Effects of Different Sources of Organic Chromium on Immune Function in Weaned Pigs*

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ABSTRACT : A five-week trial was conducted to evaluate the effect of organic chromium from different sources on growth performance, immune response and serum parameters of weaned pigs. One hundred and eighty Tianjin white pigs weaned at 35±1 days of age, were allotted to three treatments with six replicates and10 pigs per pen. Pigs were fed corn-soybean-whey-fishmeal basal diets with either no supplemental Cr, 200 μg/kg Cr as chromium picolinate (CrPi), or 200 μg/kg Cr as chromium yeast (Cr-yeast). To assess humoral immune response, all pigs were immunized with swine fever virus on day 21 and two pigs from each pen were immunized with pure albumin on day 14. Cell-mediated immunity was measured by determining the double skinfold thickness (DST) of two pigs from each pen before and 24h after stimulation with phytohemagglutinin (PHA) on day 28. The results indicated that: (1) diets with Cr-yeast increased average daily gain (ADG, $p<0.05$) and tended to increase average daily feed intake (ADFI, $p<0.10$). Diets with CrPi did not increase ADG and ADFI ($p>0.05$). (2) Dietary CrPi or Cr-yeast supplementation did not affect blood urea nitrogen, glucose, or cholesterol ($p>0.05$), but blood urea nitrogen in CrPi and Cr-yeast supplemented groups and blood glucose in the Cr-yeast supplemented group were significantly influenced by sampling days ($p<0.05$). (3) Serum proteins (TP, ALB, and GLB) were influenced by sampling days ($p<0.05$), but not by dietary Cr treatment ($p>0.10$). (4) There were no significant differences among treatments in the titers of albumin antibody and swine fever virus antibody ($p>0.05$) or DST before and after PHA stimulation ($p>0.05$), indicating that organic chromium has no significant effect on the immune function of weaning pigs. Therefore, these results agree with other research that the effects of supplemental Cr are variable in weanling pigs. *(Asian-Aust. J. Anim. Sci. 2001. Vol 14, No. 8 : 1164-1169)*

Key Words : Chromium Picolinate, Chromium Yeast, Immune Function, Weaned Pig

INTRODUCTION

Introduction

Chromium (Cr) is an essential trace mineral for swine (NRC,1998). Interest in Cr as a nutrient first stemmed from laboratory animal studies in the late 1950's when trivalent Cr was found to be a major component of the glucose tolerance factor (GTF). Glucose Tolerance Factor, a substance that is particularly abundant in Brewer's Yeast, was found to maintain normal glucose tolerance in rats (Schwarz and Mertz, 1959). Studies have shown a high activity of insulin in the presence of GTF. Chromium plays an active role in carbohydrate, protein and lipid metabolism by potentiating the action of insulin (Anderson, 1987). Chromium performs a positive role in dealing with stress problems and immunological functions of cattle (Burton, 1995).

During weaning, pigs are subjected to environmental, behavioral and nutritional stresses. Moreover, at this stage, pig immunity status is at its lowest level due to the change from passive immunity acquired from colostrum to active immunity (Pluske et al., 1995). Stress and disease increase urinary excretion of Cr (Pekarek et al., 1975; Anderson et al., 1988), which may exacerbate a marginal Cr deficiency. This study was conducted to investigate the effects of two sources of supplemental Cr (CrPi and Cr-yeast) on growth performance, blood parameters, and immune function in weaning pigs.

