Effects of organic chromium sources on growth performance, lipid metabolism, antioxidant status, breast amino acid and fatty acid profiles in broilers

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Abstract

BACKGROUND: Trivalent chromium (Cr) is involved in carbohydrate, lipid, protein and nucleic acid metabolism in animals. This study evaluated the effects of different organic Cr forms with Cr methionine (CrMet), Cr picolinate (CrPic), Cr nicotinate (CrNic), and Cr yeast (Cr-yeast) at the level of 400 ∼ g kg −1 Cr, on growth performance, lipid metabolism, antioxidant status, breast amino acid and fatty acid profiles of broilers. In total, 540 one-day-old Arbor Acres male broilers were randomly assigned to five treatments with six replicates (18 broilers per replicate) until day 42.

RESULTS: The results showed growth performance was not affected by Cr sources. The Cr-yeast group had lower serum cortisol levels than the CrNic group (P < 0.05). Besides, Cr-yeast increased methionine and cysteine content in breast compared with the control group. Liver malondialdehyde content was lower in the CrMet group than the CrPic group on day 42 (P < 0.05). The n-3 polyunsaturated fatty acid (PUFA) values were increased, but the n-6/n-3 PUFA ratio was decreased in both CrMet and CrNic groups (P < 0.05). There were no significant effects on broilers’ serum antioxidant status and breast total essential amino acid content among all treatments.

CONCLUSIONS: Diets supplemented with organic Cr could regulate lipid metabolism, and improve amino acid and fatty acid profiles in broiler breast. Moreover, Cr-yeast was the most effective source in improving methionine and cysteine content, whereas CrMet was more effective than CrNic in increasing n-3 PUFA value and decreasing n-6/n-3 PUFA ratio in breast meat and effectively strengthened liver antioxidant ability than CrPic.

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Keywords: organic chromium; lipid metabolism; antioxidant status; fatty acid and amino acid profiles; broiler

INTRODUCTION

In recent years, the total consumption of chicken meat has increased with the rapid development of the poultry industry. 1,2 Poultry meat generally contains not only nutrients but also enriches health-promoting substances such as amino acids (AA) and n-3 polyunsaturated fatty acids (PUFA). 3,4 The characteristics of AA affect the umami and sweet flavor of meat. 5 The n-3 PUFA members are precursors of many inflammatory lipid mediators and have been shown to exert anti-inflammatory functions. 1,6 The n-6/n-3 PUFA ratio is a vital index for the pathogenesis of some diseases, such as cardiovascular diseases, cancer and autoimmune diseases. 7 Moreover, n-3 PUFA is an important factor contributing to selection of chicken meat by consumers. However, it is susceptible to quality deterioration and a short meat shelf life by lipid oxidation due to the lack of antioxidants in the meat. 8,9 The double bond in unsaturated fatty acids can be easily attacked by free radicals during the oxidation process, thus producing malondialdehyde (MDA) during lipid peroxidation. 9 In order to improve the antioxidant status, extend the meat shelf life and provide consumers with nutritious broiler meat, we added trivalent chromium (Cr) to broiler complete feed. Cr is also an antioxidant additive that may be used as a dietary supplement in a nutritional strategy to accumulate the desired PUFA, improve antioxidant capacity and finally strengthen broiler health.

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Trivalent Cr is involved in the metabolism of insulin by stimulating glucose consumption and increasing mRNA expression of the insulin receptor and glucose transporter-4. Cr may regulate lipid metabolism by decreasing the enzymatic activity of the fatty acid synthase or hormone-sensitive lipase. In addition, Cr is also vital for chondromodulin to stimulate the activity of the insulin receptor protein tyrosine kinase, which is required for stabilizing proteins and nucleic acid formation. Cr might act as an indirect antioxidant, by reducing high insulin levels and preventing glucose auto-oxidation. Supplementation of Cr in broilers has been shown to improve growth performance, regulate lipid metabolism, enhance immune status and alleviate heat or transport stress. However, other studies have reported opposite or even toxic results.

Organic Cr chelates, known as Cr methionine (CrMet), Cr picolinate (CrPic), Cr nicotinate (CrNic), Cr-yeast, Cr propionate and Cr histidinate, have higher bioavailability and lower toxicity than inorganic Cr forms. Chromium methionine (CrMet) has been reported to increase PUFA effects in animals. Each group containing six replicates (18 birds per replicate). The 540 one-day-old male broilers (Arbor Acres) were weighed and had ad libitum access to water and feed during the entire experimental period. The rearing room temperature was consistent at 35 °C (the first 3 days), after which the room temperature was gradually reduced by 3 °C each week until the temperature reached 24 °C. This temperature was maintained until the end of the experiment on day 42. The relative humidity of the rearing room was maintained between 50% and 70%. On days 7 and days 21, all broilers were inoculated with an inactivated Newcastle disease vaccine, and on days 14 and 28 broilers were given an inactivated infectious bursal disease vaccine.

MATERIALS AND METHODS

Animals

The protocol used in this experiment was approved by the Institutional Animal Care and Use Committee of China Agricultural University. Throughout the study, animal experiments were performed following the National Institutes of Health Guidelines for the care and use of experimental animals. This study was conducted on 540 one-day-old Arbor Acres male broilers (initial body weight 48.62 ± 0.72 g), which were purchased from Arbor Acres Poultry Breeding Co. (Beijing, China).

