Effects of Chromium Supplementation and Lipopolysaccharide Injection on Physiological Responses of Weanling Pigs[a]

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ABSTRACT : Sixteen specific pathogen free 4-wk-old crossbred weanling pigs were allotted into a 2×2 factorial design to evaluate chromium picolinate (CrPic) on growth and physiological responses. Two factors included (1) no Cr or 400 ppb Cr supplementation from chromium picolinate and (2) lipopolysaccharide (LPS) injection on day 21 (d 21) and 35 (d 35) compared to saline application. Plasma samples and rectal temperature were obtained from all piglets before (h 0) and at 2 h (h 2), 4 h (h 4), 8 h (h 8), and 24 h (h 24) after LPS injection (200μg/kg BW, intraperitoneally). The rectal temperature on d 21 was significantly decreased ($p<0.05$) of about 0.36℃ with Cr supplementation before LPS injection. After LPS injection, the daily gain of piglets was decreased during d 35-38. Supplementation of Cr had no effect in general on growth performance particularly after LPS injection. The plasma glucose, triglycerides and urea nitrogen concentrations were changed in different ways after LPS injection. Plasma cortisol level was significantly elevated at h 2 after LPS injection on d 21 and d 35. The supplementation of Cr in the diet can delayed plasma cortisol release on d 35. The results suggest that 400 ppb Cr supplementation from CrPic may modulate the physiological response during immune stress in weanling pigs. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 4 : 528-534*)

Key Words : Chromium, Lipopolysaccharide, Physiological Response, Weanling Pig

INTRODUCTION

Chromium (Cr) is an important factor in carbohydrate, fat, and protein metabolism (Mertz, 1993). Dietary supplementation of Cr has increased glucose disappearance rate and reducing plasma triglycerides (TG) in growing-finishing pigs (Amoikon et al., 1995; Lien et al., 1996; Min et al., 1997). In addition to its role in metabolic processes, Cr has been reported to reduce plasma cortisol content and rectal temperature and to improve immune response when organic forms of Cr are supplemented to stressed feeder calves (Chang and Mowat, 1992; Moonsie-Shageer and Mowat, 1993) and to dairy cows (Burton et al., 1993). van Heugten and Spears (1997) found that supplementation with Cr was no effect in alleviating the depression in growth due to lipopolysaccharide (LPS, an endotoxin), but Cr-fed piglets tended to improve ($p<0.10$) the daily gain and feed intake in piglets not challenged with LPS. However, there is no information related to the effect of Cr on physiological responses in weanling pigs so far.

The weaning process of piglets incurs the decrease in growth rate and non-homeostatic physiological response due to stress from environmental and nutritional changes (Blecha et al., 1983; Crenshaw et al., 1986; Haye and Kornegay, 1979). Stress (Klasing and Johnstone, 1991; Schrauzer et al., 1986) and disease (Pekarek et al., 1975) increase urinary excretion of Cr and may exacerbate a marginal Cr deficiency. Lipopolysaccharide has been virtually found to be a principal component of gram-negative bacteria and plays a major role in causing the pathophysiological changes in weanling pigs (Harel et al., 1991). Lipopolysaccharide also leads to an increase in plasma glucose flux into the liver and spleen which are rich in mononuclear phagocytes (Lang and Dobrescu, 1991; Meszaros et al., 1987) and to enhance glucose utilization (Fong et al., 1994). The plasma triglycerides and urea nitrogen (PUN) levels are significantly increased after LPS injection in growing pigs (Spurlock et al., 1997). In addition, plasma cortisol levels and PUN are dramatically increased at 4-8 hour in piglets injected intraperitoneally (i.p.) with LPS 5 μg/kg body weight (BW) (Webel et al., 1997).

This study was conducted to investigate the effect of Cr supplementation on growth and physiological response of weanling pigs and to determine whether Cr treatment could overcome the stress response to LPS injection.

