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Effects of Dietary Chromium Methionine on Growth Performance, Carcass Composition, Meat Colour and Expression of the Colour-related Gene Myoglobin of Growing-finishing Pigs

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ABSTRACT: To investigate the effect of dietary chromium (Cr) as Cr methionine (CrMet) on growth performance, carcass traits, pork quality, meat colour and expression of meat colour-related genes in growing-finishing pigs, 189 crossbred Duroc×(Landrace×Yorkshire) growing-finishing pigs (male, castrated, average initial BW 74.58±1.52 kg) were selected and randomly allocated into four groups. Dietary treatments per kg of feed were as follows: 0 (CT), 0.3 mg/kg (T1), 0.6 mg/kg (T2) and 0.9 mg/kg (T3) Cr (in the form of CrMet; as-fed basis), and each treatment was replicated five times with 8 to 10 pigs per replicate pen. During the 28 d of the experiment, both the ADG and the ADFI increased linearly ($p<0.05$) as the level of dietary Cr increased. The F/G ratio decreased linearly ($p<0.05$). As dietary Cr increased, loin muscle areas (linear, $p = 0.013$) and average backfat thickness (linear, $p = 0.072$) decreased. Shear force (linear, $p = 0.070$) and Commission Internationale de I'Éclairage (CIE) redness (quadratic, $p = 0.028$) were increased. In addition, CIE Lightness (quadratic, $p = 0.053$) were decreased as dietary Cr increased. As dietary Cr increased, total myglobin (Mb) content (quadratic, $p = 0.015$) and the *mb* mRNA levels (quadratic, $p = 0.046$) in *longissimus* muscles of pigs were up-regulated. In conclusion, supplementation of dietary Cr improved growth and meat colour, but increased shear force and decreased IMF reduced palatability of *longissimus* muscles. Moreover, the increasing total Mb content and *mb* mRNA levels indicated that CrMet dietary supplementation may improve meat colour via up-regulating expression of the *mb* gene. (**Key Words:** Growing-finishing Pigs, Chromium Methionine, Meat Colour, Myoglobin)

INTRODUCTION

Chromium (Cr) is a trace mineral involved in the metabolism of carbohydrates, lipids, proteins and nucleic acids. Trivalent Cr plays important roles in human diet and medicine, e.g. regulating glucose and lipid metabolism in humans and laboratory animals (Mertz, 1993; Haldar et al., 2009; Krzysik et al., 2011). A recent study also reported that maternal Cr restriction significantly decreased the percent of lean body mass and fat-free mass in rat offspring (Padmavathi et al., 2010). The effects of Cr have been investigated for potential use as feed additives in animal production. However, the results were not always consistent; e.g. dietary Cr propionate (CrProp) supplementation was reported to decrease 10th-rib backfat thickness in crossbred Yorkshire gilts (Jackson et al., 2009), but in another report the 10th-rib backfat thickness was not affected in the same crossbred gilts fed the same dose CrProp (Matthews et al., 2005). Previous examinations of Cr supplementation of swine diets have been primarily conducted with Cr picolinate (CrPic), Cr nicotinate (CrNic), CrProp or Cr chloride ($CrCl_3$), whereas investigations using Cr methionine (CrMet) are less common. Moreover, chromium methionine chelate could directly cross the intestinal cell membrane and be metabolized without any prior digestion due to being chelated with amino acids (Ohh and Lee, 2005). Based on these reasons above, bioavailability of CrMet may be higher than those of other organic chromium compounds.