Material

Chromium methionine (CrMet, 9.54% Cr), chromium picolinate (CrPic, 12.20% Cr), chromium nicotinate (CrNic, 12.30% Cr) and chromium yeast (Cr-yeast, 0.07% Cr) were purchased from different Chinese companies (Harbin DeBon Xinjin Bio-tech Co. Ltd (Heilongjiang, China), Mianyang Sinyiml Chemical Co. Ltd (Sichuan, China) and Alltech Co. Ltd (Tianjin, China)).

Experimental protocol

The 540 one-day-old male broilers (Arbor Acres) were weighed and then randomly divided into five experimental groups, with each group containing six replicates (18 birds per replicate). The control group received corn–soybean meal basal diets. Experimental broilers were fed basal diets supplemented with 400 μg kg⁻¹ Cr in the form of CrNic, CrMet, CrPic and Cr-yeast, respectively. The organic Cr sources were premixed with corn flour and then added to each experimental diet. All experimental diets were fed in mash form. As shown in Table 1, the basal diet was a corn–soybean meal diet, which was formulated to meet or exceed the suggested nutrient requirements of broiler chickens.

Broilers were fed with starter diets (days 1–21) and grower diets (days 22–42). Broilers were reared in wire-floored cages, in a controlled rearing room environment. Broilers were exposed to 23 h lighting and had ad libitum access to water and feed during the entire experimental period. The rearing room temperature was consistent at 35 °C (the first 3 days), after which the room temperature was gradually reduced by 3 °C each week until the temperature reached 24 °C. This temperature was maintained until the end of the experiment on day 42. The relative humidity of the rearing room was maintained between 50% and 70%. On days 7 and days 21, all broilers were inoculated with an inactivated Newcastle disease vaccine, and on days 14 and 28 broilers were given an inactivated infectious bursal disease vaccine.

Growth performance

On days 21 and 42, the body weight (BW) and feed consumption of all broilers in all replicates were recorded after a 12 h fasting period. At the end of the experiment, each replicate was selected (30 birds per replicate) and dissected. The longissimus thoracis muscle, which is one of the most important muscle groups for meat production, was taken from the right side of the breast. The whole breast muscle was rapidly chilled and dissected, and the visceral fat was carefully removed to determine the fat content. The fat and protein contents were determined by the acid soxhlet method and the Kjeldahl method, respectively.

Table 1. Ingredients and chemical composition of the basal diet (as-fed basis, %)

| Item                  | Starter phase (days 1–21) | Grower phase (days 22–42) |
|-----------------------|---------------------------|---------------------------|
| Ingredients           |                           |                           |
| Corn                  | 60.13                     | 61.53                     |
| Soybean meal          | 32.50                     | 31.70                     |
| Fish meal             | 2.00                      | 0.00                      |
| Soybean oil           | 1.50                      | 3.00                      |
| Dicalcium phosphate   | 1.50                      | 1.70                      |
| Limestone             | 1.34                      | 1.15                      |
| α-Methionine (98%)    | 0.23                      | 0.12                      |
| Sodium chloride       | 0.30                      | 0.30                      |
| Vitamin–mineral premixa | 0.50                    | 0.50                      |
| Total                 | 100.00                    | 100.00                    |
| Nutrient levels b     |                           |                           |
| ME (MJ kg⁻¹)          | 12.60                     | 13.23                     |
| Crude protein         | 21.54                     | 20.02                     |
| Ether extract         | 3.90                      | 6.10                      |
| Calcium               | 1.02                      | 0.91                      |
| Total phosphorus      | 0.68                      | 0.66                      |
| Lysine                | 1.21                      | 1.09                      |
| Methionine + cysteine | 0.93                      | 0.79                      |

* Provided per kilogram of diet: vitamin A 9000 IU; vitamin D₃ 3000 IU; vitamin E 24 IU; vitamin K₁ 1.8 mg; thiamine 2.0 mg; riboflavin 5.0 mg; pyridoxine 3.0 mg; cobalamin 0.1 mg; nicotinic acid 40 mg; pantothenic acid 15 mg; folic acid 1.0 mg; biotin 0.05 mg; choline chloride 500 mg; iron (from FeSO₄) 80 mg; copper (from CuSO₄) 20 mg; zinc (from ZnSO₄) 90 mg; manganese (from MnSO₄) 80 mg; iodine (from KI) 0.35 mg; selenium (from Na₂SeO₃) 0.30 mg.

b All nutrient levels were analyzed except metabolizable energy.

c ME, metabolizable energy.
period. The growth performance parameters of the chickens for the periods between days 1–21, 22–42 and 1–42 were calculated as average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) for each replicate.

Sample collection
One broiler that was visually approximated the average BW in each replicate was selected for blood and tissue sampling on days 21 and 42. Blood was collected into 5 mL anticoagulant-free vacutainer tubes. Serum was collected and stored at −80 °C for later analysis. Serum from days 21 and 42 was prepared to detect the lipid metabolites, related hormones and antioxidant parameters. Liver and breast meat samples from days 21 and 42 were used to detect antioxidant indices. The remaining breast meat sample from day 42 was freeze-dried for 72 h and used to analyze the amino acid and fatty acid profiles.

