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The Effect of Various Levels of Chromium on Growth, Carcass, Immunity, Blood, Liver enzymes, Cecal Microbiota, Sensory Quality, and Fatty Acid Profile Traits in Broiler Chicks

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Abstract

This experiment was conducted to investigate the effects of three levels of chromium (Cr) (0, 700, and 1400 µg/kg of diet) on growth performance, carcass characteristics, immunity, blood parameters, cecal microbial flora, meat taste, and fatty acid profile of Arbor Acres commercial broilers. 120 one-day-old male chicks with four replicates and 10 chicks per replicate were used in a completely randomized design for 42 days. The results of the experiment showed that the lowest cost per kg of live chicken and the best European production index, without statistically significant difference, was demonstrated in Cr700. The growth performance in the whole period (1-42d) was not affected by Cr ($P > 0.05$). The amount of antibody titer against Newcastle virus, influenza, and sheep red blood cells was affected by experimental treatments ($P < 0.05$). The data showed that the percentage of saturated fatty acids such as myristic acid, palmitic acid, and stearic acid decreased and the amount of unsaturated fatty acids increased in Cr700 and Cr1400 compared to the control group. The fat content, juiciness, color, chewing ability, elasticity, oral sensation, and general acceptance were affected by using Cr ($P < 0.05$). Also, the data showed an improvement in cecal microbial flora in broilers fed Cr. In conclusion, it is recommended to feed 700 µg/kg Cr Arbor Acres farming.

Introduction

The broiler industry plays a key role in producing meat and providing protein to humans. Considering that the cost of fed represents 60-70% of broiler production costs, it is very important to pay attention to nutritional programs and preparing rations that lead to the maximum profit from broiler production (Zhao *et al*., 2010). Although the requirement for some nutrients such as mineral elements is low, their deficiency leads to disruption of essential reactions, disruption of the metabolic balance of the body, reduction of the qualitative value of nutrients, and reduction of feed efficiency (Oviedo-Rondon *et al*., 2013). Since some of the minerals in commercial diets are unavailable to birds due to the presence of anti-nutritional substances such as phytate (Yan *et al*., 2001), low-requirement minerals may be added to the diet in the form of oxide, sulfate, carbonate, or complexed with amino acids to facilitate the growth potential of birds (Bao *et al*., 2010; Zhao *et al*., 2010). These forms of mineral elements in the diet are excreted in the environment due to the low level of absorption and lead to environmental pollution (Vieira, 2008). Therefore, the use of chelated forms of these elements, in addition to solving the challenges of their mineral forms, will lead to increasing the availability of nutrients in the diet and, in turn, increasing the performance of broilers (Świątkiewicz *et al*., 2014).

Chromium (Cr) is one of the ultra-trace minerals required for birds, although the requirement of broilers for this element has not been reported by the National Research Council (NRC, 1994), but its necessary role in insulin function and improving the efficiency of protein, fat and glucose metabolisms have been proven by researchers (Pechova and Pavlata, 2007). Supplemental dietary Cr as Cr-picolinate (CrPic) alters glucose metabolism and reduces mortality in broiler chicks. Cr is necessary for the synthesis of low molecular weight Cr binder (LMWCr), which after conversion to chromodulin, activates the insulin

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signaling cascade, which leads to greater cell response to insulin and subsequently has a positive effect on the metabolism of lipids, proteins and carbohydrates (Moeini *et al*., 2011). By increasing the activity of insulin, chromium leads to the absorption of more amino acids into the cell for protein production (Li *et al*., 2013). In addition, Cr supplementation increases serum total protein, albumin, and insulin and decreases the concentration of corticosterone and cholesterol in the blood (Moeini *et al*., 2011). Corticosterone reduction has a positive effect on carcass quality in broilers because corticosterone can affect protein synthesis in muscles (Uyanik *et al*., 2002). It has been reported that different sources of chromium, including chromium methionine, cause an increase in feed intake, feed efficiency, and body weight in broilers (Safwat *et al*., 2020). Cr-methionine as an amino acid complex is a source of Cr with high bioavailability which results in an increased rate of glucose clearance in animals (Emami *et al*., 2015). In addition, it increases the performance and decreases corticosterone and cholesterol in broilers exposed to heat stress (Li *et al*., 2013; Silva *et al*., 2019). In addition, previous studies showed that Cr supplementation significantly improved growth performance, carcass characteristics, and antioxidant capacity in broilers (Toghyani *et al*., 2012; Huang *et al*., 2016). Cr deficiency causes an increase in corticoids and thus decreases serum protein concentration and immune system function, increases glucose concentration, and decreases the use of glucose by peripheral tissues. Therefore, Cr supplementation causes the secretion of corticosteroids and increases glucose metabolism by increasing glycogenolysis and accelerating glucose transport (Rosebrough and Steele, 1981). In many studies, the beneficial effect of chromium supplementation on improving the immune system, growth rate, improving meat and carcass quality has been shown (Borgs and Mallard, 1998; Sahin *et al*., 2003; Toghyani *et al*., 2007; Rouhalamini *et al*., 2014).