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MATERIALS AND METHODS

Animals and treatments

A total of 16 specific pathogen free 4-wk-old crossbred weanling pigs (sire was Duroc and sow was Yorkshire-Landrace cross, average body weight 6.67 kg) were allotted into one of four treatments based on weight, sex, and litter origin. Pigs were housed in a nursery room with four piglets per pen. Temperature in the nursery room was maintained around 26℃ with heating supply during the first 2 weeks. Each treatment consisted of four pigs. A 2×2 factorial arrangement was used in a random design. Factors included (1) no Cr supplementation or 400 ppb Cr supplementation (provided by chromium picolinate, CrPic, from Prince Agri Products, Inc., USA) and (2) with LPS injection or saline injection. Pigs were injected i.p. with either 1 mL of LPS solution containing LPS 200 μg/kg BW or with saline successively on d 21 and d 35 of the experiment. Lipopolysaccharide was obtained from *Escherichia coli* (serotype 055: B5; Sigma Chemical Co., USA).

The experimental diet (table 1) mainly consisted of corn, skimmilk, and isolated soybean protein to meet or exceed NRC requirements for all nutrients (NRC, 1988). Feed and water were freely available. Body weight and feed intake were determined before and 3 days after each LPS injection and weekly thereafter. Rectal temperature was measured with an animal thermometer. Concentration of Cr in the basal diet was 1300 ppb as determined by atomic absorption spectrophotometry (Perkin-Elmer, 5100PC, USA).

Blood collection and analysis

Blood samples were obtained from each pig by venipuncture in the cervical vena before (h 0) and at 2 h (h 2), 4 h (h 4), 8 h (h 8), and 24 h (h 24) after LPS injection. The blood was centrifuged at 1,000×g for 20 min at 4℃, and the plasma was collected and frozen at -20℃ for further analysis.

Plasma glucose, TG, and PUN concentrations were measured by an automated clinical chemistry analyzer (Cobas Mira Plus, Japan). Plasma insulin, cortisol, and insulin-like growth factor-1 (IGF-1) were measured by radioimmunoassay (RIA) methods. The content of insulin was determined according to the method described by Ho et al. (1983). Guinea pigs anti-porcine insulin antiserum was generously provided by Dr L. T. Ho (Veterans General Hospital, Taipei). ^{125}I-human insulin (Amersham, IM-166, BK) was used as a tracer. Porcine insulin (24 IU/mg, Sigma Chemical Co.) was measured as a standard for calculating the concentration of unknown samples. The assay of IGF-1 followed the method described by Evock-Clover et al. (1993). And the IGF-1 concentrations were determined by using RIA kit (Amersham, BK). Plasma cortisol level was determined as described previously by McCauley and Hartmann (1983). Rat anti-serum and ^{3}H-cortisol (generously provided by Dr. P. P. Huang, Academia Sinica, Taipei) were used as the antibody and tracer, respectively. Hydrocortisone was used as a standard (Sigma Chemical Co.) to calculate the cortisol concentration of unknown samples.

Table 1. The composition of basal diet

Ingredients (%)	
Corn	79.46
Isolated soybean protein[a]	11.09
Skimmilk	5.00
Soybean oil	1.77
Dicalcium phosphate	1.29
Limestone, pulverized	0.69
Salt	0.40
Vitamin premix[b]	0.20
Trace mineral premix[c]	0.10
Calculated nutrient composition	
Metabolizable energy (kcal/kg)	3250
Crude protein (%)	19.00
Lysine (%)	1.15
Calcium (%)	0.86
Phosphorus (%)	0.65
Analyzed nutrient composition	
Chromium (ppb)	1300

[a] Pro Fam 972, ADM, Holland.
[b] Supplied per kg diet: vitamin A, 8,000 IU; vitamin D, 1,200 IU; vitamin E, 40 IU; vitamin K_3, 4 mg; vitamin B_2, 8 mg; pantothenic acid, 24 mg; niacin, 80 mg; vitamin B_{12}, 40 μg and choline-HCl, 700 mg.
[c] Supplied per kg diet: Cu, 20 mg; Zn, 100 mg; Fe, 140 mg; Mn, 4 mg; Se, 0.1 mg and I, 0.2 mg.