Dietary chemical composition analysis
The nutritional value of the experimental diets was analyzed according to the methods of the Association of Official Analytical Chemists29 for total phosphorus (995.11), calcium (927.02), ether extract (920.39) and crude protein (988.05). Methionine and lysine contents were determined using acid hydrolysis with 6 mol L−1 HCl (994.12) and measured using an amino acid analyzer (Hitachi L-8800, Tokyo, Japan). Performic acid oxidation was performed prior to acid hydrolysis to determine the methionine concentration. The Cr content in the diets was determined by inductively coupled plasma mass spectrometry (model 7500, Agilent Technologies, Inc., Palo Alto, CA, USA) according to the method reported by Lindemann et al.29

Assay of serum lipid metabolites and hormones
On days 21 and 42, the serum contents of total triglyceride (TG), high-density lipoprotein cholesterol (HDLc), low-density lipoprotein cholesterol (LDLc) and cholesterol (CHOL) were analyzed using an automatic biochemical analyzer (Hitachi 7160, Hitachi High-Tech Corp., Tokyo, Japan). The serum contents were quantified using commercial kits from Jiancheng Biochemical Reagent Co. (Nanjing, China) and the methodology followed the manufacturer’s instructions.

Assay of amino acid profiles in breast
The amino acid compositions in freeze-dried breast samples were hydrolyzed with 6 mol L−1 HCl at 110 °C for 24 h except for methionine, cysteine and tryptophan, and then detected by an amino acid analyzer (Hitachi L-8900). In addition, sulfur amino acids (such as methionine and cysteine) were analyzed as methionine sulfone and cysteic acid after cold performic acid oxidation overnight and hydrolyzed with 6.8 mol L−1 HCl at 110 °C for 24 h before being detected using an amino acid analyzer (Hitachi L-8800). Tryptophan content was hydrolyzed by lithium hydroxide at 110 °C for 22 h, and then detected using high-performance liquid chromatography (Agilent 1200 Series).

Assay of fatty acid profiles in breast
Freeze-dried breast samples (200 mg) were used to detect fatty acid composition by gas chromatography (Agilent 6890 Series) following the method described by Pritam et al.30 Fatty acids were expressed as a percentage of the sum of the identified fatty acids (% w/w).

Statistical analysis
All experimental data were analyzed by one-way analysis of variance (ANOVA) for a completely randomized design using the general linear model (GLM) procedure of SAS (v 8.0, Inst. Inc., Cary, NC, USA). Differences among all treatments were examined with Tukey’s post hoc test. The replicate served as the experimental unit. P < 0.05 was considered statistically significant.

RESULTS
Chromium analysis of diets
The analyzed Cr content in the starter and grower diets are presented in Table 2. The Cr content in the control diets during the starter and grower phases was 454.04 and 426.49 μg kg−1, respectively. The analyzed Cr concentrations in diets supplemented with 400 μg kg−1 Cr each in the form of CrNic, CrMet, CrPic and Cr-yeast were 837.65, 850.27, 836.30 and 872.82 μg kg−1 for the starter diets, and 840.43, 896.51, 827.98 and 859.76 μg kg−1 for the grower diets, respectively. The Cr content in diets supplemented with different organic Cr sources agreed with the expected values.

Growth performance
The effects of organic Cr sources on the growth performance of broilers are provided in Table 3. Diets supplemented with different forms of organic Cr did not affect the growth performance parameters of the broilers. In all experimental intervals (21 and 42 days), the ADG, ADFI and FCR were similar among all groups.

| Table 2. Chromium content in experimental diets (μg kg−1) |
|----------------------------------------------------------|
| Items | Control | CrNic | CrMet | CrPic | Cr-yeast |
|-------|---------|-------|-------|-------|----------|
| Starter phase | 454.04 | 837.65 | 850.27 | 836.30 | 872.82 |
| Grower phase | 426.49 | 840.43 | 896.51 | 827.98 | 859.76 |
| CrNic, chromium nicotinate; CrMet, chromium methionine; CrPic, chromium picolinate; Cr-yeast, chromium yeast. |
The effects of the different organic Cr sources on broiler serum lipid metabolites and hormones are listed in Table 4. On day 21, diet supplemented with CrNic significantly increased serum HDLC content ($P < 0.05$) compared with the control group and the CrMet-treated group. There were no impacts on serum HDLC among CrNic, CrPic and Cr-yeast groups. Compared with the control group, diets supplemented with different organic Cr sources did not influence serum COR levels, whereas, compared with the CrNic group, the CrPic and Cr-yeast-treated diets significantly reduced serum COR levels ($P < 0.05$). Conversely, no differences in the serum COR levels were observed among the CrMet, CrPic and Cr-yeast groups. On day 42, compared with the control group, diets supplemented with CrPic and Cr-yeast significantly reduced serum COR levels ($P < 0.05$).