Also, Madamanchi *et al*. (2005) stated that the increase of free radicals causes oxidation and destruction of biological cells. Therefore, it can cause many disturbances in the vital tissue (Sahin *et al*., 2003; Ocak *et al*., 2008). Antioxidants, including the chelated form of Cr, with their ability to effectively scavenge free radicals, may reduce problems associated with intestinal disorders and improve functional characteristics (Wang *et al*., 2012). Therefore, the effect of various sources of Cr with antioxidant properties on carcass characteristics, growth performance and digestive organs, immune system, blood parameters, cecal microbial flora and breast meat fatty acid profile can be different in various physiological conditions. There are conflicting reports about the effect of antioxidant compounds on the stated factors and limited reports about the application of the chelated form of Cr in the broiler diet. Therefore, the purpose of this study is to study the effect of different levels of Cr on growth performance, carcass characteristics, digestive organs, immunity, blood parameters, liver enzymes, cecal microbial flora, evaluation of meat taste, and fatty acid profile of breast meat of Arbor Acres commercial strain broilers.

Materials and Methods

This study was conducted on a broiler farm in Rasht, Iran. In total, 120 one-day-old male chicks of the commercial strain of Arbor Acres with an average weight of 45±2 grams were completely randomized to three treatments, four replications with 10 chickens per replication in a broiler rearing farm. Treatments included (1) control without Cr supplementation (Cr0), (2) 700 µg/kg DM Cr (Cr 700), and (3) 1400 µg/kg DM Cr (Cr1400). The chromium supplement was chromium complexed with methionine which is highly available.

A basal diet was prepared according to Arbor Acres broiler nutrition specifications (2019) for starter, grower, and finisher periods (Table 1). Broiler chicks were reared in 1 × 1 m^2 cages with cellulose pellet litter. Each cage contained a separate drinker and feeder. The temperature gradually decreased from 33°C to 23°C over an 18th day and remained constant thereafter. The humidity of the room was maintained at 65-70% during the study period and the chicks were exposed to light for 23 hours and dark for one hour. Feed and water were available *ad-libitum* throughout the study. All birds were vaccinated against infectious bronchitis (1st and 12nd days of age), Newcastle disease (1st, 7th, 21st, and 35th days of age), infectious bursal disease (16th days old), and influenza (1st day of age). All vaccines were from Razi Vaccine and Serum Institute (Karaj, Iran).

The body weight of broilers was measured at 1, 10, 24, and 42 days of age by a digital scale (A&D GF-300, A&D Weighing Design, and Manufacture, San Jose, California) with an accuracy of ± 0.1 gr. At the end of starter (1-10d), grower (11-24d), and finisher periods (25- 42d), the amount of feed remaining in each feeder was weighed. The feed conversion ratio was calculated by dividing the feed intake by the weight gain for each of the three periods and for the whole period. The European production index (EPI) was calculated as follows (Tavakoli *et al*., 2021):

$$\text{EPI} = \frac{\text{Mean live weight (gr)} \times \text{Survival percentage}}{\text{Feed conversion ratio} \times \text{Number of rearing days} \times 10}$$

The cost of feed consumed per kg of live chicken (C/kg LW) was calculated as follows (Tavakoli *et al*., 2021):

$$\text{C/kg LW} = \frac{\text{Price of feed consumed during 42d for each chicken in Iranian Rials}}{\text{Weight of a chicken per kg at 42d}}$$

The cost of Cr at that time was estimated for each diet separately and included in the formula.