Meat colour, one of the most important factors in

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determining consumer choice (Mancini and Hunt, 2005; Hoek et al., 2011), is the result of complex interactions among animal genetics, antemortem and postmortem conditions, fundamental muscle chemistry and many factors related to meat processing, packaging, distribution, storage, display and final preparation for consumption (Hunt et al., 2004; Gandolfi et al., 2011). Recent studies have shown that dietary Cr supplementation is increasingly well recognized as a potential means of improving meat colour. There were a series of experiments in which various levels of Cr were fed to pigs, and reported that the subjective colour score was increased by 16.5% by adding 0.2 mg/kg Cr as CrProp (Shelton et al., 2003). Additionally, different levels of total muscular pigment in the ribeye area were significantly improved ($p<0.05$) in growing-finishing pigs fed CrPic (Zhang et al., 2011). Furthermore, the research of Matthews et al. (2005) indicated that CIE L*, a*, b* of the loin muscle were not affected ($p<0.62$ to 0.99) by CrProp. Myoglobin (Mb), a water-soluble protein, is commonly found in three forms, oxymyoglobin, deoxymyoglobin and metmyoglobin, the relative proportions of which determine the colour of fresh meat (Wallace et al., 1982). However, few studies have examined the effects of supplementation with CrMet on the related gene *mb*.

With increasing focus on meat quality in the swine industry and lacking the published data regarding the potential effects of Cr on meat quality, particularly meat colour, clearly defining the effects of Cr on pork quality is necessary. Thus, the purpose of this study was to evaluate the effects of CrMet as Cr source on growth, carcass composition of growing-finishing pigs and on the meat quality of the pork by focusing on the expression of a gene related to meat colour. We hypothesized that Cr supplementation might improve growth, carcass traits and meat quality, and induce some alterations in meat colour via the *mb* gene expression in growing-finishing pig fed with CrMet.

MATERIALS AND METHODS

Animal selection and allotment

In total, 189 crossbred Duroc×(Landrace×Yorkshire) growing-finishing pigs (male, castrated, average initial BW 74.58±1.52 kg were selected from a breeding pig farm of Changzhou Yongkang Agricultural and Animal Husbandry Technology Co., Ltd (Jintan, Jiangsu, China) where the purebred Yorkshire, Landrace and Duroc had already been in closed bred for nearly 10 yrs. The pigs were randomly divided into four groups. The experimental pigs were housed in a curtain-sided finishing piggery and raised in pens (2.7 m×3.0 m) with a concrete-slotted floor in a single row confinement house with a 1.2-m-wide Northern-alley. Each treatment group had five pens with 8 to10 animals in each pen. The pigs had free access to food, as well as water from nipple drinkers within a transverse aeration-cooling system. The house received natural light for approximately 14 h daily. The ambient temperature of the inner house ranged from 16.1±2.6°C (06:00 to 07:00 h) to 10.7±3.4°C (14:00 to 15:00 h) with 18.5±3.5°C on average. The relative humidity ranged from 63.9±5.6% (06:00 to 07:00 h) to 67.4 ±7.5% (14:00 to 15:00 h) with 64.9%±6.49% on average. No vaccination was administered during the experimental period according to the immunization rules of the experimental farm. Faeces were cleaned from pens twice. At the end of the trial, growth performance was determined from 189 pigs, and five pigs per treatment (one pig per pen) were selected by having a body weight at the median for the group and used to evaluate carcass traits, pork quality, total myoglobin (Mb) content and the relative quantity of the Mb gene (*mb*) mRNA. The study and animal care protocols followed the guidelines from the Laboratory of Animal Management Association of Nanjing Agricultural University.

The experimental pigs were allotted to four dietary treatments on the basis of weight, and ancestry was equalized across treatments in a randomized complete block design. Pigs in the control group were fed a basal diet (control treatment, CT), whereas the experimental groups were fed basal diets supplemented with Cr in the form of CrMet (Hing Ka Bio-Engineering Co. Ltd) representing the following dietary proportions: 0.30 mg/kg Cr (treatment 1, T1), 0.60 mg/kg Cr (treatment 2, T2) and 0.90 mg/kg Cr (treatment 3, T3). The diet met or exceeded NRC (1998) recommendations for nutrients except digestible energy and was analyzed to provide 15.73% CP, 0.71% calcium, 0.58% phosphorus, 0.80% lysine and was calculated to provide digestible energy concentration of 3,151 kcal/kg. Basal diet composition and nutrient contents are shown in Table 1. Nutritional analysis showed the content of methionine and cysteine in the basal diet was 0.63%, which is higher than that of requirements for 50 to 120 kg growing pigs (NRC, 1998). Thus, a modest methionine in CrMet could not be expected to have an effect. Diets were mixed and sampled weekly. Feed samples from each treatment were collected and analyzed for dry matter, N, Ca and P according to the Association of Official Analytical Chemists procedures (AOAC, 1997).