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MATERIALS AND METHODS

Animals and diets

A total of 180, mixed-sex, 35d-old crossbred (Landrace×Native North-East) weanling pigs (average initial weight of 9.16±1.29 kg) were allotted to one of three treatments based on weight and sex. Pigs were housed ten per pen (5 male and 5 female) in a half-open nursery with solid concrete floors. Temperature in the nursery ranged from 15~29°C. There were six replicates per treatment, and treatments were arranged in a one-factor randomized complete block design. Pigs were allowed feed and water ad libitum. The basal diet (table 1) consisted of corn, soybean meal, dried whey, and the Five Star Booster pack, formulated to contain 1.4% lysine for the early two weeks (Phase 1) and 1.3% lysine for the last three weeks (Phase 2). The Five Star Booster pack is a unique complex of highly digestible sugars, oligosacchrides, organic acids and natural flavors that improves pig starter feeds in five ways (see table 1 for nutrient analysis). The diets were supplemented with minerals and vitamins to meet or exceed requirements (NRC, 1998). The three dietary treatments consisted of the

Table 1. Composition of diets and their nutrient levels

Ingredients (%)	Phase 1	Phase 2
Corn	56.3	55.39
Soybean meal	15.53	26.05
Five Star Booster[a]	5	5
Dried whey	5	3
Soy isolated protein	3.5	0
Spray dried plasma protein	3	0
Corn gluten meal	0	2.8
Soy oil	3.6	2
Fish meal	4	1.5
Limestone	0.92	0
Shell meal	0	0.93
Dicalcium phosphate	1.07	1.18
Salt	0.3	0.3
Premix[b]	1	1
Lysine	0.38	0.54
Methionine	0.24	0.19
Threonine	0.16	0.12
Nutrient levels*		
DE(MJ/kg)	13.79	13.75
CP	19.03	18.49
Ca	0.84	0.8
Total phosphorus	0.67	0.65
Total Lysine	1.4	1.3
Methionine	0.55	0.53
Threonine	0.88	0.88

[b]Premix provided per Kg of complete diet: Vitamin A, 5,512 IU; Vitamin D, 3351 IU; Vitamin E, 66.1 IU; Vitamin B_{12}, 22.76 ug; Menadione, 2.2 mg; Riboflavin, 5.5 mg; d-pantothenic acid, 13.8 mg; Niacin, 30.3 mg; Choline,551 mg; Mn, 100 mg; Zn, 100 mg; Fe 100 mg; Cu, 250 mg; I,0.3 mg; Co, 1 mg; Se, .0.3mg.

* CP, Ca, and total phosphorus are assayed values: others are calculated values.

[a]Nutrient Analysis of Five Starr Booster

Ingredients	%
Total sugars	80.0
Oligosacchrides	12.5
Crude protein	1.0
Crude fat	1.0
Crude fiber	0.5
Moisture	4.5
Ash	0.5
ME kcal/kg (Calculated)	3,830
PH (in 10% solution)	3.5

All the above nutrient data were the analyzed results except total sugars, oligosacchrides, and ME, which were derived from the technique brochure of International Ingredient Corporation, US.

basal diet, the basal diet supplemented with chromium picolinate (providing 200 μg/kg Cr) from Zhe Jiang Huang Yan Chemical Company, P.R China, and the basal diet supplemented with Chromium yeast (providing 200 μg/kg Cr) from Alltech, USA. Chromium picolinate was mixed with limestone, which was then incorporated into the premix, then into the diet. Chromium yeast was mixed with premix and incorporated into the diet. Phase 1 diets were pelleted and Phase 2 diets were fed as meal.

Experiment procedure

Body weight and feed intake were determined on d 0, 14 and 28 of the experiment. Also on d 0, 14 and 28 of the experiment, approximately 15 ml of blood were taken from the anterior vena cava using vacutainer tubes in two randomly selected pigs per pen. Blood centrifuged (Heraeus Biofuge 22R Centrifuge) at 3,000 rpm for 15 minutes to get plasma. Plasma was stored and frozen at -30°C for analysis of glucose (GLU), blood urea nitrogen (BUN) cholesterol (CHOL), total protein (TP), globulins (GLB).

On d 21 of the study, in vivo cellular immune response was determined using a PHA skin test (Heugten and Spears, 1997). Two randomly selected pigs per pen were injected subcutaneously with 0.5 ml PHA (250 μg/ml) in the left flank and 0.5 ml physiological saline in the right flank. Double skinfold thickness (DST) was determined at 0 h and 24 h after injection of PHA and physiological salt.