Table 1. Ingredients, chemical composition, and energy of basal diet

Experimental diet	Starter diet (1st-10th days of age)	Grower diet (11st -24th days of age)	Finisher diet (25th-42nd days of age)
Corn	47.03	59.60	65.99
Wheat	5.58	5.00	5.00
Soybean meal (44% crude protein)	29.02	16.15	10.28
Corn gluten	10.00	11.48	11.50
Soy Oil	3.50	3.40	3.09
Limestone	1.45	1.23	1.00
Di-calcium phosphate	1.95	1.80	1.83
Salt	0.20	0.20	0.20
Vitamin and mineral supplements[1]	0.50	0.50	0.50
DL-Methionine	0.52	0.58	0.57
L-Lysine hydrochloride	0.25	0.06	0.04
Calculated analysis			
Metabolizable energy (kcal/kg)	2950	3000	3050
Crude protein (%)	22	20	19
Lysine (%)	1.3	1.2	1.1
Methionine (%)	0.56	0.54	0.52
Met+Cys (%)	0.92	0.90	0.88
Threonine (%)	0.861	0.804	0.722
Tryptophan (%)	0.2	0.185	0.169
Arginine (%)	1.39	1.284	1.177
Isoleucine (%)	0.861	0.800	0.743
Leucine (%)	1.41	1.302	1.187
Valine (%)	1.014	0.936	0.869
Available Phosphorous (%)	0.52	0.47	0.41
Calcium (%)	1.04	0.95	0.92

1. The amount of vitamins and minerals per kg of the final diet: vitamin A, 9000 IU; vitamin D3, 3000 IU; vitamin E, 18 IU; vitamin K3, 3 mg; vitamin B1 (Thiamine), 1. 8 mg; vitamin B2 (Riboflavin), 6 mg; vitamin B6 (Pyridoxine), 3 mg; vitamin B12 (Cyanocobalamin), 0.012 mg; vitamin B3 (Niacin), 30 mg; vitamin B9 (Folic acid), 1 mg; vitamin H3 (Biotin), 0. 24 mg; vitamin B5 (Pantothenic acid), 10 mg; Choline, 100 mg; Mn, 100 mg; Zinc, 80 mg; Iron, 10 mg; Cu, 1 mg; I, 0.2 mg.

Growth performance and economic efficiency

Carcasses characteristics, intestinal components, and digestive organs

At the end of the experiment (42d), two birds from each replicate with a weight near the pen average were selected and slaughtered. The weights of organs were recorded (Shabani *et al*., 2015). Organ Weights taken included; eviscerated carcass, relative weights of breast, drumsticks, thigh, digestive organs (gizzard, heart, fat) abdomen, duodenum, jejunum and ileum, and also the length of duodenum, jejunum, and ileum.

Blood plasma components and liver enzymes

To measure the components of blood plasma and liver enzymes on the 42nd day, three birds with close to the average weight were randomly selected from each replicate, 5 mL of blood was taken from the wing vein at the same time, and the samples were mixed and divided into portions for whole blood using EDTA anticoagulant and portions for serum. Serum separation was performed using a centrifuge, (Sorvall super T21, Du Point Co. USA) in which the samples were transferred to micro-tubes, and the final samples were kept for 12 hours at room temperature and centrifuged for 3 minutes (before separating the serum) at a speed of 2991 × g were centrifuged. The serum was stored in a freezer at -20°C (Emersun freezer, NRF3292D Emersun Co., Tehran, Iran) until further analysis. Analyzes were determined using commercial kits of Pars Azmoun and by autoanalyzer (Hitachi 917, Hitachi; Tokyo, Japan; reagents from Roche Diagnostics, Mannheim, Germany), according to the instructions of commercial kits (RANDOX Laboratories Ltd. Ardmore, Diamond Road, Crumlin, Co. Antrim, UK, BT29 4QY) (Golrokh *et al*., 2016).

The measured metabolites were included the number of white blood cells, the number of red blood cells, hematocrit, hemoglobin, the average volume of red blood cells (MCV), the average hemoglobin in one unit of red blood cells (MCH), the average concentration of hemoglobin per erythrocyte (MCHC), heterophil, lymphocyte, monocyte, eosinophil, triglyceride, cholesterol, total protein, albumin, low-density lipoprotein-cholesterol (LDL-c), very low-density lipoprotein-cholesterol (VLDL-c), high-density lipoprotein-cholesterol (HDL-c), lactate dehydrogenase, and creatine phosphokinase.

Immune responses

Broiler chicks were vaccinated against sheep red blood cells (SRBC) by Lerner's method (Lerner *et al*., 1971) to investigate humoral immunity. Blood samples were collected from three sheep and combined into a glass container containing EDTA to make the suspension. After that 2% SRBC suspension was prepared in PBS and injected into three birds in each replication using one hundred microliters of 2% SRBC solution intravenously through the wing vein at 28 and 36 days.

