats

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Abstract: The experiment was conducted to study the effect of supplementing Small Peptide Chelate Chromium (SPCr) in diets on growth performance, organ development and serum traits in Spargue-Dawley rats. Seventy two SD rats with initial body weight about (65±5 g) were randomly assigned to six dietary treatment, basal diet, basal diet supplemented with 100, 200, 500 or 1000 μg/kg Cr in the form of SPCr and 200 μg/kg Cr in the form of chromium picolinate (CrPic). Each treatment had 6 replicates. The duration of the study was 35 days. The results showed that: Supplementation of Cr with different types at low-level (below 500 μg/kg) increased daily gain and feed efficiency. Supplementation of 500 μg/kg SPCr increased ADG ($p<0.05$), decreased feed:gain ratio ($p<0.05$) compared with control group. Serum cholesterol and triglyceride was decreased($p<0.05$) fed diets with SPCr at low-level. Supplementation of Cr with different types increased serum high density lipoprotein ($p<0.05$), also decreased serum glucose and insulin compared with control group. Addiction of Cr with different types increased the relative weights of liver ($p<0.05$). It was concluded that SPCr had effect to improve performance and serum lipids.

Key words: Small peptide chelate chromium; SD rats; feed efficiency; serum traits

INTRODUCTION

As people's standard of living and food safety consciousness improved, it is becoming a focus in research to seek for new hypoglycemic and lipid lowering functional factors. Chromium is generally considered an essential nutrient for animals, it can influence carbohydrate metabolism (Steele *et al*., 1977; Mertz, 1993; Bunting *et al*., 1994), lipid metabolism (Steel and Rosebrough, 1981; Abraham *et al*., 1991) and protein absorption and metabolism (Okada *et al*., 1983; Kornegay *et al*., 1997) in various species. Even though, its specific function is not clear, chromium is thought to research as a cofactor with insulin (Nielsen *et al*., 1994), but the quantitative requirement is not known (NRC, 1998). As a key factor of Glucose Tolerance Factor (GTF) to increasing insulin activity, previous experiments indicated that supplementation of Cr in diets improved growth performance (Page *et al*., 1993), increased longissimus muscle area and decreased backfat of carcasses (Evock-Clover *et al*., 1993; Amoikon *et al*., 1995), it also has been shown to enhance reproduction and immunological function (Shelton *et al*., 2003; WANG, 2004), but there were few reports about the effect of Cr on organ development and serum traits. Small peptide chelate chromium (majority dipeptide and tripeptide chelate Chromium) is a newly available organic chromium source whose bioavailability has not been previously determined in animals (Qiao, 2004). The objective of the present research was to investigate the effect of different level of small peptide chelate chromium in diets on growth performance, organ development and serum traits in Spargue-Dawley rats.

MATERIALS AND METHODS

Animals, housing and experimental design: The protocol for this experiment was approved by the Institutional Animal Care and Use Committee of Sichuan Agricultural University. Seventy two SD rats with initial body weight about (65±5 g), without regard to their sex, were randomly assigned to six dietary treatments, with 12 rats per treatment and 6 replicates each, raised in stainless steel cages. The whole experiment lasts 35 days. The rats had free access to diet and triple-distilled, deionized water which contained 0.1 ppm vanadium and molybdenum (as vanadyl sulfate and sodium molybdate) throughout the trial. During the whole trial, rats were housed in a closed building in which, the temperature was controlled and maintained in the range 22-25°C.

Experimental diets: The corn-soybean-wheat basal diet used (Table 1) was formulated according to the recommendations for rats proposed by Laboratory animals-Mice and rats formula feeds (GB 14924.3, China, 2001) and it met or exceeded the requirements for minerals and vitamins. The chromium content of the basal diets was 380 μg/kg, measured by atomic absorption spectroscopy at Sichuan Academy of

Table 1: Composition and nutrient levels of basal diets (air-dry basis, %)

Ingredients	Content	Nutrient levels[3]	Content
Corn	34.0	Crude protein	21.4
Soybean	25.0	Crude fat	4.2
Wheat starch	25.0	Crude fibre	5.0
Wheat bran	5.0	Crude ash	3.2
Fish meal	5.0	Gross energy(kJ/kg)	1676.2
Yeast meal	1.0		
NaCl	1.0		
Soybean oil	1.5		
Mineral mix[1]	1.5		
Vitamin mix[2]	1.0		
Total	100.0		

[1]Provided per kg of diets: Cu 10 mg; Zn 30 mg; Fe 120 mg; Mn 75 mg; Se 0.1 mg; I 0.5 mg. [2]Provided per kg of diets: VA 14,000 IU; VD 1,500 IU; VE 120 IU; VK 5 mg; VB_1 13 mg; VB_2 12 mg; niacin 60 mg; D-pantothenic acid 24 mg.
[3]The measured value of Cr is 380 μg/kg