### Table 3. Effects of organic chromium source on growth performance of broilers

| Items      | Control | CrNic | CrMet | CrPic | Cr-yeast | SEM  | $P$-value |
|------------|---------|-------|-------|-------|----------|------|-----------|
| Body weight|         |       |       |       |          |      |           |
| day 1 (g)  | 48.84   | 48.76 | 48.91 | 48.30 | 48.27    | 0.18 | 0.76      |
| day 21 (kg)| 0.70    | 0.71  | 0.70  | 0.71  | 0.71     | 0.01 | 0.97      |
| day 42 (kg)| 2.11    | 2.13  | 2.12  | 2.18  | 2.12     | 0.02 | 0.80      |
| ADG (g)    |         |       |       |       |          |      |           |
| day 1–21   | 31.22   | 31.69 | 31.22 | 31.72 | 31.50    | 0.26 | 0.96      |
| day 22–42  | 66.74   | 67.41 | 67.56 | 69.72 | 67.24    | 0.68 | 0.74      |
| day 1–42   | 48.98   | 49.55 | 49.39 | 50.72 | 49.37    | 0.42 | 0.79      |
| FCR        |         |       |       |       |          |      |           |
| day 1–21   | 1.36    | 1.36  | 1.29  | 1.32  | 1.36     | 0.01 | 0.27      |
| day 22–42  | 1.79    | 1.82  | 1.81  | 1.83  | 1.80     | 0.01 | 0.87      |
| day 1–42   | 1.65    | 1.67  | 1.66  | 1.68  | 1.66     | 0.01 | 0.91      |

CrNic, chromium nicotinate; CrMet, chromium methionine; CrPic, chromium picolinate; Cr-yeast, chromium yeast; SEM, standard error of means; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio.

### Table 4. Effects of organic chromium sources on serum metabolites and hormones of broilers

| Items      | Control | CrNic | CrMet | CrPic | Cr-yeast | SEM  | $P$-value |
|------------|---------|-------|-------|-------|----------|------|-----------|
| Day 21     |         |       |       |       |          |      |           |
| TG (mmol L$^{-1}$) | 0.30 | 0.31 | 0.25 | 0.28 | 0.29     | 0.01 | 0.42      |
| HDLC (mmol L$^{-1}$) | 2.26b | 2.92a | 2.31b | 2.47ab | 2.43ab    | 0.07 | 0.02      |
| LDLC (mmol L$^{-1}$) | 0.33 | 0.39 | 0.33 | 0.34 | 0.39     | 0.02 | 0.81      |
| CHO (mmol L$^{-1}$) | 4.64 | 5.18 | 4.60 | 5.03 | 4.78     | 0.09 | 0.14      |
| INS ($\mu$IU ml$^{-1}$) | 4.95 | 4.77 | 5.24 | 5.09 | 4.81     | 0.10 | 0.62      |
| IGF-I (ng mL$^{-1}$) | 134.66 | 140.51 | 170.48 | 134.13 | 129.72   | 4.85 | 0.06      |
| COR ($\mu$g dL$^{-1}$) | 2.82ab | 2.94a | 2.50ab | 2.18b | 2.20b     | 0.09 | <0.01     |
| Day 42     |         |       |       |       |          |      |           |
| TG (mmol L$^{-1}$) | 0.27a | 0.20ab | 0.19ab | 0.12b | 0.13b     | 0.02 | 0.02      |
| HDLC (mmol L$^{-1}$) | 1.91a | 1.55ab | 1.49ab | 1.02ab | 0.86b     | 0.12 | 0.03      |
| LDLC (mmol L$^{-1}$) | 0.43 | 0.29 | 0.31 | 0.20 | 0.21     | 0.03 | 0.23      |
| CHO (mmol L$^{-1}$) | 4.00a | 3.17ab | 3.14ab | 2.12ab | 1.80b     | 0.24 | 0.02      |
| INS ($\mu$g ml$^{-1}$) | 5.33 | 6.61 | 5.97 | 6.19 | 6.79     | 0.20 | 0.15      |
| IGF-I (ng mL$^{-1}$) | 158.48 | 139.95 | 172.75 | 145.93 | 172.40   | 5.41 | 0.18      |
| COR ($\mu$g dL$^{-1}$) | 2.06ab | 2.28a | 2.17ab | 2.05ab | 1.90b     | 0.04 | 0.05      |

Values with different letters (a, b) in the same row differ significantly ($P < 0.05$).
CrNic, chromium nicotinate; CrMet, chromium methionine; CrPic, chromium picolinate; Cr-yeast, chromium yeast; SEM, standard error of means; TG, total triglyceride; HDLC, high-density lipoprotein cholesterol; LDLC, low-density lipoprotein cholesterol; CHO, cholesterol; INS, insulin; IGF-I, insulin-like growth factor I; COR, cortisol.

### Lipid metabolism

The effects of the different organic Cr sources on broiler serum lipid metabolites and hormones are listed in Table 4. On day 21, diet supplemented with CrNic significantly increased serum HDLC content ($P < 0.05$) compared with the control group and the CrMet-treated group. There were no impacts on serum HDLC among CrNic, CrPic and Cr-yeast groups. Compared with the control group, diets supplemented with different organic Cr sources did not influence serum COR levels, whereas, compared with the CrNic group, the CrPic and Cr-yeast-treated diets significantly reduced serum COR levels ($P < 0.05$). Conversely, no differences in the serum COR levels were observed among the CrMet, CrPic and Cr-yeast groups. On day 42, compared with the control group, diets supplemented with CrPic and Cr-yeast significantly reduced serum COR levels ($P < 0.05$).
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decreased serum TG content (P < 0.05) and diet supplemented with Cr-yeast significantly declined serum HDLC and CHO levels (P < 0.05), whereas there were no differences observed in TG, HDLC and CHO content in groups treated with different Cr sources. However, compared with broilers fed a diet supplemented with CrNic, broilers fed a Cr-yeast diet had lower COR levels (P < 0.05). In addition, there were no obvious differences in the levels of serum LDL, INS and IGF-I among all groups.