Then, on the 35th and 42nd days, blood samples were taken from three birds in each replication and pooled and transferred to the laboratory (Seidavi *et al*., 2014). By using Van derzipp hemagglutination method, antibody titer against SRBC was measured (Pourhossein *et al*., 2015).

Newcastle disease antibody titers (NDVs; Live LaSota strain; Vetrina, Zagreb, Croatia) and influenza were measured at 28 and 42 days of age. In this way, blood was collected from three broilers in each replication and collected and pooled. Based on OIE standard, samples were tested for hemagglutination inhibition (HI). 96 well microplates were used for the experiment. After adding 25 µL of PBS to each well, 25 µL of blood serum was poured into the first well of a 96 well plate and its dilution continued until the last well.

In the next step, 25 µL of influenza and Newcastle antigens were added to the wells. A mechanical shaker was used to shake the microplate well for 1 minute and incubated at 25°C for 30 minutes. 25 µL of 1% red blood cells were added into all wells. The microplate was placed on a mechanical shaker for 15 seconds and after that, was kept at 25°C for 30 minutes. The data were recorded to perform the hemagglutination inhibition (HI) test, and a 4-unit antigen (Pasouk, Iran) was used (Seidavi *et al*., 2014). Titers were estimated as log2 of the highest dilution indicating agglutination. 1% red blood cell was obtained from pathogen-free birds. Blood samples were collected from three birds per replicate on 42d. The samples were mixed and tested for white blood cell count and resulting differences. Also, in each repetition, two birds whose weight was close to the average weight were selected and slaughtered (Shabani *et al*., 2015).

Cecal microbial flora

To investigate cecal microbial flora, two birds of each treatment were slaughtered on day 42. The abdominal cavity was opened and the left and right cecum were opened with sterile scissors. Immediately, 1 gr of the cecal contents was removed using a sampler. The contents of each treatment were mixed with 9 mL of diluted physiological serum and transferred to a sterile container. The samples were taken to the laboratory to count *Escherichia coli*, *Coliform*, *Lactobacillus*, and *Clostridium* (Dibaji *et al*., 2014).

To determine the total bacterial counts, VRBL (Violet Red Bile Lactose Agar), WC (Wilkins Chalgren), MRS (Material Research Society Agar), and EMB (Eosin Methylene Blue Agar) agar media were used. Serial dilution in distilled water was used to make 10^{-5}, $10^{-6,}$ and 10^{-7} dilutions to count Escherichia coli, Coliforms, and Lactobacillus bacteria. Dilutions of 10^{-2}, $10^{-3,}$ and 10^{-4} of the cecum contents were used to count clostridia. Afterward, 300 microliters of each dilution, were inoculated on eosin methylene blue agar and then incubated for 24 hours at 37°C to determine the colonies based on colony forming units (CFU). Data were expressed as log2 before statistical analysis (Dibaji *et al*., 2014).

Fatty acid profile of breast meat

To measure the characteristics of fatty acids on the 42nd day, one bird from each treatment was slaughtered and its breast meat sample was taken to the laboratory. At first, the chest muscle was separated and after grinding, they were stored in a freezer at -20°C (Emersun freezer, NRF3292D Emersun Co, Tehran, Iran). The fat content of the muscle was extracted by the Folch method (Folch *et al*., 1957).

Meat sensory taste evaluation

The method of Kim *et al*. (2007) was used for sensory evaluation of meat samples. Based on a combination of indicators of fat content, juiciness, tenderness, color, odor, chewing ability, elasticity, oral sensation, and general acceptance using a five-point hedonic method with a score of 5 points (5; points very good, 4; good, 3; average, 2; bad and 1; very bad). For the sensory evaluation of the samples, a 10-person evaluator group of sensory evaluators and food industry experts was used (Baston and Barna, 2010). The first step was to thaw the samples overnight in the refrigerator, and then from all the repetitions of each experimental treatment, three pieces of meat were cut in dimensions (10×10×10 mm) and cooked. The oven (Electric oven, SI4 854 C IX - F096799, Hamburg, Germany) was used at a temperature of 75 °C. It should be mentioned that to increase the precision and accuracy of the samples, no additives such as salt were added to the samples, and also to neutralize the sensory senses between the samples, bread, and water at normal temperature were used (Komprda *et al*., 2003).

Statistical analysis

Statistical analysis was done using SPSS (2012) statistics software (IBM Corp., New York, USA). The comparison between groups was obtained in the form of a completely randomized design using one-way analysis of variance (ANOVA) and then Duncan's multi-range test $P < 0.05$ was used (Duncan, 1955).