Growth performance

Surplus feed remaining in the feeders of each pen (replicate) was cleared away and weighed daily. Feed intake was calculated and recorded daily. Pre-feeding body weight of each pig was recorded individually at the beginning of the experiment to obtain the initial body weight, and at the end of the finishing period to get the terminal weight. Average daily gain (ADG), average daily feed intake

Table 1. Ingredient composition and nutrient contents of basal diets for experimental growing-finishing pigs

Items	Amount (%)
Ingredient	
Corn	67.86
Soybean meal	15.25
Wheat bran	14.14
Monocalcium phosphate	0.63
Limestone	0.84
Salt	0.10
L-lysine·HCl	0.13
Vitamin and mineral premix[1]	1.05
Nutrition composition	
Digestible energy (kcal/kg)	3,151
Crude protein	15.73
Ca	0.71
P	0.58
Lysine	0.80
Methionine and cysteine	0.63

[1] Vitamin premix provided per kilogram of diet: vitamin A, 10,000 IU; vitamin D_3, 1,400 IU; vitamin E, 400 mg; vitamin K_3, 60 mg; vitamin B_1, 30 mg; vitamin B_2, 110 mg; vitamin B_6, 80 mg; vitamin B_{12}, 0.4 mg; niacin, 450 mg; Ca-D-pantothenic acid, 170 mg; folic acid, 11 mg; D-biotin, 0.8 mg; and choline chloride, 10 g; Mn (manganese sulphate), 850 mg; Fe (ferrous sulphate), 1,500 mg; Zn (zinc sulphate), 1,500 mg; Copper (copper sulphate), 150 mg; I (ethylene-diamine dihydriodide), 7 mg; Selenium (sodium selenite), 0.25 mg.

(ADFI), and feed gain ratio (F/G) were then calculated. The experimental period lasted approximately 28 d.

Any episodes of diarrhoea or death were recorded daily and compiled across the study. Three inspections were performed each day, in the early morning before cleaning, during feeding and after feeding. If watery faeces were found on the floor, the anus of each pig in the pen would be checked and judged. An episode of diarrhoea was accounted if the faeces was watery or was adherent to the anus. The incidence of diarrhoea was calculated as follows: incidence of diarrhoea (%) = (total head of diarrhoea pigs/number of experimental pigs)×100%. When death occurred, the BW of the dead pig was recorded.

Carcass evaluation

At a mean block weight of 100 kg, 20 pigs (one pig per pen selected on the basis of body weight near the mean group body weight) were commercially slaughtered by conventional methods. Carcasses were split along the dorsal midline, weighed, and chilled in 2°C for 24 h (Gandolfi et al., 2011). Loin muscle area between the 12th and 13th sternal ribs was determined, and average backfat thickness (ABT) was measured at three locations (first rib, last rib and last lumbar vertebrate). All carcass measurements were taken from the left side and determined using the equations and procedures of National Pork Producers Council (NPPC, 2000).

Pork quality evaluation

Forty-five-minute pH (pH_1) was determined in loin between the 10th and 11th ribs with a hand-held pH meter (model HI9025, HANNA instruments, Portugal). After collection of all carcass data, loin sections from the 10th to 14th ribs and from 1st to 2nd lumbar were collected respectively. The 12th-rib chop was used to determine water binding capacity (WBC). Total percent of loin water content was measured by oven at 100°C for 16 h (AOAC, 1997). A 35-kg pressure was vertically exerted on the surface of the loin sample collected by a standard circle sampler (diameter, 25.23 mm; thickness, 10 mm) for 5 min with a soil compression device (WZ-2, Nanjing Ningxi Soil Instrument Corporation, Ltd, Nanjing, China). Initial and final weights of the loin sample were obtained before and after pressing. WBC was calculated as follows: 1-((initial loin weight (g)-final loin weight (g))/(initial loin weight (g)×total percent loin water content))×100%. The loin section from 1st to 2nd lumbars was used to determine shear force (SF) using a SF tester (XL1155, XIELI Technology Corporation, Ltd, Qinhuangdao, China) with a load cell capacity of 50 kg and fitted with a Warner-Bratzler shearing device (Honikel, 1998). Six 1.27-diameter cores were removed from the lumbars parallel to the longitudinal orientation of muscle fibers. Each core was sheared perpendicular to the long axis of the core for SF determination. Immediately after the collection of the loin, Commission Internationale de I'Éclairage (CIE) L*, a* and b* values were obtained from three orientations on the last-rib loin interface using a Minolta spectrophotometer (model CR-10; Minolta Corp., Ramsey, NJ) (Mason et al., 2005). Two minced loin meat samples of approximately 20 g divided from the loin of the 14th rib were used to determine intramuscular fat percentage (IMF) using the modified Soxhlet method (AOAC, 1997).