Antioxidant capacity
There were no differences in the antioxidant indices of T-AOC, GSH-Px and MDA levels in the serum and breast among all groups (Table 5). However, compared with the control treatment, diets supplemented with organic Cr did not affect the liver MDA levels on day 42, whereas MDA content was significantly decreased in broilers fed a diet supplemented with CrMet compared with CrPic (P < 0.05).

Amino acid profiles of broilers
Table 6 shows that methionine and cysteine levels in breast were significantly affected by Cr supplementation, unlike the content of the other amino acids in the breast. Compared with the control group, CrNic and Cr-yeast supplementation significantly increased methionine levels in the breast of broilers (P < 0.05), with Cr-yeast being superior to CrNic (P < 0.05). Besides, Cr-yeast supplementation significantly increased cysteine levels in the breast of broilers compared with the other four groups (P < 0.05). However, no differences were noted across all experimental groups in the total amount of essential amino acid (EAA).

Fatty acid profiles of breast
As shown in Table 7, there were no differences in the saturated fatty acid (SFA) contents except for C12:0 and C20:0 in the breast of broilers. Compared with the control group, diets supplemented with CrNic, CrMet and CrPic significantly decreased C12:0 content (P < 0.05). In addition, broilers fed CrPic-treated diet had lower C20:0 levels compared with the control group, CrMet group, and Cr-yeast group (P < 0.05). However, no differences were observed in the total amount of SFA, monounsaturated fatty acid (MUFA), PUFA, and n-6 PUFA among all groups. Compared with the control group, the PUFA/SFA ratios and total amount of n-3 PUFA were significantly increased with Cr supplementation, except for the Cr-yeast addition (P < 0.05). The CrNic- and CrMet-treated groups had significantly decreased ratios of n-6/n-3 PUFA compared with the control group (P < 0.05). Conversely, there were negligible differences across the various Cr-treated groups for PUFA/SFA ratios, n-3 PUFA content and n-6/n-3 PUFA ratios.

Table 5. Effects of organic chromium sources on antioxidant status in serum, liver and breast of broilers

| Item          | Control | CrNic | CrMet | CrPic | Cr-yeast | SEM   | P-value |
|---------------|---------|-------|-------|-------|----------|-------|---------|
| **Day 21**    |         |       |       |       |          |       |         |
| Serum         |         |       |       |       |          |       |         |
| GSH-Px (μM)   | 703.98  | 730.97| 714.69| 705.31| 760.62   | 25.84 | 0.96    |
| T-AOC (μM)    | 10.50   | 10.50 | 9.75  | 9.70  | 10.11    | 0.23  | 0.70    |
| MDA (μM)      | 3.96    | 4.19  | 3.73  | 3.80  | 4.54     | 0.15  | 0.48    |
| Liver         |         |       |       |       |          |       |         |
| GSH-Px (μM)   | 714.28  | 588.29| 612.01| 663.58| 558.12   | 23.00 | 0.22    |
| T-AOC (μM)    | 7.87    | 8.09  | 8.23  | 7.84  | 8.52     | 0.23  | 0.89    |
| MDA (μM)      | 2.45    | 2.41  | 2.32  | 2.24  | 2.29     | 0.05  | 0.73    |
| Breast        |         |       |       |       |          |       |         |
| GSH-Px (μM)   | 426.77  | 390.59| 413.03| 438.78| 441.58   | 7.44  | 0.16    |
| T-AOC (μM)    | 5.06    | 5.20  | 5.69  | 5.71  | 4.79     | 0.19  | 0.52    |
| MDA (μM)      | 3.59    | 2.95  | 2.75  | 2.36  | 3.00     | 0.14  | 0.07    |
| **Day 42**    |         |       |       |       |          |       |         |
| Serum         |         |       |       |       |          |       |         |
| GSH-Px (μM)   | 782.89  | 815.19| 783.48| 809.59| 816.96   | 10.48 | 0.74    |
| T-AOC (μM)    | 9.54    | 9.21  | 8.69  | 8.98  | 8.49     | 0.22  | 0.61    |
| MDA (μM)      | 4.95    | 3.68  | 3.89  | 4.20  | 4.48     | 0.20  | 0.31    |
| Liver         |         |       |       |       |          |       |         |
| GSH-Px (μM)   | 729.07  | 620.46| 653.63| 653.15| 675.12   | 19.70 | 0.57    |
| T-AOC (μM)    | 7.82    | 7.23  | 7.58  | 6.27  | 7.20     | 0.19  | 0.08    |
| MDA (μM)      | 3.15ab  | 2.95ab| 2.24b | 3.47a | 2.60ab   | 0.13  | 0.02    |
| Breast        |         |       |       |       |          |       |         |
| GSH-Px (μM)   | 3.62    | 3.42  | 3.65  | 3.23  | 3.00     | 0.11  | 0.32    |
| T-AOC (μM)    | 421.02  | 413.94| 414.98| 396.47| 461.78   | 8.38  | 0.13    |
| MDA (μM)      | 3.86    | 4.19  | 3.84  | 4.45  | 5.07     | 0.19  | 0.25    |

Values with different letters (a, b) in the same row differ significantly (P < 0.05). CrNic, chromium nicotinate; CrMet, chromium methionine; CrPic, chromium picolinate; Cr-yeast, chromium yeast; SEM, standard error of mean; GSH-Px, glutathione peroxidase; T-AOC, total antioxidant capacity; MDA, malondialdehyde.

