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Effects of Chromium Picolinate Supplementation on Growth Hormone Secretion and Pituitary mRNA Expression in Finishing Pigs*

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ABSTRACT : The purpose of the present study was to investigate the effects of chromium picolinate (CrPic) on growth hormone (GH) secretion and pituitary GH mRNA expression in finishing pigs. Forty eight crossbred pigs with an initial body weight of 65.57 kg (SD = 1.05) were blocked by body weight and randomly assigned to two treatments with three replicates. Each group was fed the diet supplemented with or without 200 μg/kg chromium from CrPic for 40 days. The results showed that average daily gain of pigs was increased by 9.84% ($p<0.05$), and longissimus muscle area was increased by 17.29% ($p<0.05$) with the supplementation of CrPic. The results of GH dynamic secretion showed that supplemental CrPic increased the mean level and peak value of GH by 36.58% ($p<0.05$) and 26.60% ($p<0.05$), respectively, while there was no significant effect on basal value, peak amplitude and peak duration. Pituitary mRNA expression of GH was not significantly influenced by supplemental CrPic. These results indicated that CrPic increased pigs GH secretion without change of pituitary GH mRNA expression. (**Key Words :** Chromium Picolinate, Pigs, Growth Hormone)

INTRODUCTION

Chromium (Cr) has been considered as an essential nutrient for humans and animals for approximately 50 years. It is involved in carbohydrate, lipid, protein and nucleic acid metabolism, and is necessary for optimal insulin function and glucose uptake by insulin-sensitive cells (Anderson, 1987). Steele et al. (1977) reported that a synthetic glucose tolerance factor containing Cr potentiated insulin activity and that this glucose tolerance factor was "biologically active" in pigs. Research with supplementation of Cr chloride ($CrCl_3$) (Mooney and Cromwell, 1997), Cr Picolinate (CrPic) (Lindemann et al., 1995; Xi et al., 2001), Cr yeast (Guan et al., 2000), Cr propionate (CrProp) (Shelton et al., 2003) and Cr nanocomposite (Wang and Xu, 2004; Wang et al., 2007) to pigs has been reported. CrPic is the most generally accepted organic source of Cr, and its effects have been widely studied on growth, carcass characteristics, reproduction performance in pigs. CrPic has been shown to increase loin eye area and decrease fat thickness (Page et al., 1993; Lindemann et al., 1995), and to increase the rate of lean and decrease the rate of fat deposition (Boleman et al., 1995; Mooney and Cromwell, 1995). However, others reported no responses in carcass leanness to supplemental CrPic (Ward et al., 1995; Mooney and Cromwell, 1996). The lack of a consistent response may be related to Cr level of the diets, Cr status of pigs and amino acid levels of diets (NRC, 1997).

It is well-accepted that growth hormone (GH) level influences growth performance and carcass composition of pigs. However, the effects of Cr on hormones except insulin have not yet been widely investigated. Therefore, the following experiment was conducted specifically to study the effect of CrPic on GH pulsatile secretion and mRNA expression in the pituitary of finishing pigs.

MATERIALS AND METHODS

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

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

Ingredients	%
Corn	64.5
Soybean meal	21.5
Rapeseed meal	4.0
Wheat bran	6.0
Limestone	1.3
Calcium phosphate	1.5
Salt	0.3
Mineral premix[2]	0.7
Vitamin premix[3]	0.2
Chemical composition[1]	
Digestible energy (MJ/kg)	13.40
Crude protein (%)	17.10
Calcium (%)	0.76
Phosphorus (%)	0.62
Lysine (%)	1.05
Methionine (%)	0.45

[1] All data were analyzed values except digestible energy which was calculated using swine NRC (1998) values.

[2] Contained per kg of diet: Cu, 10 mg from $CuSO_4 \cdot 5H_2O$; Zn, 100 mg from $ZnSO_4 \cdot 7H_2O$; Fe, 140 mg from $FeSO_4 \cdot H_2O$; Mn, 40 mg from $MnSO_4 \cdot 5H_2O$; Se, 0.1 mg from $Na_2SeO_3 \cdot 5H_2O$; I, 0.3 mg from KI.

[3] Contained per kg of diet: vitamin A, 6,000 IU; vitamin D_3, 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 B_{12}, 22 μg; D-biotin, 300 μg; folic acid, 2.5 mg.

in Anji Zhengxin Breeding Farm. Forty eight crossbred pigs (Duroc×Landrace×Yorkshire), with an average body weight of 65.57 kg (SD = 1.05), were blocked by initial weight, equalized for sex and ancestry and randomly allotted to one of the following dietary treatments: control and control diet supplemented with 200 μg/kg chromium from CrPic, with three replicate pens per treatment and eight pigs per pen. The CrPic was provided by Zhejiang Huangyan Kanda Animal Health Co., Ltd., China. All experimental treatments used the same corn-soybean meal basal diet formulated to meet or exceed NRC (1998) recommendations for all nutrients except digestible energy (Table 1).