Total Mb content analysis

After the collection of the loin section, approximately 5 g *longissmus* muscle sample from the 14th rib were collected and stored at -20°C for subsequent Mb content analysIs. Mb were extracted in duplicate from *longissimus* muscle samples by using Tris-EDTA buffer (3 mM/L $MgCl_2$, 5 mM/L EDTA, 75 mM/L Tris, pH 7.2, 20°C). The solutions containing Mb were obtained by centrifugation of the *longissimus* muscle at 10,000×*g* and 4°C for 10 min, and total Mb concentration was assayed at OD 525 nm (Krzywicki, 1982). The total Mb content was expressed as mg Mb per gram wet sample.

RNA isolation and quantitative real-time PCR

Within 30 min following exsanguination, approximately

2 g *longissmus* samples were removed from the 14th rib of each carcass, frozen in liquid nitrogen and stored at -80°C for subsequent RNA analysis. Total tissue RNA was extracted from *longissimus* muscle using the TRIzol reagent (TIAN GEN Biotech Co. Ltd., Beijing, China) according to the manufacturer's instruction. The RNA was resuspended in diethyl pyrocarbonate-treated water and quantified by measuring optical density (OD) at 260 nm (NANODROP-1000, Thermo, Shanghai, China). The purity of the RNA preparation was estimated using the OD260/280 ratio, and RNA integrity was verified by 1.5% agarose, 2.2 M formaldehyde gel electrophoresis. All RNA samples used in quantitative real-time PCR analysis exhibited OD260/280 ratios >1.8. Strand cDNA was synthesized by reverse transcription of 2 μg of total RNA using TIANScript (TIAN GEN Biotech Co. Ltd., Beijing, China) to obtain first-strand complementary DNA. The cDNA was amplified using a PCR optimization kit (TIAN GEN Biotech Co. Ltd., Beijing, China). Primers for quantitative real-time PCR amplification of the glyceraldehyde-3-phosphate dehydrogenase gene (*gapdh*) and *mb* were designed using Primer V5 software. Gene-specific primers for *gapdh* (forward, 5'-GGACTCATGACCACGGTCCA-3'; reverse, 5'-TCAGATCCACAACCGACACGT-3'; GenBank sequence No. U48832) and *mb* (forward, 5'-ATGCCACCAA GCACAAG-3'; reverse, 5'-CAAACCCTACAGCTACA GGA-3'; GenBank sequence No. M14433.1) were synthesized by Invitrogen Co. (Shanghai, China). The expression levels of genes were detected using an ABI 7300 Mastercycler (Applied Biosystems, USA) with qPCR mix, SYBR Premix Ex TaqTM (Takara Biotechology Co., Ltd., Dalian, China) according to the manufacturer's instructions. A water and a no-RT as controls were included to detect possible contamination in reaction. Each cDNA generated by reverse transcription was used as a temple for PCR using a 20 μl reaction mixture, in triplicate. The real-time PCR cycling conditions used were 95°C for 3 min, followed by 40 cycles at 95°C for 15 s and 57°C for 15 s, then 1 cycle at 72°C for 30 s. The amplification efficiency for two genes were investigated by gradient dilution of *longissimus* muscle cDNA and found to be more than 90%. The values were normalized using *gapdh* as an endogenous standard. The relative concentration of mRNA was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The amplified product was applied to a 2% agarose/ethidium bromide gel for electrophoresis.