To determine humoral immune response, two randomly selected pigs per pen were injected i.m. with egg albumin at a dose of 1 mg/kg BW on d14 of the study. Antibody titers against egg albumin were measured 0, 7, 14 and 21 d after antigen injection. All pigs were injected i.m. with swine fever virus (1 ml/pig) on d 21 of the study, and antibody titers against swine fever virus were measured on d 0 and d 14 after antigen injection.

Chemical analysis

The samples of diets were analyzed for the proximate principles (AOAC, 1990). Serum glucose, blood urea nitrogen, cholesterol, total protein, and globulins were measured using a fully-automatic biochemical analyzer (Technicon RA1000). Glucose was analyzed by a hexokinase method (Leon et al., 1997). Blood urea nitrogen was analyzed by the enzymatic method of Tiffany et al. (1976). Cholesterol was identified by an enzymatic method for glycerol after hydrolysis with lipoprotein lipase, using glycerol-phosphate-oxidase, which releases H_2O_2 (Fossati and Precipe, 1982). Total protein was analyzed by the biuret method (Skaggs and Hochstrasser, 1964). Test reagents for glucose, blood urea nitrogen, cholesterol, total protein, and globulins were manufactured by Beijing Zhongsheng Hightech Bioengineering Co., Beijing, China. Serum chromium (Cr) was measured using an atomic absorption spectrophotometer (Z-5000, Hitchi). The relative egg albumin antibody contents in diluted serum were estimated by a direct double-antibody enzyme-linked immunosorbent assay (ELISA) (Yang, 1996) using a 551 ELISA Reader

(Third Analytical Equipment Factory, Shanghai, China). The entire assay was performed at room temperature. All samples were tested at dilutions of 1/10, 1/200, 1/400, and 1/800. Optical density (OD) was used as the estimate of antibody content of diluted test serum. The OD of the 1/400 dilution of positive serum was used to reflect the content of antibody. The 1/400 dilution of positive control serum was chosen to standardize the assay. Swine fever virus antibody titers were tested following the same procedures above for the egg albumin antibody ELISA.

Statistical analysis

The data were statistically analyzed by one-way analysis of variance (SPSS, 1993). Duncan's Multiple Range Test determined significant different among treatments. Data on skinfold thickness before and after injection of phytagglutinin were compared using a paired sample Student's T-test.

Table 2. Effects of different sources of organic chromium on growth performance of weanling pigs

Items	Control	CrPi	Cr-yeast	SEM	P value
Initial BW (kg)	9.21	9.18	9.08	0.17	0.86
0-14 days					
ADG (kg)	0.31	0.30	0.31	0.021	0.83
ADFI (kg)	0.38	0.37	0.40	0.016	0.61
F/G	1.23	1.28	1.28	0.06	0.69
15-28 days					
ADG (kg)	0.49[ab]	0.44[a]	0.51[b]	0.021	0.052
ADFI (kg)	0.74	0.70	0.76	0.046	0.71
F/G	1.52	1.60	1.49	0.13	0.98
0-28 days					
ADG (kg)	0.40[ab]	0.37[a]	0.41[b]	0.012	0.042
ADFI (kg)	0.57	0.54	0.58	0.028	0.087
F/G	1.42	1.47	1.42	0.07	0.91

[a,b] Means in the same row with different superscripts differ significantly ($p<0.05$).

Table 3. Effects of organic chromium sources on serum biochemical index

Items	Control	CrPi	Cr-yeast	SEM	P value
Cholesterol (mg/dl)					
d 0	100.11	98	100.20	8.52	0.98
d 14	85.55	91.75	80.33	6.46	0.51
d 28	85.45	92.36	85.17	6.35	0.67
Blood urea nitrogen (mg/dl)					
d 0	16.54	13.61[a]	12.90[a]	1.58	0.25
d 14	11.87	11.09[a]	9.09[a]	2.00	0.63
d 28	14.75	17.22[b]	18.05[b]	1.53	0.30
Blood glucose (mg/dl)					
d 0	120.22	118.56	93.70[a]	5.78	0.004
d 14	102.55	117.25	112.67[b]	7.32	0.29
d 28	86.91	99.82	98.33[c]	6.29	0.30
Blood Cr (mg/ml)					
d 0	7.34	7.09	6.81	0.63	0.83
d 14	8.23	8.14	7.62	0.81	0.86
d 28	8.28	6.91	7.59	1.02	0.64

[a,b,c] Means in the same column with different superscripts differ ($p<0.05$) for each parameter measured.