Samples of the mixed basal diet were analyzed for CP by N analyzer (N×6.25); the method was based on standard procedures (AOAC, 1995). Calcium was analyzed by atomic absorption spectrophotometry after wet-ashing, and P was determined by a colorimetric procedure (AOAC, 1995). Amino acids were analyzed by ion exchange chromatography after acid hydrolysis in 6 mol/L HCl and 0.1% phenol under vacuum for 24 h at 110±2°C. Methionine was oxidized to methionine sulfone by treatment with performic acid before hydrolysis (Schram et al., 1954).

The pigs were housed in 3.25×5.25 m pens, with a nipple drinker and feeder to allow pigs *ad libitum* access to feed and water. The duration of the feeding trial was 40 days. Preceding the study, pigs were allowed a 7-day adaptation period, during which they were offered the basal diet *ad libitum*.

Blood, pituitary sampling and carcass evaluation

At the end of the feeding trial, 1h after feed removal, four pigs were selected and blood samples were taken by anterior vena cava puncture at 15 min intervals for 3 h. The samples were then centrifuged at 1,500×g at 4°C for 15 min. Serum collected from each sample was stored at -20°C until analysis.

After blood sampling, eight pigs from each treatment (2-3 pigs per pen) were selected on the basis of closer body weight (control group, 90.05±0.53 kg; CrPic treated group, 90.26±0.43 kg), and slaughtered by exsanguination after electrical stunning. Three pituitaries from each treatment were collected by opening the cranial cavity, snap-frozen in a cryovial using liquid nitrogen and stored at -80°C. Hot carcass (for determination of dressing proportion) was collected. Measurements of backfat depth and longissimus muscle area were made from a left carcass tracing taken at the tenth rib.

Assay of GH in serum

Serum growth hormone (GH) was determined using a ^{125}I RIA kit provided by National Hormone and Peptide Program HARBDE-UCLA Medical Center, USA. The minimum detectable concentration of GH was 0.1 ng/ml, and the intraassay CV was 10%.

Determination of pituitary GH mRNA level

RNA isolation : Total RNA was isolated from pituitary tissue using TRIzol (Gibco Life Technologies, Grand Island, NY). After pulverization and homogenization of the tissue, the homogenate was extracted with chloroform and then precipitated by isopropanol. The resulting pellets of total RNA were dissolved in ultra-pure water; the purity and concentration of total RNA were measured by a spectrophotometer at 260 and 280 nm.

RT-PCR : RT-PCR was performed in a thermocycler (Gene Amp PCR system 9600, Perkin-Elmer Cetus Instrument, USA). Two micrograms of RNA isolated from each sample was reverse transcribed using oligo(dT) 15 primer (Promega, Madison, USA) and an RNA PCR kit (AMV) (Promega, Madison, USA), essentially according to the manufacture's protocol. Aliquots (2 μl) from the generated cDNA were used for subsequent PCR amplification in the reaction buffer containing 3.5 μl $MgCl_2$ (25 mM), 1 μl dNTPs (10 mM), 0.5 μl Taq DNA polymerase (Promega, Madison, USA), 1 μl sense and antisense primer (20 mM) each, to a final volume of 50 μl. The PCR primer sets used are shown in Table 2. Primer

Table 2. Primer pairs used in the RT-PCR reaction

Genes	Primer sequences*	PCR product (bp)
GH	[S] 5' -GGCTG TGATG GCTGC AGGCC – 3'	658
	[AS] 5' - CTAGAAGGCACAGCTGCTCTCCACG- 3'	
18S rRNA	[S] 5' - CTAGATAGTC AAGTTCGACC - 3'	198
	[AS] 5' -TTCAGGGGA ATAATTGC - 3'	

* S = sense primer; AS = anti-sense primer.

Table 3. The effects of CrPic supplementation on the performance and carcass traits of finishing pigs[1]

	CrPic added (μg/kg)		SEM[2]
	0	200	
Growth			
Average daily gain (kg)	0.61[a]	0.67[b]	0.256
Average daily feed intake (kg)	2.13	2.22	0.091
Feed:gain	3.49	3.33	0.162
Carcass characteristics			
Carcass weight (kg)	69.8	70.5	0.650
Dressing proportion	0.73	0.75	0.022
10^{th} rib backfat (cm)	1.85	1.66	0.189
Longissimus muscle area (cm^2)	46.84[a]	54.94[b]	3.087

[1] Values are presented as means; n = 3 per treatment with eight pigs per pen contributing to a pen mean. Means in a row with different letters differ significantly ($p<0.05$).
[2] Standard error of the mean.