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DISCUSSION

In the present study, the broilers were reared under normal conditions without any obvious stress factors, similar to local industry conditions. The results indicated that supplementation with different Cr sources at a level of 400 μg kg⁻¹ in broiler diets had no impact on broiler growth performance (ADG, ADFI and FCR) throughout the experimental periods. This result is consistent with those of some previous studies. Zheng et al.³³ did not observe an effect of Cr supplementation on growth performance for Cobb 500 broilers fed diets supplemented with 400 or 2000 μg kg⁻¹ Cr in the forms of Cr propionate, Cr chloride (CrCl₃) or CrPic. In addition, Naghieh et al.³² reported no differences in body weight gain and FCR of broilers that received Cr-treated diets at the level of 600 μg kg⁻¹ Cr in the form of CrNic, CrMet or Cr-yeast. Sahin et al.³³ showed that growth performance was not affected in Ross 308 broilers fed diets containing 200 μg kg⁻¹ Cr in the form of CrPic or Cr histidinate. Sukombat et al.³⁴ demonstrated no differences in growth performance among treatments of broilers fed Cr-treated diets with 200–800 μg kg⁻¹ Cr in the form of Cr-yeast, CrPic and CrCl₃, respectively. Safwat et al.³⁵ also demonstrated no differences in Arbor Acres broiler growth performance in the CrMet group and Cr-yeast group under normal conditions. Under heat stress conditions, positive effects of Cr on broiler performance were reported in the form of CrPic and Cr histidinate, and the results of the study showed that Cr histidinate was superior to CrPic.³³ Toghyani et al.³⁶ found that Cr supplementation in the form of CrNic and CrCl₃ in broiler diets significantly increased feed intake and body mass. Conversely, FCR was not influenced by dietary Cr treatments under heat stress conditions.³³ The extensive differences in outcomes might be attributed to the Cr sources or temperature variations between the studies.

Chromium plays a pivotal role in carbohydrate and lipid metabolism since it has been effectively involved in improving glucose tolerance and alleviating insulin resistance.¹⁴,³⁷ The mechanisms through which Cr regulates glucose metabolism are not fully elucidated. It has been reported that Cr could enhance glucose transporter-4 translocation and insulin-stimulated glucose transport, which might be induced by decreasing circulating membrane cholesterol levels and thereby positively affecting membrane fluidity.³⁷,³⁸ In addition, Cr reportedly regulated lipid metabolism by decreasing the enzymatic activity of fatty acid synthase and hormone-sensitive lipase.¹⁵ Zheng et al.³¹ demonstrated that diets supplemented with Cr propionate, CrPic and CrCl₃ did not affect serum levels of TC, HDLC and LDL, but increased serum TG content in Cobb 500 broilers. Sahin et al.³³ indicated that diets supplemented with CrPic and Cr histidinate did not influence serum CHO levels under thermoneutral conditions, but decreased serum CHO levels under heat stress, with Cr histidinate proving to be superior to the CrPic group in Ross 308 broilers. Safwat et al.³⁵ reported that, compared with the control group, serum TG, CHO and low-density lipoprotein content was decreased and high-density lipoprotein content was elevated in broilers fed diets containing 500 and 1000 μg kg⁻¹ Cr in the form of CrMet or Cr-yeast, with no significant difference between the CrMet group and Cr-yeast group. In the present study, compared with the control group, the broilers fed CrNic-treated diets increased serum HDLC content on day 21, and the CrNic was more effective than CrMet. The broilers fed diets supplemented with CrPic and Cr-yeast decreased serum TG content on day 42, and

![Table 6. Effects of organic chromium sources on amino acid profiles in breast muscle of broilers (dry matter basis, %)](https://www.soci.org)
Cr-yeast decreased serum HDLC and CHO content on day 42, compared with the control group. Furthermore, on days 21 and 42, serum hormones of the INS and IGF-I contents were not affected by dietary supplementation with organic Cr. Therefore, the present study indicated that different organic Cr sources could effectively regulate lipid metabolism, but there were discrepancies in the lipid parameters. Chromium can facilitate the binding of insulin to membrane receptors and increase the insulin function. However, further research is required to explain the pathways by which Cr potentiates insulin function and contributes to lipid metabolism. In addition, cortisol, which releases from the adrenal gland, was induced by stress factors through the hypothalamus–pituitary–adrenal axis; hence cortisol is considered an indicator of stress conditions in broilers. In the present study, diets supplemented with Cr-yeast decreased broiler serum COR content on days 21 and 42, being obviously superior to the CrNic group, which confirmed that the diet supplemented with Cr-yeast could alleviate the detrimental effects of physiological stress. Cr-yeast was superior to other Cr forms in the reduction of serum COR content. This result could be related to the fact that the Cr-yeast is produced by culturing yeast cells in a trivalent Cr-containing medium. Yeast may have positive effects on the regulation of inflammatory processes.