Statistical analysis

Data were analyzed by univariate analysis of variance procedures using the general linear model of SPSS version 16.0 (Statistical Product and Service Solutions Incorporation, 2008), where a polynomial contrast was constructed to determine linear and quadratic effects of increased dietary Cr levels. Carcass data were analyzed with final body weight as a covariate. The pen of pigs served as the experimental unit for all data. The experimental data were presented as means.

RESULTS

Growth performance analysis

The duration of the trial was 28 d. One pig from the control treatment was removed from the study on d 12 due to extreme weakness of its forequarters. Another pig from the 0.9 mg/kg Cr treatment exhibited a vomiting reaction on d 26 without any previous indication of problems. No pigs fed the 0.3 mg/kg Cr or 0.6 mg/kg Cr diet were removed from the experiment and none of pigs subjected to the dietary treatments were treated for any type of illness. For each pen, total feed intake was recorded daily and average value of the individual feed intake could be obtained by dividing total feed intake by pig number in each pen. The effects of increasing the level of dietary Cr on growth performance of the experimental pigs are shown in Table 2. A quadratic effect of dietary Cr supplementation levels on final body weight was detected ($p = 0.037$). Feeding increasing levels of dietary Cr affected the ADG, ADFI and F/G across the whole 28 d experiment. A linear effect of dietary Cr levels on ADG ($p = 0.044$), ADFI ($p = 0.034$) and F/G ratio ($p = 0.005$) was detected with the increased levels of dietary Cr.

Table 2. Effects of chromium supplementation on the growth performance of the growing-finishing pigs during the 28 d experiment[1]

Item	Cr level (mg/kg)				SEM[2]	p-value	
	0	0.3	0.6	0.9		Linear	Quadratic
IBW (kg)	75.79	74.82	73.44	74.81	0.48	0.504	0.202
FBW (kg)	99.74	99.74	101.23	103.68	1.86	0.076	0.037
ADG (g)	855.4	890.1	992.6	1,031.1	41.5	0.044	0.282
ADFI (kg)	3.002	3.177	3.613	3.794	0.18	0.034	0.252
F/G	3.51	3.57	3.64	3.68	0.04	0.005	0.069

[1] IBW = Initial body weight; FBW = Final body weight; ADG = Average daily gain; ADFI = Average daily food intake; F/G = Food gain ratio. Pen was considered as an experimental unit for growth performance (n = 8 to 10).

[2] Standard error of the mean.

Table 3. Effects of dietary chromium methione on carcass composition of finishing pigs[1]

Item	Cr level (mg/kg)				SEM[2]	p-value	
	0	0.3	0.6	0.9		Linear	Quadratic
DP (%)	73.68	72.97	73.90	73.58	0.40	0.636	0.843
ABT (cm)	2.46	2.08	1.99	1.90	0.25	0.072	0.152
LMA(cm^2)	34.21	36.69	38.72	38.68	2.13	0.013	0.158
LMP (%)	63.28	65.46	67.83	67.92	2.21	0.122	0.133

[1] DP = Dressing percentage; ABT = Average backfat thickness of the first and last rib and last lumbar fat depths; LMA = Loin muscle area; LMP = Lean meat percentage. Pen was considered as an experimental unit. Values were presented as means (n = 5).
[2] Standard error of the mean.

Carcass composition analysis

Carcass composition parameters (dressing percentage, ABT, loin muscle area and lean meat percentage) are shown in Table 3. As levels of dietary Cr increased, loin muscle area increased linearly (p = 0.013), while ABT decreased linearly (p = 0.072).

Pork quality analysis

Pork quality parameters of pH_1, WBC, SF, IMF, CIE L*, CIE a* and CIE b* are shown in Table 4. When the dietary Cr supplementation was increased from 0 mg/kg to 0.9 mg/kg, SF tended to increase in a linear response (p = 0.070) and CIE a* tended to increase in a quadratic response (p = 0.028), while CIE L* tended to decrease in a quadratic response (p = 0.053).