Table 4. The effect of CrPic supplementation on GH secretion measured after 35 days in finishing pigs[1]

	CrPic added (μg/kg)		SEM[2]
	0	200	
Mean level (ng/ml)	5.96[a]	8.14[b]	0.689
Basal value (ng/ml)	2.81	3.28	0.670
Peak value (ng/ml)	10.30[a]	13.04[b]	1.041
Peak amplitude (ng/ml)	8.49	9.76	1.452
Peak duration (min)	63.86	66.99	4.027

[1] Blood samples were collected at 15-min intervals for 3 h after feeds were removed for 1 h. Serum GH concentration was determined by an RIA kit. Values are presented as means; n = 4 per treatment. Means in a row with different letters differ significantly ($p<0.05$).
[2] Standard error of the mean.

sequence for GH and 18S rRNA housekeeping gene were designed by using the Primer Program of the Winsconsin Sequence Analysis Package (Genetics Computer Group Inc., Madison, WI, USA) based on known sequences deposited in Genebank. Amplification was carried out for 29 cycles, when the reaction was in the middle of the linear range (before reaching the amplification plateau). Each cycle consisted of denaturation at 94°C for 2 min, annealing at 59°C for 50 s and extension at 72°C for 10 min.

Statistical analysis

Pulses of GH were identified using Pulsar Analysis (Merriam and Wachter, 1982). Electrophoresis band intensities of the PCR products were quantified using Image Master VDS software (Amersham Pharmacial Biotech, Uppsala, Sweden). Mean GH mRNA levels normalized against 18S rRNA levels from pituitary of pigs from each treatment were presented in absolute integrated density. Data were analyzed for all variables using SAS software (SAS Institute, 1989). Data were subjected to *t*-test procedure to establish differences between means. For all data, the model included treatment as main effect. A probability of $p<0.05$ was considered significant.

RESULTS AND DICUSSIONS

Growth and carcass response of pigs

The effects of CrPic supplementation on growth performance and carcass traits are shown in Table 3. Average daily gain of pigs fed supplemental CrPic diet was increased by 9.84% ($p<0.05$) compared to the control group, while average daily feed intake and feed gain ratio was not affected by CrPic supplementation. Dietary CrPic supplementation increased longissimus muscle area (LMA) by 17.29% ($p<0.05$).

Research with Cr supplementation of swine diets has been primarily conducted with CrPic, $CrCl_3$, Cr nicotinate, Cr yeast or chromium propionate, with widely disparate results among studies. Statistically significant ($p\leq0.10$) improvements in growth rate of swine as a result of supplementing diets with 200 to 500 μg Cr/kg as CrPic or 500 μg to 5 mg Cr/kg as $CrCl_3$ were reported in 11 of 31 studies, and feed efficiency was improved by Cr supplementation of diets in 8 of 31 studies (NRC, 1997). When supplemental dietary $CrCl_3$ or CrPic was used, increases in carcass leanness were reported in 9 of 24 experiments and decreases in carcass fat were reported for 11 of 26 experiments (NRC, 1997). Shelton et al. (2003) reported that overall growth performance was not affected by addition of Cr in the form of CrPic or chromium propionate (CrProp). Matthews et al. (2003) confirmed that CrProp had no consistent effect on growth and carcass trait, however, CrProp did affect some aspects of pork quality. Lien et al. (2005) also found that CrProp had no effect on growth performance in weaned pigs. Although responses of swine to supplemental Cr have been inconsistent, there is an increasing amount of evidence indicating that Cr may favorably alter metabolism of swine under some circumstances, with resultant improvement in growth rate and carcass traits (NRC, 1997).