The antioxidant status is critical for maintaining animal health and can be influenced by nutrients. Antioxidant capacity includes antioxidant enzyme activities, such as GSH-Px, and non-enzymatic compounds, such as GSH. GSH-Px helps to control the level of hydrogen peroxide and lipid peroxides produced during the redox reaction of metabolic activities, and then inhibits the formation of free radicals. In addition, T-AOC and MDA are also vital indices for evaluating antioxidant status in animals. T-AOC represents the capacity of free radical scavenging by the radical-scavenging antioxidants contained in the samples and indicates the oxidative status of the whole body. MDA is another marker of oxidative damage.

### Table 7. Effects of organic chromium sources on fatty acid profiles in breast muscle of broilers (dry matter basis, %)

| Items | Control | CrNic | CrMet | CrPic | Cr-yeast | SEM | P-value |
|-------|---------|-------|-------|-------|----------|-----|---------|
| SFA   |         |       |       |       |          |     |         |
| C12:0 | 0.08a   | 0.05b | 0.05b | 0.06b | 0.07ab   | <0.01| 0.02    |
| C14:0 | 0.33    | 0.32  | 0.31  | 0.33  | 0.32     | 0.01| 0.90    |
| C15:0 | 0.10    | 0.09  | 0.10  | 0.11  | 0.10     | 0.00| 0.10    |
| C16:0 | 22.00   | 21.68 | 21.32 | 21.51 | 21.73    | 0.14| 0.66    |
| C17:0 | 0.03    | 0.03  | 0.03  | 0.03  | 0.03     | <0.01| 0.40    |
| C18:0 | 12.90   | 12.35 | 12.82 | 12.33 | 12.46    | 0.29| 0.96    |
| C20:0 | 0.39a   | 0.28ab| 0.37a | 0.20b | 0.39a    | 0.02| 0.01    |
| C21:0 | 0.83    | 0.82  | 0.88  | 0.91  | 0.92     | 0.03| 0.80    |
| C22:0 | 0.23    | 0.19  | 0.20  | 0.18  | 0.17     | 0.01| 0.21    |
| C24:0 | 0.12    | 0.11  | 0.13  | 0.12  | 0.12     | 0.01| 0.95    |
| MUFA  |         |       |       |       |          |     |         |
| C16:1 | 1.63    | 1.83  | 1.69  | 1.62  | 1.79     | 0.09| 0.93    |
| C18:1n9c | 24.35  | 24.14 | 23.54 | 24.00 | 24.37    | 0.40| 0.97    |
| C20:1 | 0.28    | 0.24  | 0.25  | 0.26  | 0.24     | 0.01| 0.24    |
| C24:1 | 0.32    | 0.25  | 0.29  | 0.28  | 0.25     | 0.01| 0.27    |
| n-3 PUFA |       |       |       |       |          |     |         |
| C18:3n3 | 1.07  | 1.35  | 1.35  | 1.30  | 1.25     | 0.05| 0.33    |
| C20:3n3 | 0.06  | 0.08  | 0.09  | 0.08  | 0.09     | 0.01| 0.59    |
| C20:5n3 | 0.32  | 0.31  | 0.36  | 0.32  | 0.40     | 0.01| 0.23    |
| C22:6n3 | 0.83  | 0.98  | 1.08  | 0.96  | 0.81     | 0.05| 0.44    |
| n-6 PUFA |       |       |       |       |          |     |         |
| C18:2n6c | 24.81 | 26.69 | 26.14 | 27.15 | 26.73    | 0.28| 0.06    |
| C20:3n6 | 1.00  | 1.02  | 0.97  | 1.00  | 1.00     | 0.03| 0.98    |
| C20:4n6 | 7.72  | 7.19  | 7.37  | 7.27  | 7.56     | 0.27| 0.97    |
| SFA   | 37.21   | 35.93 | 36.28 | 35.78 | 37.22    | 0.32| 0.48    |
| MUFA  | 26.88   | 26.46 | 26.85 | 26.16 | 27.06    | 0.44| 0.97    |
| PUFA  | 36.36   | 37.61 | 37.96 | 38.06 | 37.79    | 0.28| 0.37    |
| PUFA/SFA | 0.97b | 1.05a | 1.05a | 1.06a | 1.02ab   | 0.01| 0.01    |
| n-3 PUFA | 2.36b | 2.70a | 2.85a | 2.64a | 2.62ab   | <0.01| 0.01    |
| n-6 PUFA | 34.07 | 34.90 | 35.11 | 35.41 | 34.75    | 0.24| 0.54    |
| n-6/n-3 PUFA | 14.26a | 12.94b | 12.36b | 13.43ab | 13.46ab | 0.18| <0.01    |