Agricultural Sciences, China. The six treatments consisted of the basal diet supplemented with 0, 100, 200, 500 or 1000 μg/kg Cr in the form of Small Peptide Chelate Chromium (SPCr) and 200 μg/kg Cr in the form of Chromium Picolinate (CrPic). These levels of Cr was chosen based on research by Gu *et al.* (2007), which suggested that 200 μg/kg of Cr was the efficacious dose in improving growth performance in rats. SPCr was offered by Animal Nutrition Institute, Sichuan Agricultural University, China. CrPic was offered by Mianyang Sinyiml Chemical Co. Ltd, China. The analyzed concentrations of Cr in the form of SPCr and CrPic were 1.17 and 12.36%, respectively, measured by atomic absorption spectroscopy at Sichuan Academy of Agricultural Sciences, China.

Performance and blood sampling: All rats were weighed at the beginning of the trial. At the end of the experiment, the rats were fasted overnight, feed intake and weight of the rats were recorded. Average Daily Gain (ADG), Average Daily Feed Intake (ADFI) and Feed:Gain ratio (F:G) were calculated per replicate. Blood samples were collected from all experimental rats via abdominal aortic approach into 10 mL heparin-free glass tubes after anaesthesia, which were centrifuged at 3,500 rpm (Centrifuge Model 0406-1, Shanghai Medical Instruments Corp. Ltd., Shanghai, China) for 10 min. Supernatant was gathered into Eppendorf tubes, respectively and then immediately stored at -20°C for later analysis. After the collection of blood samples, the rats were sacrificed, heart, liver, spleen, kidney and testis were collected and weighed, then immediately stored in liquid nitrogen for later analysis.

Analytical and statistical procedures: Serum samples were analyzed for glucose, cholesterol, triglyceride and High Density Lipoprotein (HDL) concentrations, determined with SHIMADZU CL8000 Clinical Chemistry Analyzer, using standard assay kits (Nanjing Jiancheng Biotechnology Co., Ltd, Jiangsu, China). Serum insulin concentrations was measured by Abbott AXSYM System using standard assay kits from Abbott AXSYM System. Relative weight of selected organs were measured as:

$$\text{Relative weight} = \frac{\text{weight of organ}}{\text{Alive weight of rat}} \times 100$$

Replicate was the experimental unit for ADFI, ADG, G:F and serum traits. Data were analyzed by General Liner Model using one-way ANOVA. Duncan's multiple comparisons were used to test the differences among each treatment group. Statistical significance was accepted at $p<0.05$ and P between 0.1 and 0.05 were interpreted as indicating a trend towards significance. Statistical analysis was performed with SPSS13.0.

RESULTS

Animal performance: There was no decease throughout the experiment. In Table 2, ADG, ADFI and F:G were presented. Supplementation of Cr with different types at low-level (below 500 μg/kg) increased daily gain and decreased the ratio of feed intake to daily gain. Supplementation of 500 μg/kg SPCr increased daily gain by 17.5% ($p<0.05$), decreased feed intake by 4.3% ($p>0.05$) and 4.9% ($p<0.05$) compared with control group and 200 μg/kg CrPic group, respectively, as a result, the feed:gain ratio decreased by 18.4% ($p<0.05$) compared with control group. However, there was no significant effect when supplementation of Cr in the form of SPCr at high-level (1000 μg/kg) on growth performance of SD rats.

Organ development: The effect of dietary treatment on organ development were shown in Table 3. Supplementation of SPCr with 200, 500 or 1000 μg/kg increased the relative weights of liver by 9.4% ($p<0.05$), 19.5% ($p<0.05$), 24.5% ($p<0.05$), respectively. Addiction of SPCr with 100 or 1000 μg/kg increased the relative weights of spleen by 21.2% ($p<0.05$) and 45.4% ($p<0.05$), respectively. The relative weights of testis tended to increase by supplementation of SPCr in diets, but the CrPic group tended to decrease the relative weight of testis. Furthermore, Addiction of Cr tended to decrease the relative weights of kidney compared with control group, except 200 μg/kg SPCr group. And the relative weights of heart were not affected by dietary Cr, except 100 μg/kg SPCr group.