Statistical analysis

Data were analyzed according to a 2×2 factorial design. Analysis of variance was performed by using the GLM procedure of SAS (1987). If a given ANOVA was significant at $p<0.05$, comparison of means was performed with t-test. The means of feed intake and feed/gain were not performed statistically, because 4 piglets in each treatment were housed in one pen.

RESULTS AND DISCUSSION

Growth performance

The effects of supplemental Cr and LPS injection on growth performance of weanling pigs are shown in table 2. After LPS injection, the daily gain was only reduced ($p<0.05$) at d 35-38. Feed intake at d 0-21 was appeared to be reduced in Cr supplemental groups, but there was no effect on the daily gain or

Table 2. Effect of supplemental chromium and lipopolysaccharide (LPS) injection on growth performance of weanling pigs

Chromium added (ppb)	0		400	
LPS injection	Saline	LPS	Saline	LPS
Initial weight (kg)	6.58±0.30*	6.73±0.49	6.67±0.32	6.70±0.31
Final weight (kg)	19.79±0.93	17.83±2.13	18.94±1.62	17.51±0.93
Daily gain (kg/d)				
D0-21	0.22±0.02	0.18±0.01	0.17±0.04	0.18±0.02
D21-35	0.50±0.02	0.48±0.07	0.49±0.04	0.43±0.03
D35-38	0.54±0.07[a]	0.21±0.12[b]	0.62±0.06[a]	0.32±0.07[b]
D0-38	0.35±0.02	0.29±0.04	0.32±0.04	0.28±0.02
Feed intake (kg/d)*				
D0-21	0.42	0.41	0.34	0.34
D21-35	0.94	0.91	0.91	0.79
D35-38	1.10	0.44	1.21	0.67
D0-38	0.66	0.60	0.62	0.54
Feed/gain (F/G)*				
D0-21	1.91	2.25	2.03	1.86
D21-35	1.89	1.90	1.84	1.86
D35-38	2.03	2.06	1.96	2.12
D0-38	1.91	2.03	1.91	1.88

* Values are means±SE; [a,b] LPS effect ($p<0.05$); * Statistical analysis not performed.

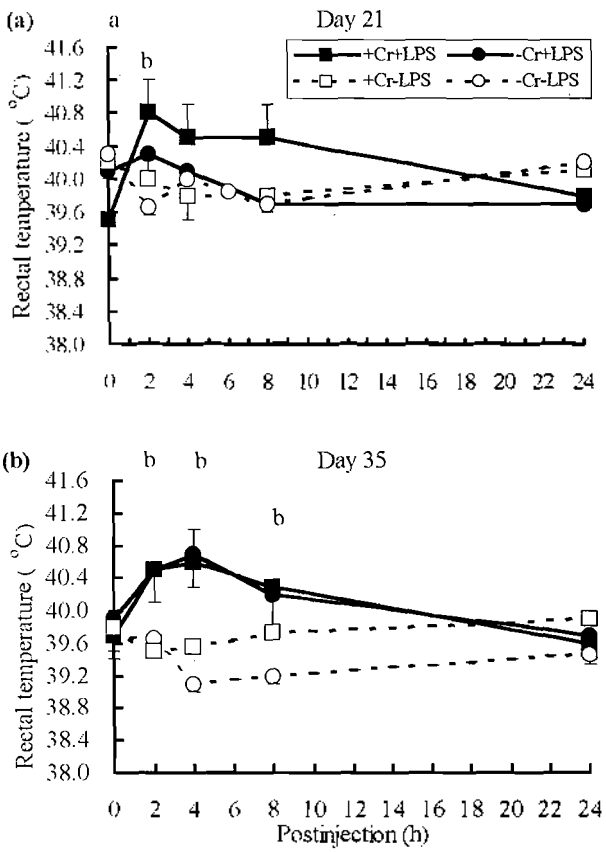

Figure 1. Effects of supplemental chromium and lipopolysaccharide (LPS) injection at day 21 (a) and day 35 (b) on rectal temperature in weanling pigs. Each point is the mean±SE. a, b: Meaning the significant difference ($p<0.05$) of Cr and LPS, respectively.