Fatty acid concentrations were expressed as percentages of the total identified fatty acids. CrNic, chromium nicotinate; CrMet, chromium methionine; CrPic, chromium picolinate; Cr-yeast, chromium yeast; SEM, standard error of means; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Values with different letters (a, b) in the same row differ significantly (P < 0.05).
of oxidative stress, which is one of the final products of polyunsaturated fatty acid peroxidation in the cells. The level of MDA was negatively correlated with the activity of GSH-Px. Chromium has positive effects on antioxidant status. The enhanced antioxidant status in animals with Cr supplemented diets has previously been reported by Rao et al., who supplemented organic Cr in Cobb 400 broiler diets, and Machac et al., who supplemented Cr histidine in cat diets. Tang et al. identified that diets supplemented with 200 μg kg⁻¹ Cr in the form of Cr-yeast, Cr-Pic or CrMet did not affect the GSH-Px capacity and MDA content in broiler breast and leg muscles. Tang et al. found that the T-AOC content increased in the leg muscle of broilers fed diets containing 200 μg kg⁻¹ Cr from CrMet, which was much more effective than Cr-yeast and CrPic in the leg. Safwat et al. demonstrated that serum MDA content was decreased in CrMet- or Cr-yeast-supplemented groups of broilers. In the current study, GSH-Px, T-AOC and MDA content did not vary in the serum and breast of broilers fed diets supplemented with organic Cr; conversely, lower MDA level on day 42 was lower in the CrMet group than the CrPic group. This could be attributed to the chelation of methionine, which, as an antioxidant, eliminated reactive oxygen species by methionine residues or through GSH synthesis. Therefore, it was recognized that diets supplemented with CrMet could improve the liver antioxidant status of broilers. However, it is unclear whether Cr and methionine had synergistic effects on the body antioxidant reaction. Therefore, the interaction of metal Cr and chelation in the body mechanism remains unknown.

Chromium plays an important role in protein metabolism. It has been demonstrated that Cr could elevate amino acid incorporation and transportation in rat heart or skeletal muscle cells. Tian et al. reported that diet supplemented with CrMet had no impact on amino acid composition in growing–finishing pig muscle. Tian et al. showed that, compared with the control group, diet supplemented with CrPic decreased alanine content in the breast of broilers, and broilers fed diets supplemented with Cr-yeast had much higher content of aspartic acid, glycine and valine in broiler breast compared to broilers fed diets supplemented with CrPic. In the present study, no significant differences were observed in the amino acid content in breast, except for methionine and cysteine, among all broiler groups. However, broilers fed a diet supplemented with Cr-yeast had increased methionine and cysteine levels compared with the control group, and Cr-yeast was significantly effective than CrNic in increasing methionine levels. The possible reason for the Cr-yeast group having higher content of methionine and cysteine may be that the active composition of yeast had synergistic effects on Cr, greatly stimulating glucose consumption. Moreover, it is likely that Cr-yeast showed a similar absorption mechanism to that of selenium yeast, which was absorbed by an active transport mechanism via methionine transporters and then entered the body’s methionine pool.

The fatty acid content in meat usually determines the meat nutritive value and potentially influences its storage or further processing. Meat enrichment with n-3 PUFA or the balance of n-6/n-3 PUFA in broiler chickens is essential for consumer acceptance. It was demonstrated that excessive n-6 PUFA and SFA increased the risk of pathogenesis of many diseases, such as cardiovascular diseases and cancer incidence, which could be reduced by higher n-3 PUFA levels (or a lower n-6/n-3 ratio). The n-3 PUFA members mainly include α-linolenic acid (C18:3n3), eicosapentaenoic acid (C20:5n3) or docosahexaenoic acid (C22:6n3), which could enhance body immune functions.

Nejad et al. indicated that diet supplemented with CrMet did not affect the SFA content, unsaturated fatty acid (UFA) content or the ratios of UFA/SFA, MUFA/SFA and PUFA/SFA, but increased PUFA value in loin side beef in Holstein steers. In finishing pigs, Tian et al. showed that diets supplemented with 100–800 μg kg⁻¹ Cr from CrMet did not affect PUFA values in belly fat. Conversely, Jin et al. observed that diet containing 200 μg kg⁻¹ Cr from CrMet increased PUFA content in the longissimus thoracis muscle. To the best of our knowledge, the available information on broiler tissues is limited to the effect of Cr supplementation on the fatty acid profiles of broilers. Diets supplemented with CrPic had no influence on PUFA content but decreased the n-6/n-3 PUFA ratio in broiler breast. In the present study, the breast n-3 PUFA values were significantly increased by 14.41%, 20.76% and 11.86% in broilers fed diets containing CrNic, CrMet and CrPic, respectively. The n-6/n-3 PUFA ratios significantly declined by 9.26% and 13.32% in the breast of broilers provided with CrNic and CrMet in their diets, respectively. Although there were no effects on PUFA/SFA ratios, n-3 PUFA content and n-6/n-3 PUFA ratios among the four Cr-treated groups, the CrMet group was much more effective both in increasing n-3 PUFA and decreasing the n-6/n-3 PUFA ratio. Therefore, the superiority of CrMet could be attributed to the fact that the synergetic effects between methionine and Cr might be much more effective in regulating lipid metabolism. However, the underlying mechanism remains unclear and requires further research.

CONCLUSIONS

Diets supplemented with organic Cr at a dosage of 400 μg kg⁻¹ could regulate lipid metabolism and improve the amino acid and fatty acid profiles in the breast meat of Arbor Acres broilers. Moreover, Cr-yeast was an effective source in improving methionine and cysteine content, whereas CrMet was more effective than CrNic in increasing the n-3 PUFA value and decreasing the n-6/n-3 PUFA ratio in the breast meat and was effectively strengthen liver antioxidant ability than CrPic.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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