RESULTS

Pig performance

Four-week growth performance is shown in table 2. For the overall 4 week period, supplemental Cr-yeast significantly improved average daily gain ($p<0.05$) and tended ($p=0.08$) to increase average daily feed intake. Also, there was a tendency ($p=0.052$) for Cr-yeast to improve gain during the last 2 weeks of the trial. No differences in feed conversion were observed at any point during the experiment. Supplemental CrPi did not elicit a positive response in any performance parameter measured.

Blood parameters

Blood parameter data are shown in table 3. Blood urea nitrogen was significantly influenced ($p<0.05$) by sampling days in both CrPi and Cr-yeast supplemented groups, and the highest concentrations were observed at the 4 wk bleeding. Blood glucose was significantly influenced ($p<0.05$) by sampling day (higher levels at 2 week and lower levels at 4 week) in Cr-yeast supplemented groups. Time of blood sampling had no effect on blood glucose in the CrPi supplemented group, nor did time of blood sampling have an effect on blood cholesterol levels in any of the treatments. Blood urea nitrogen, glucose, cholesterol, or Cr concentrations was not affected by Dietary CrPi or Cr-yeast supplementation.

Immune response measurements

Neither Cr supplementation nor time of blood sampling (0 d, 7d and 21d after egg albumin injections) had any effect ($p>0.10$) on antibody titers against egg albumin (table 4). Antibody response to swine fever virus (table 5) when measured on d 0 and d 14 after antigen injection was not significantly influenced ($p>0.10$) by injection of antigen and different treatments. Skin thickness response to PHA (table 6) was increased ($p<0.05$) 24 h after PHA injection. No differences were observed among treatments ($p>0.10$).

Serum proteins

Levels of serum total protein (table 7) were significantly higher ($p<0.05$) in CrPi supplemented piglets (28 d) and Cr-yeast supplemented piglets (d 14 and d 28) compared to values observed on d0 of experiment. However, no significant differences were found between treatments. ($p>0.10$)

Serum albumin ($p<0.05$) was significantly higher in Cr-yeast supplemented piglets on d 28 when compared with

Table 4. Effects of different organic chromium on serum albumin antibody

Items	Control	CrPi	Cr-yeast	SEM	P value
Before antigen injection	1.85	1.59	1.73	0.22	0.71
1 wk after injection	2.13	2.14	2.35	0.08	0.11
3 wk after injection	2.09	2.13	1.95	0.19	0.78

Table 5. Effects of different sources of organic chromium on swine fever antibody

Items	Control	CrPi	Cr-yeast	SEM	P value
Before antigen injection	0.11	0.11	0.12	0.012	0.94
14 d after injection	0.09	0.10	0.17	0.037	0.43

Table 6. Effects of different organic chromium on PHA stimulation

Double skinfold thickness	Control	CrPi	Cr-yeast	SEM	P value
Before injection					
Left flank (mm)	2.80	2.82	2.73	0.07	0.93
Right flank (mm)	2.71	2.87	2.72	0.05	0.64
After injection					
Left flank (mm)	4.06	4.29	4.05	0.17	0.82
Right flank(mm)	3.09	3.28	3.01	0.12	0.62
Increase					
Left flank (mm)	1.26[a]	1.47[a]	1.32[a]	0.25	0.77
Right flank(mm)	0.38[a]	0.41[b]	0.29[b]	0.16	0.83
Difference[c] (mm)	0.88	1.06	1.03	0.24	0.68

[a,b] Means in the same column with different superscripts differ ($p<0.05$) for each parameter measured.
[c] Between left and right flank.

value obtained on the initial day of experiment, with no difference among treatments ($p>0.10$). Serum globulin was significantly increased ($p<0.05$) in both CrPi and Cr-yeast supplemented piglets on d 28 when compared with concentrations at the beginning of experiment. However, no significant between treatments differences were found, nor were serum globulin/albumin ratios affected by dietary treatment ($p>0.10$).