Serum traits: Serum cholesterol and triglyceride was decreased fed diets with SPCr at low-level (below 500 μg/kg) (Table 4). Supplementation of SPCr with 100, 200 or 500 μg/kg decreased serum cholesterol by 29.6% ($p<0.05$), 20.0 and 10.4%, also decreased serum

Table 2: Influence of SPCr on growth performance of SD rats

	Level of Cr							
Items	Basal	100 μg/kg SPCr	200 μg/kg SPCr	500 μg/kg SPCr	1000 μg/kg SPCr	200 μg/kg CrPic	SEM	p- value
ADG(g/day)	4.00^{b}	4.20^{b}	4.40^{ab}	4.70^{a}	4.10^{b}	4.40^{ab}	0.21	0.069
DFI(g/day)	16.20^{ab}	16.10^{ab}	15.90^{ab}	15.50^{b}	16.10^{ab}	16.30^{a}	0.32	0.237
Feed:Gain ratio	4.02^{a}	3.79^{ab}	3.65^{ab}	3.28^{b}	3.91^{a}	3.77^{ab}	0.22	0.084

Values with different small letter superscripts in the same column indicate significant difference ($p<0.05$). The same as below.

Table 3: Influence of SPCr on relative weight of selected organs in SD rats (%)

	Level of Cr							
Items	Basal	100 μg/kg SPCr	200 μg/kg SPCr	500 μg/kg SPCr	1000 μg/kg SPCr	200 μg/kg CrPic	SEM	p- value
Heart	0.426^{b}	0.534^{a}	0.424^{b}	0.421^{b}	0.424^{b}	0.431^{b}	0.015	<0.001
Liver	3.219^{c}	3.376^{bc}	3.520^{b}	3.845^{a}	4.008^{a}	3.500^{b}	0.115	<0.001
Spleen	0.227^{c}	0.275^{b}	0.266^{bc}	0.252^{bc}	0.330^{a}	0.263^{bc}	0.017	<0.001
Kidney	0.764^{ab}	0.740^{bcd}	0.775^{a}	0.708^{d}	0.727^{cd}	0.742^{bc}	0.015	0.002
Testis	1.086	1.035	1.114	1.125	1.170	0.930	0.122	0.478

Table 4: The effect of SPCr on serum traits in SD rats

	Level of Cr							
Items	Basal	100 μg/kg SPCr	200 μg/kg SPCr	500 μg/kg SPCr	1000 μg/kg SPCr	200 μg/kg CrPic	SEM	p- value
Cholesterol (mmol/L)	1.25^{ab}	0.88^{c}	1.00^{bc}	1.12^{b}	1.38^{a}	1.23^{ab}	0.10	0.006
Triglyceride (mmol/L)	0.60^{b}	0.36^{d}	0.41^{cd}	0.44^{c}	0.87^{a}	0.63^{b}	0.02	<0.001
HDL (mmol/L)	0.67^{c}	0.69^{c}	0.84^{b}	1.04^{a}	0.85^{b}	0.84^{b}	0.04	<0.001
Glucose (mmol/L)	7.02^{a}	6.14^{a}	4.68^{b}	5.80^{ab}	6.65^{a}	6.02^{ab}	0.62	0.039
Insulin (μU/mL)	42.68^{a}	34.54^{abc}	25.96^{c}	32.22^{bc}	38.27^{ab}	33.48^{abc}	4.20	0.031

triglyceride by 40.0% ($p<0.05$), 31.7% ($p<0.05$) and 26.7% ($p<0.05$) compared with control group, respectively. Supplementation of Cr with different types increased serum high density lipoprotein. Addiction of SPCr with 500 μg/kg increased serum high density lipoprotein by 55.2% ($p<0.05$) compared with control group. Addiction of Cr with different types resulted in lower serum glucose and insulin concentrations. Supplementation of SPCr with 200 μg/kg decreased glucose and insulin concentrations by 33.3% ($p<0.05$) and 39.2% ($p<0.05$) compared with control group, respectively.

DISCUSSION

This experiment was conducted to evaluate the influence of supplementing SPCr in diets on growth performance, organ development and serum traits in SD rats. Previous experiments have demonstrated results in growth rate and feed: gain ratio in animals fed diets supplemented with Cr. Page (1993) reported an increase in growth rate but with no change in feed efficiency. Lindemann *et al.* (1995) observed no change in growth rate but found an improvement in feed:gain ratio with the addition of Cr in the form of CrPic at 200, 250, or 500 μg/kg in pigs. Harper and Kornegay (1996) reported increase both in growth rate and feed efficiency in Weaned Piglets fed diets supplemented with 0.2 mg/kg. Gu *et al.* (2007) also, demonstrated that dietary Cr as Cr Nanoparticulated increased ADG and feed:gain ratio of SD rats. In the present investigation, fed diets with SPCr at low-level (below 500 μg/kg) increased ADG and feed:gain ratio of rats, unexpectedly, a decrease($p<0.05$) in ADFI was found fed diets with 500 μg/kg SPCr, Suggested that SPCr may had effect of saving nutrients, this maybe the characteristic that distinguish the SPCr from other forms of Cr.