feed/gain. The finial body weight of pigs were not affected by LPS or Cr supplementation. Pigs treated with LPS showed a significant reduction in growth performance as observed by van Heugten et al. (1994) and van Heugten and Spears (1997). Our previous CrPic supplemental experiment showed no difference in feed intake when weanling pigs were given Cr from 0 to 800 ppb (Lee et al., 1997). However, supplementation with organic Cr 200 ppb tended to improve ($p<0.10$) the gain and feed intake (van Heugten and Spears, 1997).

Physiological response

Significantly increases in rectal temperature were observed at h 2 to h 8 after LPS injection at d 21 and d 35, respectively (figure 1). On d 21, the rectal temperature was significantly reduced in Cr-fed pigs of about 0.36℃ ($p<0.05$) at h 0. Lofgreen (1983) found that newly arrived feedlot calves with elevated temperature had a higher morbidity. Supplementation with Cr reduced rectal temperature and morbidity were observed in following improves performance and humoral immune function in stressed steer calves (Moonsie-Shageer and Mowat, 1993).

Plasma glucose concentrations appeared to be reduced and thereafter recovered at h 24 after LPS injection (figure 2a). A significant interaction ($p<0.05$) between LPS injection and Cr supplementation in plasma glucose concentration was observed at h 2 on d 21 according which the plasma glucose concentration was increased in Cr-fed pigs without LPS injection. However, the level of plasma glucose was decreased after LPS injection in Cr-fed pigs. In the end phase of response (h 24) to the second LPS injection on d 35,

