

Effects of Chromium Propionate Supplementation on Growth Performance, Serum Traits and Immune Response in Weaned Pigs

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ABSTRACT : This study investigated the effects of chromium propionate on growth performance, serum traits and immune response in weaned pigs. Twenty-four 4 wk-old crossbred weanling pigs (initial body weight about 9.52 ± 0.48 kg) were randomly allotted into one of two groups, a control group (basal diet), chromium propionate group (diet supplemented with $200 \mu\text{g kg}^{-1}$ (ppb) of chromium propionate). This experiment was conducted over nine weeks. *Escherichia coli* lipopolysaccharide (LPS) $100 \mu\text{g kg}^{-1}$ BW was used as the stress-inducing agent in the middle (4 wks) and final (8 wks) periods. The experimental results indicated that chromium propionate had no effect on growth performance ($p > 0.05$). Chromium propionate supplementation reduced the percentage of LDL+VLDL (low and very low-density lipoprotein) and increased HDL (high-density lipoprotein), but did not affect other serum traits. Pigs supplemented with chromium propionate had higher antibody titers specific for sheep red blood cells (SRBC) and serum total globulin relative to the control during the final period ($p < 0.05$). A challenge with LPS increased white blood cells in the chromium propionate group in both experimental periods ($p < 0.05$). The chromium propionate group exhibited higher IgG and γ -globulin than the control during the middle experimental period ($p < 0.05$). Moreover, the PHA (phytohemagglutinin) challenge result in the chromium propionate group was better than the control group ($p = 0.056$). Greater neutrophil activity was displayed than in the control ($p < 0.05$). This suggests that chromium propionate supplementation benefited the weaned pigs in lipoprotein and immune response. (*Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 3 : 403-408*)

Key Words : Chromium Propionate, Serum Traits, Immune Response, Pigs

INTRODUCTION

Trivalent chromium, a recognized cofactor of insulin, is involved in carbohydrate (Rosebrough and Steele, 1981), lipids (Press et al., 1990; McCart, 1991), amino acids and nucleic acids metabolism (Okada et al., 1984; Ohba et al., 1986; Page et al., 1993). Chromium plays a prominent physiological role in humans (Evans, 1989, 1992; Press et al., 1990) and animals (Lien et al., 1996, 1998, 1999a, 1999b, 2001), and is considered an essential trace element.

Organic trivalent chromium with low toxicity (Evans, 1989; Walker, 1993), has been shown to reduce blood glucose, cholesterol, body fat and stimulate muscle development in humans (Anderson, 1986; Evans, 1989; Press et al., 1990). Thus, the NRC (1989) has recommended that humans consume 50-200 ppb trivalent chromium daily.

Stress, disease and morbidity are costly economic problems in weanling pigs. Enhancing the immune response of weaning pigs by nutritional means might improve pig health and reduce the cost of medical treatment. Additionally, stress and disease increase chromium urinary excretion (Mertz, 1993), and might exacerbate marginal chromium deficiencies.

Improvements in immune response have been observed

following organic chromium supplementation to stressed feeder calves (Chang and Mowat, 1992; Moonsie-Shageer and Mowat, 1993), and dairy cows (Burton et al., 1993). Thus, the objective of this work was to investigate the chromium supplementation on the immune response of normal and stress conditions of weaned pigs.

Chromium propionate is another organic form of chromium. In a previous investigation (unpublished), we confirmed that chromium propionate could be as effective on serum traits as chromium picolinate in pigs. This work, therefore, investigates organic chromium propionate supplementation effects on growth, serum traits and immune response in normal and stress condition of weaned pigs.

MATERIALS AND METHODS

Animal and treatment

Twenty-four 4 wk-old crossbred (Landrace×Duroc) weanling pigs with both sexes in equal numbers (initial body weight approximately 9.52 ± 0.48 kg) were randomly allotted into one of two groups; control (basal diets, as listed in Table 1) and chromium propionate groups (diet supplemented with $200 \mu\text{g kg}^{-1}$ (ppb) of chromium propionate) (Kemin Industry, Singapo). The pigs were housed in four pens in an environmental nursery. Feed and water were provided *ad libitum*. This experiment was conducted for 9 weeks. The animals used in this experiment were cared for under the guidelines stated in the *Guide for*

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Table 1. Composition of basal diets on as fed basis