The total Mb content and *mb* expression in *longissimus* muscle

Table 4 shows the total Mb concentrations among different treatments in the *longissimus* muscle. A quadratic effect of dietary Cr supplementation levels on total Mb content was detected (p = 0.015). The effect of dietary Cr supplementation on the expression of *mb* in *longissimus* muscle was investigated using quantitative real-time PCR (Table 4). A quadratic effect of dietary Cr supplementation levels on *mb* mRNA levels was detected (p = 0.046).

DISCUSSION

The effect of dietary CrMet on growth performance

Recent studies showed that dietary Cr supplementation affected protein (muscle mass) and fat deposition (Wang and Xu, 2004; Jackson et al., 2009). It has been suggested that there is a muscle deposition peak of pigs near 60 to 90 kg body weight and thereafter fat deposition becomes the main part of the body weight gain (Gu et al., 1991; Wagner et al., 1999). In our study, growing-finishing pigs whose initial weight was approximately 75 kg were selected to investigate the effect of dietary Cr as CrMet.

The present results showed that increasing dietary Cr as CrMet significantly increased ADG, ADFI and F/G in a linear fashion (p<0.05), indicating that dietary Cr supplementation does affect ADG, ADFI and F/G in growing-finishing pigs as body weights grow from 75 to 100 kg over about one month. Increased levels of Cr supplementation increased AFDI significantly, which resulted in increased ADG. However, the efficiency of feed utilization of growing-finishing pigs decreased linearly (p =

Table 4. Effects of dietary chromium methione on pork qualities, the total myoglobin (Mb) content and the relative *mb* mRNA level in the *longissimus* muscle of growing-finishing pigs[1]

Item	Cr level (mg/kg)				SEM[2]	p-value	
	0	0.3	0.6	0.9		Linear	Quadratic
pH_1 (%)	6.21	6.20	6.21	6.28	0.04	0.232	0.140
WBC (%)	68.87	68.85	68.82	68.86	0.02	0.641	0.478
SF (kg/cm^2)	3.78	3.95	4.25	4.23	0.23	0.070	0.186
IMF (%)	3.02	2.52	2.14	2.06	0.44	0.106	0.128
CIE L*	48.36	46.65	45.51	45.49	1.35	0.069	0.053
CIE a*	3.64	4.55	4.92	4.88	0.60	0.113	0.028
CIE b*	10.42	10.82	10.46	10.59	0.18	0.969	0.971
Mb (mg/g)	0.262	0.363	0.423	0.434	0.08	0.055	0.015
mb mRNA[3]	1.00	1.22	1.30	1.29	0.14	0.116	0.046

[1] pH_1 = Initial pH measured 45-min post-slaughter; WBC = Water binding capacity; SF = Shear force; IMF = Intramuscular fat percentage; CIE = Commission Internationale de l'E´ clairage; L* = Measurement of lightness to darkness, with a higher value indicating a lighter colour; a* = Measurement of greenness to redness, with a higher value indicating a redder colour; b* = Measurement of blueness to yellowness, with a higher value indicating a more yellow colour. Pen was considered as an experimental unit. Values were presented as means (n = 5).
[2] Standard error of the mean.
[3] The mean value in the control (CT) group was set as 1. Quantities of mRNA are normalized on the basis of *gapdh* expression.

0.005). Taking into account this reduced feed conversion efficiency and the single pig from the 0.9 mg/kg Cr treatment exhibiting a vomiting reaction, we think that the most appropriate dietary Cr supplementation level for growing-finishing pigs from 70 to 100 kg falls between 0.6 mg/kg and 0.9 mg/kg. Mertz (1993) concluded that the effects of Cr on growth performance and carcass traits are inconsistent. Previous reports suggest that Cr supplementation improved some aspects of growth performance (Lindemann et al., 1995; Mooney and Cromwell, 1995). Conversely, other reports indicated that Cr supplementation did not affect growth performance nor some aspects of carcass traits (Evock-Clover et al., 1993; Matthews et al., 2003). Page et al. (1993) reported that dietary Cr supplementation displayed a significantly cubic increase in the ADG. Evock-Clover et al. (1993) reported that young growing pigs given Cr showed no improvement in growth rate, feed efficiency or composition of gain. Finally, Zhang et al. (2011) reported that a supplementary dose of 0.2 mg/kg Cr as chromium picolinate significantly improved daily weight gain during the finishing period, which was similar to our results. We demonstrated that dietary Cr supplementation increased ADG but that the feed utilization of growing-finishing pigs decreased with the increasing Cr supplementation. The various outcomes reported in the literature may be explained by factors such as differences in the feeding period as well as the energy concentration of the diets.