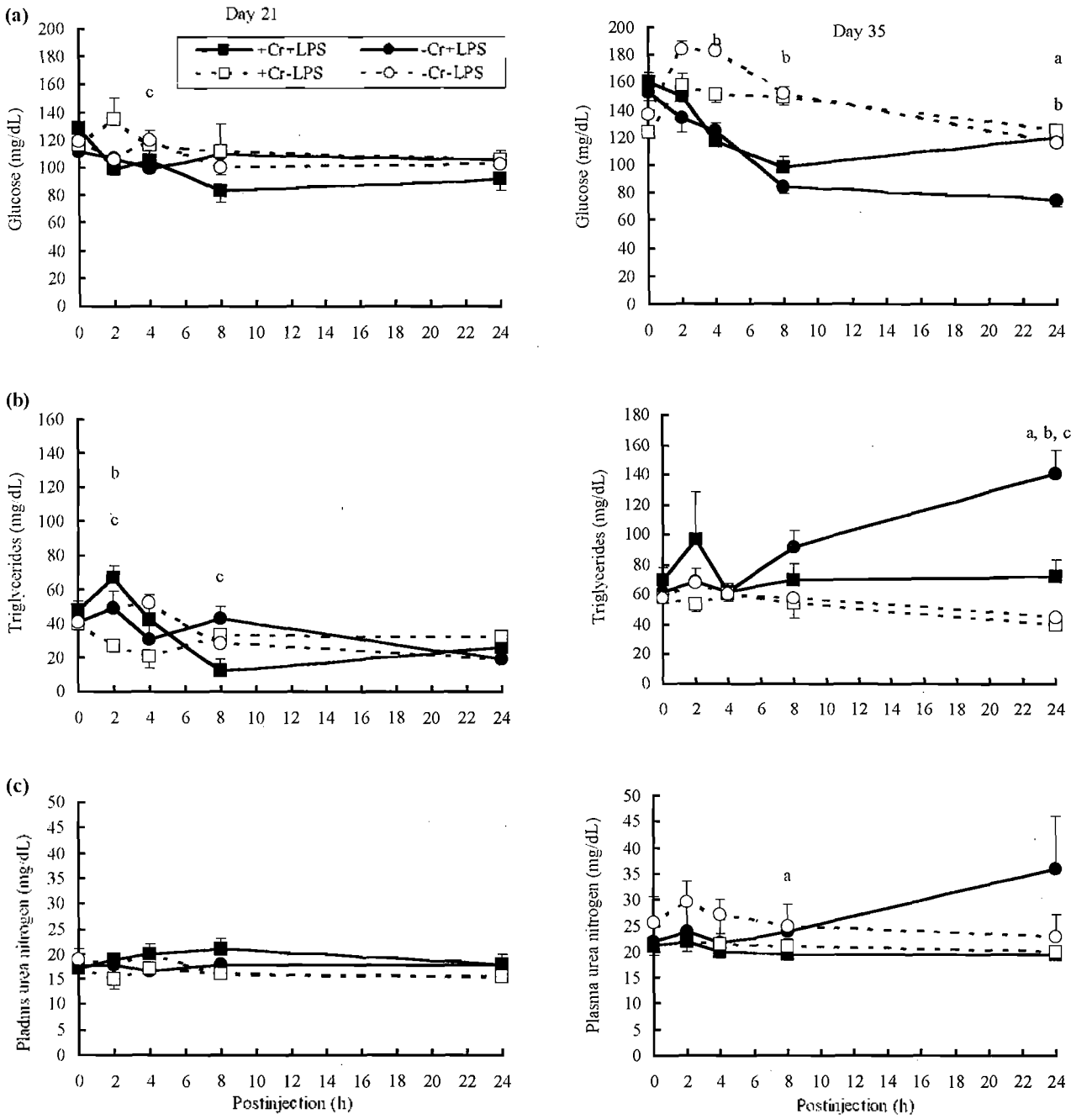

Figure 2. Effects of supplemental chromium and lipopolysaccharide (LPS) injection at day 21 and 35 on the concentrations of glucose (a), triglycerides (b), and urea nitrogen (c) in the plasma of weanling pigs. Each point is the mean ± SE. a, b, c: Meaning the significant difference ($p<0.05$) of Cr, LPS, and the interaction, respectively.

glucose concentrations were 119 and 77 mg/dL in Cr-fed and control pigs, respectively. The response of glucose content to LPS injection at d 35 was recovered more rapidly in Cr-fed pigs than those control pigs. As indicated by Amoikon et al. (1995), Cr might implicate in homeostasis of blood glucose during glucose tolerance test for growing-finishing pigs. The plasma glucose content affected by the administration of LPS might be due to the changes in the concentration of various cytokines (Elsasser et al., 1995). Myers et al. (1995) found that peripheral blood mononuclear cells (PBMC) from CrPic-fed pigs produced more interleukin-2 than the PBMC from control groups.