Ingredient	kg/1,000 kg	
	9-25 kg	25-55 kg
Yellow corn meal	791.6	752.4
Isolated soybean protein	110.9	-
Skim milk, dried	50.0	-
Soybean meal	-	165.0
Fish meal (60%)	-	20.0
Soyabean oil	17.7	34.9
Limestone, pulverized	6.9	3.9
Calcium phosphate, dibasic	12.9	13.8
Iodined salt	4.0	5.0
Vitamin premix ^a	3.0	3.0
Mineral premix ^b	2.0	2.0
DL-methionine	1.0	-
Total	1,000.0	1,000.0
Calculated value:		
Crude protein (%)	19.00	15.00
ME (MJ/kg)	13.40	13.40
Calcium (%)	0.86	0.70
Phosphorus (%)	0.65	0.60
Lysine (%)	1.15	0.75
Methionine+cystine (%)	0.55	0.49

^a Vitamin premix supplied the following per kilogram of diet: vitamin A, 8,000 IU; vitamin D₃, 1,200 IU; vitamin E, 40 IU; vitamin K₃, 4 mg; vitamin B₁, 0.6 mg; vitamin B₂, 8 mg; vitamin B₁₂, 40 mg; niacin, 80 mg; pantothenic acid, 34 mg.

^b Mineral premix supplied the following per kilogram of diet: Fe (FeSO₄·7H₂O, 20.09% Fe), 140 mg; Cu (CuSO₄·5H₂O, 25.45% Cu), 17 mg; Mn (MnSO₄·H₂O, 32.49% Mn), 3.3 mg; Zn (ZnSO₄, 80.35% Zn), 75 mg; Se (NaSeO₃, 45.56% Se), 0.17 mg; Co (CoSO₄·H₂O, 32% Co), 0.7 mg.

the Care and Use of Agricultural Animals in Agricultural Research and Teaching. Pigs were vaccinated twice for classical swine fever, pseudorabies, and foot and mouth disease.

Escherichia coli lipopolysaccharide (LPS), an endotoxin, was used as the stress-inducing agent at dose of 100 µg kg BW⁻¹ injection by i.m. during the middle (4 wks) and final (8 wks) periods of the experiment.

Determination traits and methods

Growth performance based on averaged daily gain, feed intake and feed efficiency were determined. Blood was sampled at 0 and 6 or 24 h after LPS injection during the final and middle periods of the experiment, respectively. White blood cells were measured using a microcell counter (Sysmex F-800, Japan) following LPS challenge.

Neutrophil activity was examined at final period of experiment as described by Morrow-Tesch and Andersson (1994) to determine cellular immune response. Serum immunoglobulin G (IgG) was determined in triplicate at 4 and 8 weeks of experiment using the method described by Leslie and Frank (1989), with purified porcine IgG used as the standard.

Serum total cholesterol, triacylglycerol (TG), creatinine

Table 2. Effects of chromium propionate on growth performances of pigs

Item	Control	Cr propionate
Averaged daily gain (kg)	0.62±0.02	0.60±0.04
Daily feed intake (kg)	1.27±0.26	1.28±0.23
Feed:gain ratio	2.05±0.06	2.13±0.16

Means±SE (n=12).

and urea were enzymatically measured by employing commercial kits with an autoanalyzer (Roche Co., Switzerland). The high-density lipoprotein (HDL), low-density lipoprotein (LDL), total globulin and γ-globulin levels were measured using electrophoresis. The percentage of each gel band was measured using a densitometer (Helena Co., 8JF00105, USA). Classical swine fever antibody titer was determined using an ELISA kit (JENO, Korea). To examine antigen of ovalbumin response, 1 ml of a solution with 0.5 mg ovalbumin in a mixture of 0.5 ml Freund's incomplete adjuvant was injected by i.m. at 5 week of the experiment, the blood samples was taken after 2 weeks. The ovalbumin antibody titer was measured as the described by Van Heugten et al. (1997) in duplicate. For measure antibodies against sheep red blood cell (SRBC), the SRBC was isolated from black belley sheep, and 2 ml, 0.25% SRBC was injected two times at 5 and 6 week of the experiment, the blood samples was taken at 7 week. Antibodies titer against SRBC was measured in duplicate followed application of the Van Heugten et al. (1997) procedure for investigating humoral immune response. To examine the mitogen of phytohemagglutinin (PHA) skin swelling response, 150 µg ml⁻¹ PHA was injected at 7 weeks of the experiment by s.c. and measured the ear skin thickness of swelling after 48h injection as described by Kegley and Spears (1995).

Statistical analysis

The SAS system was used to analyze the variance between groups, with the significance determined with T test (SAS, 1990). According to the model, Cr was the main effect.

$$Y = \mu + Cr_i + e_{ij}$$

Where Y is the dependent variable, µ is the mean and e is the random residual error term.