Social stress disturbs the normal immune system and endocrine system under commercial environments, thereby inducing the depression in food intake and reduced weight gain (Morrow-Tesch et al., 1994; Black et al., 2001). *In vitro* cellular immune response was increased in pigs fed supplemental Cr from $CrCl_3$, or Cr-picolinate and the gain and feed intake tended to improve (Van Heugten and Spears, 1997). Also, Cr supplementation benefited the immune response in weaned pigs following an LPS-induced stress by up-regulating IgG and γ-globulin levels (Lien et al., 2005). In broiler chicks, Cr supplementation can improve growth performance, carcass traits and meat lipid oxidation through alleviating the negative effects of heat stress (Toghyani et al., 2012). Stresses impaired immune function and thereby resulting in a reduction of feed intake and growth rate in weaning pigs (Van Heugten et al., 1996; Oh et al., 2010). As mentioned above, in present study, increased ADFI may attribute to the enhanced immune system and improved ability against stress by CrMet supplementation, which consequently led to the increased ADG.

The effect of dietary CrMet on carcass composition

Mooney and Cromwell (1995) reported that muscle accretion rates increased and fat accretion rates decreased with Cr supplementation. Additionally, carcass fat percentage and ABT decreased by 13.5% and 11.1%, respectively (O'Quinn et al., 1998). Wang and Xu (2004) reported that pigs fed chromium nanoparticles had higher carcass lean meat percentages, greater loin muscle areas and lower backfat thickness. Jackson et al. (2009) reported that the addition of Cr decreased 10th backfat thickness and increased the percentage of muscle. Moreover, CrPic resulted in a significant reduction of ABT especially in castrated pigs, which was in accordance with our results (Kim et al., 2010). Previous studies elucidated that Cr partly contributed to the insulin signaling auto-amplification mechanism and thereby increasing protein accumulation via enhancing glucose utilization and regulating fat acid and fat synthesis (Vincent et al., 2000; Wong et al., 2010). Specifically, our results showed that ABT decreased linearly with the increasing dietary Cr supplementation, which was in agreement with previous reports (O'Quinn et al., 1998; Wang and Xu, 2004; Jackson et al., 2009). Since it is generally believed that lean red meat with less fat is beneficial to human health (Li et al., 2005), and since CrMet supplementation is no higher in cost than other types of organic chromium complexes, then CrMet supplementation may be an economical means to improve the nutritional profile of pork. However, some studies have produced conflicting results. For example, dressing percentage and backfat thickness at the 10th rib and last rib did not differ between treatment groups in pigs fed CrPic (Kornegay et al., 1997). Additionally, Matthews et al. (2005) reported that loin muscle area, 10th-rib backfat thickness, ABT, dressing percentage and percent lean meat were not affected by CrProp. Such inconsistent results in terms of ABT, loin muscle area and lean meat percentage might be attributable to genetic factors, absorption ratio of different forms of Cr, or both. These alternative factors can be clarified but it will require additional studies to examine the effect of dietary CrMet on the carcass composition with different experimental conditions.

The effect of dietary CrMet on pork quality

Reports in the literature regarding the effects of supplemental Cr on *longissimus* muscle quality have been inconsistent. Matthews et al. (2005) reported that 45-min pH was not affected, but that 24-h pH was higher in pigs fed CrProp. Our data agree that increasing levels of dietary Cr supplementation had no effect on 45-min pH. O'Quinn et al. (1998) reported that drip loss percentage in gilts increased with increased CrNic supplementation. Zhang et al. (2011) reported that no change occurred in water-holding capacity, which was similar to our results. The SF was impacted by several factors such as pH, IMF and storage time of pork *longissimus* muscle (Van Laack et al., 2001). We found that increasing dietary Cr supplementation