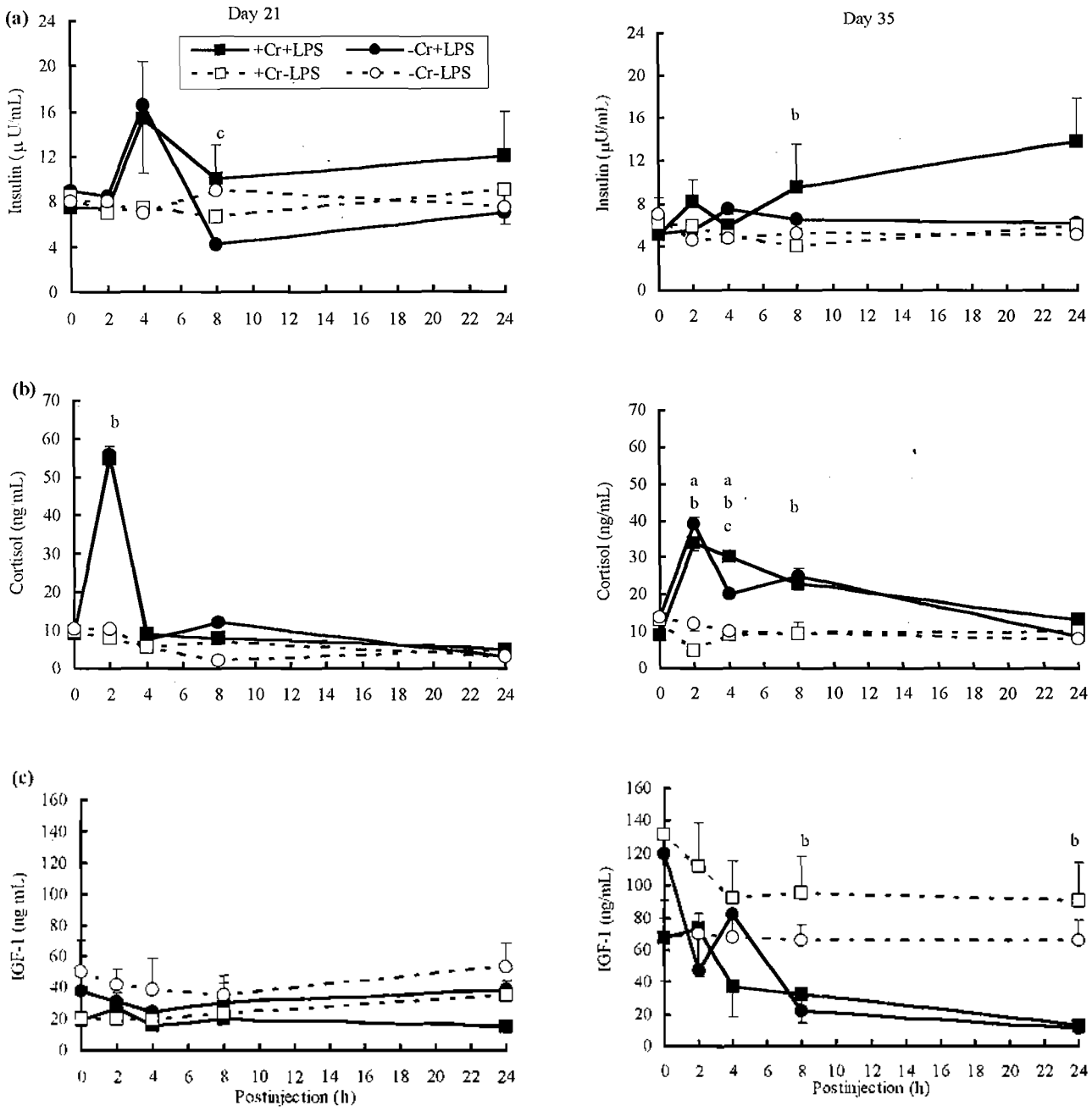

Figure 3. Effects of supplemental chromium and lipopolysaccharide (LPS) injection at day 21 and 35 on the concentrations of insulin (a), cortisol (b), and insulin-like growth factor-1 (c) in the plasma of weanling pigs. Each point is the mean±SE. a, b, c: Meaning the significant difference ($p<0.05$) of Cr, LPS, and the interaction, respectively.