DISCUSSION

Growth performance

Trivalent chromium is an essential trace mineral for growing livestock. While plants are the major source of dietary Cr for pigs, the content of chromium in plants is very low. The level of chromium in conventional pig diets for pigs does not meet the requirement for maximum growth of pigs and must be supplemented with a bioavailable source of chromium (Page et al., 1993). The chromium content of corn-soybean meal diets varies from

Table 7. Effects of different sources of organic chromium on serum total protein, albumin, globulin, and globulin/albumin ratio

Items	Control	CrPi	Cr-yeast	SEM	P value
Total protein(mg/dl)					
d 0	6.33	6.35[a]	6.08[a]	0.28	0.74
d 14	6.81	7.05[ab]	7.01[b]	0.34	0.85
d 28	6.99	7.54[b]	7.62[b]	0.29	0.28
Albumin (mg/dl)					
d 0	4.16	3.73	3.33[a]	0.25	0.08
d 14	3.95	4.28	3.93[ab]	0.23	0.49
d 28	3.85	4.22	4.15[b]	0.19	0.38
Globulin (mg/dl)					
d 0	2.49	2.62[a]	2.75[a]	0.18	0.76
d 14	2.87	3.09[ab]	3.08[ab]	0.24	0.78
d 28	3.18	3.51[b]	3.47[b]	0.27	0.52
Globulin/Albumin					
d 0	0.65	0.71	0.88	0.07	0.19
d 14	0.73	0.78	0.79	0.07	0.77
d 28	0.84	0.90	0.84	0.04	0.80

[a,b] Means in the same column with different superscripts differ ($p<0.05$) for each parameter measured.

750 to 1500 μg/kg (NRC, 1998), most of which is unavailable to the animal therefore, it is necessary to supplement digestible chromium.

In this study, the main components of the diets were corn and soybean meal. The diets were supplemented with fishmeal, limestone, dicalcium phosphate and other ingredients, which may contain a low content of chromium. So, the variables that were measured (blood parameters, immunological parameters) may have been confounded with basal levels of chromium present in above ingredients. The utilization efficiency of chromium is highly related to its chemical structure of chromium. The utilization efficiency of inorganic chromium is only 1~3% compared to the 10~25% of organic chromium (Anderson 1987). Dietary chromium picolinate supplementation increased average daily gain in weaning pigs (Harris et al., 1995; Harper et al., 1995). Contrary to those results, Lee et al. (1997) reported no significant improvement in growth performance when diets for weaned piglets were supplemented with CrPi. Average daily gain and feed intake in weaning piglets were not influenced by Cr-yeast supplementation (Baldi et al., 1999). However, in this study, pigs fed diets supplemented with Cr-yeast had larger average daily gains while pigs fed diets with supplemental CrPi did not exhibit any improvement in growth performance.

Dietary lysine level influences the effects of chromium on piglets. Ward et al. (1997) reported that chromium supplementation at 400 μg/kg of diet throughout the growing-finishing phases increased average daily gain and feed:gain ratio in pigs fed a diet providing 80% of lysine

requirement. However, when pigs were fed diets containing 120% of the lysine requirement, the supplemental Cr decreased average daily gain and gain: feed. In this study, the lysine contents in diets are respectively 1.4% and 1.3% in phase 1 and phase 2, higher than the 1.15% and 0.95%, the lysine requirement for 10~20 kg and 20~50 kg pigs (NRC, 1998). This may explain the inconsistency in positive performance response to supplemental Cr observed in this study.