Liver is the main organ of glycogen synthesis. Mertz (1969) and Amoikon *et al.* (1995) found that chromium could promote synthesis of glycogen. Wayne *et al.* (1988) reported that addition of Cr increased concentrations of hepatin in rats. Steele and Rosebrough (1981) demonstrated that dietary Cr as $CrC1_3$ increased conversion of glucose to acetyl-coA and did not affect acetate incorporation in turkey poult liver tissue. These authors concluded that chromium may had the effect to promote hepatic storage of glucose and increase glucose utilization. In the present study, the relative weight of liver increased by addition of Cr with different types might be the result of increasing of hepatic glycogen. Furthermore, liver is the main organ of lipoprotein synthesis too, the increasing of serum high density lipoprotein in the current study

indicated that chromium may accelerate the synthesis and secretion of HDL and this is also the key factor to

enhance the clearance of blood cholesterol(WANG, 1990). Spleen is the main immune organ of cellular immunity and humoral immunity. The trend of increasing on the relative weight of spleen was found by addition of SPCr in the present study, indicated that SPCr may had effect on immune function in rats. In addition, the trend of increasing on the relative weight of testis may indicated that SPCr had effect on breeding performance in rats, remains to be elucidated. Kidney is the principal route of excretion of chromium. Chromium compounds pose a toxic effect on organs, excessive ingestion of Cr might induce renal and hepatic damage (Michael *et al*., 1983). The trend of decrease on the relative weights of kidney could not explain in the present study, but no pathological changes were found in organs, indicated that supplementation of SPCr had no adverse influence on growth of rats.

The role of chromium in maintaining normal glucose tolerance has been demonstrated in man and laboratory animals (Mertz, 1967). Chromium seems to be essential for optimal insulin sensitivity and glucose uptake by insulin-sensitive cells (Anderson and Kozlovsky, 1985). Steele *et al*. (1977) demonstrated that Cr-containing glucose tolerance factor increase insulin sensitivity in pigs. These authors concluded that Cr-containing glucose tolerance factor is biologically active in animals and that it potentiated the insulin-insulin receptor interaction. Amoikon *et al*. (1995) found that Cr supplementation could lower blood insulin concentrations and increase insulin concentrations by other tissues resulting in increasing glucose utilization, this is because Cr binding is influenced by glucose concentrations differently in insulin-sensitive and insulin-insensitive tissues (Morris *et al*., 1993). Insulin could promote the transportation of glucose and amino acid into muscle cell (Hill and Millner, 1985), it also, enhance the effect of growth hormone (Golde *et al*., 1980). Indeed, the decrease of serum insulin concentrations and the improvement of performance in rats supplemented with SPCr in the current study may implicates this relationship.

Chromium can influence lipid metabolism (Steel and Rosebrough, 1981) in various species. The effect of chromium in decreasing blood triglyceride and maintaining normal blood cholesterol has been demonstrated in animals (Chang and Mowat, 1992; Page *et al*., 1993; Matthews *et al*., 2001), it was also observed in our trials by supplemental SPCr (Table 3). Previous study found that chromium could regulate the synthesis and clearance of cholesterol in liver to influence lipid metabolism (Abraham *et al*., 1980), decreasing deposition of fat to the body (Evans, 1989). There is a compact correlation between cholesterol and cholesterol/HDL ratio and

insulin resistance, higher cholesterol/HDL ratio usually be associated with insulin resistance (Zhang *et al*., 2006), if Cr increases insulin sensitivity, blood cholesterol and triglyceride should be decreased and cholesterol/HDL inhibited, theoretically resulting in improved lipid metabolism, this might indicate that lipid metabolism is as sensitive as carbohydrate metabolism to insulin by fed Cr in diets. In the present study, an increase in serum HDL concentrations was found in rats supplemented with SPCr (Table 3). HDL could promote transportation of cholesterol from blood and surrounding tissues to liver to degradation. Liu *et al*. (1991) demonstrated that the activities of Lipoprotein Lipase (LPl) and Lecithin Cholesterol Acyltransferase (LCAT) was increased by supplementation of chromium, these enzymes participate in synthesis of HDL, consequently increase HDL concentrations in blood to enhance clearance of cholesterol, resulting in decreasing of blood lipids, in associating with the current study, indicated that SPCr may had effect to enhance lipolysis, furthermore, the potential function of SPCr to protect blood vascular system by improving resistance of atherosclerosis should be concerned.

Conclusion: In the current study, SPCr supplementation with low-level (below 500 μg/kg) to diets can increase daily gain and it was more effective than CrPic to improve feed efficiency. In addition, there were positive responses of serum lipids metabolism in rats when fed SPCr in diets.

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