RESULTS

Table 2 displays the chromium propionate supplementation effects on pig performance. No significant effect was noted on average daily weight gain, daily feed intake and feed conversion ratio (p>0.05).

Table 3 presents the chromium propionate supplementation effects on the serum traits of pigs during

Table 3. Effects of chromium propionate on the serum traits of pigs during the middle (4 wks) and final (8 wks) of experimental period

Item	4 wks		8 wks	
	Control	Cr propionate	Control	Cr propionate
Urea (mg/dl)	12.24±0.62	12.86±0.75	12.33±0.95	11.26±0.66
Creatinine (mg/dl)	2.99±0.15	3.20±0.25	3.19±0.12	2.89±0.10
Cholesterol (mg/dl)	65.83±5.42	64.00±4.98	51.08±2.68	51.09±1.82
Triacylglycerol (mg/dl)	34.49±1.43	36.77±1.79	29.25±1.83	29.32±1.78
Glucose (mg/dl)	53.36±6.07	41.33±4.39	57.20±3.97	56.10±2.33
HDL (%)	33.68±0.91	41.54±0.62 *	36.66±0.59	37.60±0.89
LDL+VLDL (%)	66.32±0.91	58.46±0.62 *	63.34±0.57	62.41±0.89

Means±SE (n=12). * Mean in the same row control V.S. Cr propionate is significantly different (p<0.05).

Table 4. Effects of chromium propionate on the blood immune response of pigs during the middle (4 wks) and final (8 wks) of experimental period

Item	4 wks		8 wks	
	Control	Cr propionate	Control	Cr propionate
IgG (mg/ml)	3.42±0.46	6.26±0.57 *	8.61±0.66	9.52±0.82
Total globulin (%)	53.01±1.25	56.77±1.93	46.47±0.76	50.94±1.60 *
γ-globulin (%)	22.79±0.51	26.89±1.01 *	21.47±0.45	21.83±0.65
Increased white blood cell (10 ⁶ /ml)	15.16±1.53	20.62±1.48 *	6.35±1.63	11.93±0.45 *

Means±SE (n=12). * Mean in the same row control V.S. Cr propionate is significantly different (p<0.05).

Table 5. Effects of chromium propionate on the immune response of pigs

Item	Control	Cr propionate
Classical swine fever antibody (log ₂)	406.67±55.58	436.36±50.92
Neutrophil activity (%)	42.58±4.27	74.17±7.18 *
SRBC antibody (log ₂)	2.42±0.14	3.08±0.10 *
Ovalbumin antibody (log ₂)	162.26±10.03	155.00±6.22
PHA skin swelling test, mm ^a	9.26±0.24	12.49±1.73

Means±SE (n=12).

* Mean in the same row is significantly different (p<0.05).

^a The P value in control V.S. chromium propionate group is p=0.056.

the middle (4 wks) and final (8 wks) of experimental periods. Supplementation with chromium propionate could reduce LDL+VLDL percentage, meanwhile, increased HDL percentage at middle period (p<0.05). The effects on the other parameters among groups were insignificant (p>0.05).

Table 4 displays the chromium propionate effects on pig blood immune response during the middle (4 wks) and final (8 wks) of experimental periods. The table reveals that IgG and γ-globulin were higher in the chromium propionate group than in the control group at middle period (p<0.05). The chromium propionate group displayed elevated blood total globulin compared to the control group at final period (p<0.05). Moreover, the chromium propionate group displayed a significant increase in white blood cells after LPS challenge than the control group at both periods (p<0.05).

Table 5 shows the chromium propionate effects on pig immune response. The chromium propionate groups exhibited higher sheep red blood cell antibodies than the control group (p<0.05). The neutrophil activity in the chromium propionate group was also markedly greater than

that in the control group (p<0.05). The skin thickness after the PHA challenge in the chromium propionate group was better than that in the control group (p=0.056).

DISCUSSION

This study found that chromium propionate supplementation did not improve pig growth performance. This was consistent with our previous study with chromium picolinate (Lien et al., 1998). However, the chromium supplementation effect on pig growth performance was inconsistent.

Chromium is considered to function as an insulin cofactor. Chromium stimulates insulin activity by boosting insulin receptor synthesis or binding activity (Anderson et al., 1987,1991; McCarty, 1991; Morris et al., 1992; Ward et al., 1994). Increased insulin activity stimulates lipoprotein lipase activity (Howard et al., 1993), consequently increasing VLDL metabolism. Chromium propionate supplementation thus appears to reduce the VLDL+LDL percentage during the middle experimental period. Simultaneously, chromium supplementation increased the HDL percentage. This result was consistent with our previous study with pigs (Lien et al., 2001). Numerous previous chromium studies have shown that chromium supplementation reduces serum cholesterol, triacylglycerol and glucose of pigs (Amoikon et al., 1995; Lien et al., 1998,2001). However, this study failed to find any such effect.