Lipopolysaccharide injections increased plasma TG concentrations on d 35 and Cr supplementation significantly reduced plasma TG concentration at h 24 on d 35 with LPS injection (figure 2b). This result was similar to previous reports showing reduced TG concentration and nonesterified free fatty acids in growing-finishing pigs under normal feeding conditions (Amoikon et al., 1995; Lien et al., 1996). The pathophysiological responses of increasing fat tissue degradation was associated with the acute TG increases during animal inflammation (Kenison et al., 1991). Webel et al. (1997) indicated that 12 kg body weight pigs treated with LPS 5 μg/kg BW exhibited a 10-fold increase in TNF-α and a 100-fold increase

in IL-6 at h 2 after injection. Both TNF-α and IL-6 have been shown to induce proteolysis and lipolysis (Evock-Clover et al., 1993).

Plasma urea nitrogen, an indication of protein calabolism in animals, was tended to increase at h 8 on d 21 ($p<0.10$) and then recovered at h 24 after LPS injection (figure 2c). After the second LPS injection on d 35, the PUN levels were tended to increase by the injection of LPS at h 24. Changes in PUN after LPS administration also noted in consistent with an increase in the oxidative degradation of amino acids in muscle, and with a decrease in protein synthesis in liver (Fong et al., 1994). Supplementation with Cr was effective in alleviating the increase in PUN due to LPS at h 8 on d 35.

The effects of Cr supplementation and LPS injection on the changes in plasma insulin, cortisol, and IGF-1 concentrations are shown in figure 3. On d 21, plasma insulin levels seemed to be increased in Cr-fed and LPS-injection piglets. However, this response to Cr supplementation was only significant in pigs with LPS at h 4 (Cr*LPS, $p<0.05$). The temporary elevation on insulin concentration following LPS injection has also been seen in calves (Kenison et al., 1991). The level of insulin was also elevated at h 8 after LPS injection on d 35 but had less extent. The Cr treatment had no effect on insulin level on d 35. Significant elevations in plasma cortisol content were noted at h 2 after LPS injection with a six- and four-fold increases on d 21 and 35, respectively. LPS injection to pigs (Norimatus et al., 1995) and to calves (Elsasser et al., 1995; Elsasser et al., 1996) resulting in increased plasma cortisol concentrations were noted from one to four hours after injection. Chromium supplementation did not affect cortisol concentrations changed in the plasma after the first LPS injection on d 21. However, cortisol level was significantly decreased at h 2 and significantly increased at h 4 with LPS injection on d 35 ($p<0.05$) for Cr-fed pigs. The data showed that Cr might delay cortisol secretion on d 35 after the second LPS injection. It has been reported that serum cortisol was decreased by Cr supplementation in stressed feeder cattle (Chang and Mowat, 1992; Moonsie-Shageer and Mowat, 1993). However, supplementation of Cr did not affect cortisol level before or after ACTH injection of pigs (van Heugten and Spears, 1997). Further studies need to be elucidated the effect of Cr on cortisol levels in pigs.

There was no effect of LPS injection on plasma IGF-1 response on d 21. On d 35, LPS injection reduced plasma IGF-1 concentrations at h 8 and h 24, the concentration of IGF-1 in LPS injected pigs was 1/3 of the control pigs. However, the concentrations of IGF-1 were not affected by Cr supplementation. Elsasser et al. (1995) also reported a suppression of IGF-1 and IGF-binding protein-3 concentrations in calves after administration of LPS.

The present data demonstrate that piglets injected with LPS have changed plasma glucose and TG. These may indicate the change in carbohydrate and lipid metabolism. Furthermore, chromium has been implicated in affecting insulin sensitivity in pigs (Amoikon et al., 1995) and cortisol levels in daily cows (Chang et al., 1996). Supplementation of Cr caused a reduction of cortisol release after LPS challenged suggests that the effect of Cr supplementation on nutrient metabolism may be mediated by hormones.

IMPLICATIONS

The results suggest that 400 ppb chromium supplementation from picolinate can reduce rectal temperature, and affect cortisol release after lipopolysaccharide injection. This research has implications that chromium can be modulate the physiological response during immune stress in weanling pigs. However, further evaluation of chromium effects on growth and physiological responses under chronic stress conditions are necessary.

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