exerted a linear decline on SF. As shown in Table 4, the pigs in the 0.9 mg/kg and in the 0.6 mg/kg treatment groups exhibited lower IMF than those of the other treatment groups. The increased SF may be attributed to the decreased IMF induced by increased Cr supplementation. This may suggest that pigs have a limited capacity to maintain IMF with increasing dietary Cr addition, and that pork quality might be compromised by high Cr supplementation. Given that Cr addition may influence the metabolism of fat, it is plausible that Cr is a nutritional supplement that would affect fat deposition, including intramuscular fat deposition. In the present study, pigs fed increasing dietary Cr exhibited a significant quadratic effect of CIE a*, which was consistent with previous reports (Shelton et al., 2003; Zhang et al., 2011). CIE a* increased as dietary Cr supplementation increased, particularly following the 0.6 and 0.9 mg/kg Cr treatments, suggesting that increasing Cr supplementation could increase meat redness. Customers in many parts of the world prefer to purchase well-coloured pork if marbling and tenderness are no worse than medium (Norman et al., 2003; Fortomaris et al., 2006; Ngapo et al., 2007). In this case, meat colour can be regulated by dietary Cr and classified into several grades according to customer preference. Taken together, CrMet would be more beneficial since it is able to improve the meat colour, decrease fat percentage and increase muscle mass. Matthews et al. (2003; 2005) reported that CrProp had no effect on colour scores. The inconsistent effect might have been due to the supplementation level of dietary Cr, which was 0.9 mg/kg in our case, compared to 0.2 mg/kg for the previous research.

The effect of dietary CrMet on total Mb concentration and mRNA expression of *mb*

The biochemical basis of red colour in meats is well established and depends on the concentration and redox state of Mb, haemoglobin and cytochromes in meat. Due to the exposure to oxygenic environment during exsanguinations, most of the muscle haemoglobin is lost and ultimately accounts for between 6% and 16% of total fresh meat pigment. Cr supplementation improved anti-oxidative ability and attenuated oxidative stress in broilers and rats (Rama Rao et al., 2012; Sundaram et al., 2012). Mb is the primary protein pigment responsible for meat colour and constitutes between 80% and 90% of total fresh meat pigment (Giddings and Solberg, 1977). Thus, the total Mb concentration in the *longissimus* muscle of the pig is a very important contributor to pork colour. Cr is essential for normal glucose metabolism, functioning as a part of the insulin signaling auto-amplification mechanism and improves insulin-stimulated amino acid uptake and protein synthesis and gene expression (Vincent et al., 2000; Peng et al., 2010). The present results showed the CIE a* increased linearly with increasing total Mb content. Quantitative real-time PCR was used to analyze differentially expressed proteins at the mRNA level, which revealed a positive correlation between the content of the Mb and the mRNA levels. CrMet influenced total Mb content and the expression levels of *mb* suggesting that Cr has a dose-related effect on Mb synthesis activity by regulating the transcription of *mb*. Zhang et al. (2011) reported that RNA/DNA increased significantly, while the content of DNA in skeletal muscle showed no marked changes with CrPic supplementation. Therefore, on the basis of these results, we hypothesize that dietary Cr supplementation may have improved anti-oxidative status and stimulated the mRNA expression level of the *mb* gene and Mb synthesis, which promoted Mb protein synthesis and improved the meat colour. However, no significant difference in *mb* mRNA expression existed among treatments in the five pigs per treatment that were analyzed. This may imply that the threshold of effect for Cr on *mb* mRNA may fall below the levels we tested. The effect of dietary CrMet on local *mb* mRNA abundance therefore needs further study.

CONCLUSIONS

CrMet, as a highly bioavailable complex, has lesser environmental hazards and would be more beneficial since it is able to improve the meat colour, decrease fat percentage and increase muscle. If some regulatory agencies could alleviate the negative effects of SF and IMF, then CrMet may become an economical means to improve meat quality and it then would be wise for pig producers to add CrMet to the diets. However, considering previous inconsistent studies and the small number of pigs analyzed in the present study, the effect of CrMet on growing-finishing pigs needs further confirmation.

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