Chromium supplementation has been reported to ameliorate body weight loss and improve the immune response in beef cattle subjected to transportation stress

(Chang and Mowat, 1992; Moonsie-Shageer and Mowat, 1993). In the current investigation, pigs SRBC antibody titers, γ -globulin at 4 wks and total immune globulin at 8 wks of experiment were elevated by chromium propionate supplementation. This agreed with the experimental results obtained by Moonsie-Shageer and Mowat (1993); namely, that calves supplemented with chromium displayed boosted serum total globulin and human red blood cell antibody titers. Furthermore, chromium nicotinate supplementation in pigs also indicated increased antibody response to SRBC (Van Heugten and Spears, 1997). Similarly, Cao et al. (2004) reported that chicks receiving Cr 5-10 mg/kg feed, the Newcastle disease antibody titer was significantly elevated. However, the classical swine fever response antibody did not differ significantly between the two groups in this study.

The PHA skin swelling test primarily measures mononuclear cell infiltration into the injection site (Blecha et al., 1984). Generally, neutrophils are the first cells to respond followed by monocytes and lymphocytes. This study showed that the chromium propionate group had the greater PHA skin fold thickness. Meanwhile, the neutrophils activity test also showed that the chromium propionate group was a greater response than the control group. These two parameters agreed with each other. Kegley et al. (1996) investigated chromium with calves and demonstrated that chromium supplementation increased skin swelling after PHA intradermal injection, and blastogenic response in lymphocytes to PHA also increased (Kegley and Spear, 1995). However, Chang et al. (1996) reported that chromium supplementation did not boost neutrophil phagocytic activity in dairy cows.

Most investigators used lipopolysaccharide, an endotoxin, as a stress-inducing agent when studying immune response. This work also used lipopolysaccharide to induce stress. The animal response to LPS depends on the dose used and the method of administration. This work wishes to stress that in preliminary test injection of LPS at a dose of 200 $\mu\text{g kg BW}^{-1}$ by i.v. on day 30 of the trial, resulted in death in half of the pigs. Interestingly though, the mortality rates differed significantly between groups, with mortality 10/12 in the control group, compared to 5/12 in the chromium propionate group. Apparently, chromium propionate supplementation could reduce the harmful effects of the endotoxin. Mowat et al. (1993) and Kegley and Spears (1995) also demonstrated that chromium supplementation could reduce morbidity of cattles. The LPS dose was then reduced to 100 $\mu\text{g kg BW}^{-1}$, with injection by i.m. and the experiment was repeated.

Animal response to LPS administration can differ with the mitogen used and the measurement time (Richardson et al., 1989; Norimatus et al., 1995). Therefore, this study took

measurements at 6 h and 24 h after LPS administration in the final and middle period of the experiment. The number of white blood cells in the chromium propionate group increased markedly in both periods. Lee et al. (2000) also indicated that chromium supplementation could increase the number of white blood cells of weaning pigs following an LPS challenge.

Burton et al. (1993) and Chang et al. (1996) reported that chromium supplementation increased the anti-ovalbumin antibody response in cattle. Conversely, Van Heugten and Spears (1997) reported decreased antibody response to ovalbumin in weaning pigs supplemented with chromium nicotinate. Moreover, another investigation on pigs by Van de Ligt et al. (2002b) showed that chromium had no effect on the antibody response to ovalbumin. This investigation also found that chromium supplementation failed to improve the response to ovalbumin. Additionally, Van de Ligt et al. (2002a) reported that serum total IgG in post-weaning pigs was not significantly affected by chromium picolinate, excepted at day 28. But, Moonsie-Shageer and Mowat (1993) indicated that steer calves supplemental with chromium could increase serum IgG titer at day 14, but no effective at day 28. In this study, chromium propionate did enhance pig serum IgG in the middle experimental period, but not in the final period. Therefore, the immune response might change at different rates over time with different mitogen. Thus, use of a number of different parameters is seem necessary to evaluate the degree of immune response *in vivo*.

CONCLUSION

The experimental results from this study indicated that chromium propionate supplementation could reduce LDL+VLDL and increase HDL percentage. Moreover, it can enhance serum total globulin, IgG, and the γ -globulin concentration. Increased SRBC antibody titer, PHA skin swelling, neutrophil activity and white blood cells following LPS challenge were also noted. We therefore suggest that chromium propionate supplementation can benefit lipoprotein and immune response in weaned pigs.

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