Blood parameters

Chromium is important in carbohydrate, fat and protein metabolism, presumably by potentiating the action of insulin (Anderson, 1987; Mertz, 1993). Glucose tolerance factor (GTF) is important in maintaining the normal concentration of glucose in blood. Glucose tolerance factor (GTF) can improve the tolerance of glucose and insulin activity and stimulate glucose absorption. Animals cannot utilize glucose when chromium is deficient in feed. Kegley and Spears (1995) observed the tendency of higher clearance rate of glucose in diets supplemented with chromium nicotinic acid as compared to the non-supplemented group. Glucose in serum significantly decreased after weaning pigs were stimulated by ACTH (Baldi et al., 1999), suggesting this response is induced by higher insulin sensitivity and higher glucose uptake.

Glucose concentrations showed significant differences among treatments at the beginning of the experiment, but this did not occur in other blood parameters. One of the reasons could be the collection time of the blood after feeding diets. Considering the difference of glucose concentrations among animals at the beginning of this experiment, concentrations of glucose on d0 and 28 were compared. The concentrations of glucose on d 28 individually were 72.39%, 84.19% and 104.94% of that on d 0 for control, CrPi and Cr-yeast groups, respectively. These results are contrary to other researchers' observations (Lee et al., 1997; Page et al., 1993).

Chromium can maintain normal concentrations of cholesterol in blood (Page et al., 1993) and affects the synthesis and decomposition of fat and cholesterol in liver. Concentration of cholesterol in blood decreased when CrPi (providing 0.25 mg/kg Cr) was supplemented to diets for lambs (Kitchlong et al., 1995). Contrary to their results, Samsell and Spears(1989) observed higher concentration of total cholesterol in blood when CrPi is supplemented to diets. Bunting (1994) did not observe a decrease in cholesterol concentration until 6 weeks into the experiment. Our present findings indicate that cholesterol levels in blood were not influenced by CrPi or Cr-yeast supplementation in weaning pigs.

Blood urea nitrogen (BUN) varies when protein and amino acid metabolism changes. Blood urea nitrogen (BUN) increases with a decrease of amino acid utilization (Baldi et al., 1999). Lindamann et al. (1995) observed same results in diets supplementation with CrPi. However, Ward et al. (1995) reported contrary results in diets containing higher levels of lysine. Levels of protein and amino acids in diets may influence effects of Cr on nitrogen metabolism. In this study, CrPi or Cr-yeast in diets did not significantly affect the metabolism of nutrients, which may be influenced by the high lysine in the diet.

Immune function

Chromium concentrations in conventional diets cannot meet the Cr requirement of pigs, and chromium is an essential precursor in the immune function of pigs (Burton, 1995). Any stress results in more excretion of Cr from urine (Pekarek et al., 1975). Glucose tolerance factor (GTF) strengthens the activity of insulin and influences the secretion of cortisol. Chromium may enhance the immune function by improving the effects of vaccine or decreasing cortisol concentration (Roth and Keberle, 1982; Munck et al., 1984). Chung and Mowat (1992) suggested that Cr promoted the synthesis of immune protein by influencing the activity of enzyme or by influencing the metabolism of copper and zinc.

Antibody titers against pseudohydrophobia, lymphocyte blastogenesis after PHA stimulation, and contents of IgG and IgM in weaning pigs were improved by CrPi supplementation (Lee et al., 1997). Many researchers have reported better immune function in ruminants with Cr supplementation in diets (Chung and Mowat, 1992; Burton et al., 1993; Wright et al., 1994). Contrary to these results, Heugten and Spears (1997) reported that lymphocyte blastogenesis and antibody titer against SRBC were improved, but the antibody titer against OVA was decreased by supplementation Cr in weaning pigs. Also, the negative effects of stimulation of lipopolysaccharide (LPS) was not alleviated. Immune function in crowded pens was not improved and the function of lymphoid leucocyte and neutrophils was not changed by CrPi supplementation. The change of skinfold thickness after PHA injection reflects the infiltration of primarily mononuclear cells (Blecha and Minocha, 1983). No effect of Cr supplementation was observed on the PHA skin test response, which agrees with the results of Heugten and Spears (1997).

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