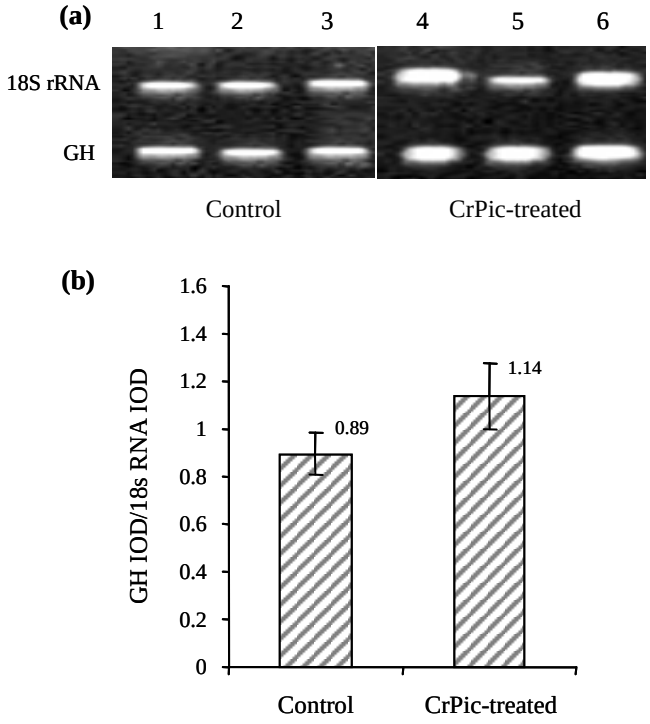

Figure 1. Effect of dietary CrPic on pituitary GH mRNA expression when pigs were analyzed by RT-PCR. (a) Amplified products obtained with mRNA after transcriptions were size-fractionated on a 1.0% agarose gel. The gel was stained with ethidium bromide, and PCR products were observed under UV light (Immage master VDS). The image of bands are shown as the expected sizes of RT-PCR products of pig pituitary GH and 18sRNA, which was used as an internal control. Three repetitions of control group are represented by 1, 2, 3, three repetition of CrPic-treated group are represented by 4, 5, 6. (b) Densitometric analysis of pig GH were normalized to 18 sRNA and were shown as GH IOD/18 sRNA IOD. IOD means the integrated optical density. Values on the columns are presented as the means of three repetitions respectively.

Pulsatile secretion of GH

As shown in Table 4, mean level and peak value of GH in serum were increased by 36.58% ($p<0.05$), 26.60% ($p<0.05$) respectively with the supplementation of CrPic. No significant effect was found on basal value, peak amplitude and peak duration with the addition of CrPic.

Page et al. (1993) found inconsistent changes in plasma GH levels in pigs fed CrPic. In calves, Bunting et al. (1994) found that CrPic had no effect on baseline GH concentrations or GH responses to GHRH. Evock-Clover et al. (1993) reported that dietary CrPic increased plasma GH in GH-injected pigs but did not affect IGF-I in un-injected or GH-injected pigs. The measurement from a single blood sample is not a sensitive enough technique to assess treatment effects on GH given the pulsatile nature of GH release in most mammals (Barb et al., 2002). In the present experiment, we investigated GH pulsatile secretion in pigs fed 200 μg/kg CrPic. The results exhibited that supplemental CrPic significantly increased serum GH mean level and peak value, which could contribute to improvements of growth performance and be responsible for the effects of CrPic on protein deposition as indicated by increased LMA. Roginski and Mertz (1969) reported that Cr supplementation increased amino acid incorporation into heart proteins and amino acid uptake into tissues of rats. No other studies of an effect of Cr on protein synthesis or turnover have been reported.

Pituitary GH mRNA level

The electrophoresis results of three pigs from each experimental group are shown in Figure 1. Electrophoresis band intensities of PCR products were quantified and analyzed for statistical difference. The current results showed that supplemental CrPic increased pituitary GH mRNA level by 27.63%, while there was no statistical difference ($p = 0.221$).

Trivalent chromium seems to be involved in the structural integrity and expression of genetic information in animals. Chromium (III) has been shown to enhance RNA synthesis in mice *in vitro* (Okada et al., 1982) and *in vivo* (Okada et al., 1983). With the use of the regenerating rat liver model, nucleic-acid-enhancing activity was associated with a 70,000 dalton protein that contained 5 to 6 chromium ions (Okada et al., 1984, 1989). In our study, the mRNA expression level of GH in the pituitary tended to be increased with the supplementation of CrPic, though no statistical difference was found. It is possible that the effect of CrPic on serum GH level might be related to GH gene expression in the pituitary. Further study needs to be conducted to confirm the pituitary GH gene expression response to CrPic supplementation, and real-time RT-RNA analysis is necessary to establish quantitatively GH mRNA in order to improve analytical accuracy.

IMPLICATIONS

In conclusion, this study indicated that chromium picolinate increased GH pulsatile secretion in finishing pigs, and the results suggest that effects of CrPic on serum GH level may be reflective of the positive effects on growth and protein anabolism. The mechanism underlying the effect is yet to be established and requires further research.

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