Results and Discussion

Growth performance

The effects of supplementation with various levels of Cr on the growth performance of broilers are reported in Table 2. No statistical difference was observed between the treatment groups on weight gain, feed intake, and feed conversion ratio. But numerically the Cr700 group showed the highest growth rate compared to the control and Cr1400 groups. In such a way that chickens receiving Cr700 showed the lowest amount of feed intake, along with the highest weight gain and the lowest feed conversion ratio in the initial, growth and final periods.

Table 2. Growth performance of broilers fed diets containing different levels of Chromium

Treatment	Feed intake (g/chick/day)	Body weight (g/chick)	Weight gain (g/chick/day)	Feed conversion ratio
		Starter period (1st-10th days of age)		
Chromium (0 µg/kg)	30.6	214.5	17.2	1.78
Chromium (700 µg/kg)	31.5	214.5	17.1	1.84
Chromium (1400 µg/kg)	31.4	219.5	17.7	1.77
SEM	0.393	4.493	0.451	0.035
P-value	0.251	0.679	0.637	0.369
		Grower period (11st-24th days of age)		
Chromium (0 µg/kg)	85.7	701.3	34.7	2.46
Chromium (700 µg/kg)	85.1	727.1	36.6	2.32
Chromium (1400 µg/kg)	85.4	717.9	35.6	2.40
SEM	1.532	13.369	0.775	0.057
P-value	0.961	0.420	0.292	0.270
		Finisher period (25th-42nd days of age)		
Chromium (0 µg/kg)	152.9	2017.5	73.2	2.09
Chromium (700 µg/kg)	141.7	2106.8	76.9	1.84
Chromium (1400 µg/kg)	148.9	2020.7	73.1	2.03
SEM	3.773	44.764	2.225	0.078
P-value	0.162	0.324	0.422	0.111
		Total period (1st-42nd days of age)		
Chromium (0 µg/kg)	100.3	2017.5	46.9	2.14
Chromium (700 µg/kg)	94.6	2106.8	48.9	1.93
Chromium (1400 µg/kg)	95.5	2020.7	46.7	2.04
SEM	2.062	44.764	1.037	0.067
P-value	0.164	0.324	0.312	0.138

SEM= Standard Error of Means.

Contrary to the results of the present study, the addition of Cr supplement improved feed consumption and weight gain and also reduced the feed conversion ratio in broiler chicks (Safwat *et al*., 2020). In accordance with the findings of the present study, in one of the studies (Spears *et al*., 2019), the use of Cr-propionate (Cr Prop) in the broiler diets reduced feed consumption and led to an increase in the weight of male broilers. It has also been reported that Cr sources increase body weight, and feed intake and decrease feed conversion ratio in broiler chicks (Safwat *et al*., 2020). Anandhi *et al*. (2006) showed that broilers fed with a diet supplemented by organic chromium at levels of 250, 500, and 750 µg/kg diet had no significant effect on feed consumption, body weight gain, and feed conversion ratio. Maybe one of the reasons for the no difference in the current study is the Cr levels, El-Kholy *et al*. (2017) showed that dietary Cr supplementation did not affect any of the performance during the whole period, which was consistent with this study.

Cr participates in insulin-sensitive tissues to augment insulin function (Brooks *et al*., 2016). The mechanism of action is that Cr is necessary for the production of low molecular weight Cr binding agent (LMWCr), which after being converted to chromodulin, increases the insulin signaling, which in turn leads to an increase in cell permeability to insulin, and subsequently it affects the metabolism of proteins, lipids, and carbohydrates. Jackson *et al*. (2008) found that supplementation of 0.80 mg/kg of Cr-propionate in the broiler chicks diet reduces feed conversion ratio from 15 to 42 days of age by enhanced glucose consumption by skeletal muscles and liver. Insulin sensitivity and glucose metabolism increase in response to the use of Cr supplements, which reduces gluconeogenesis in the liver and also leads to the growth and preservation of protein by preserving amino acids (Souza *et al*., 2011). Gursoy (2000) reported that organic Cr supplementation in broiler chicks diet improves carcass quality and feed efficiency as well as reduces fat content. In addition, Souza *et al*. (2011) observed an improvement in breast meat weight by adding 0.20 mg/kg Cr yeast to the broiler diets (Souza *et al*., 2011). Contradictory findings among the above-mentioned studies may be partially dependent on factors such as form and source of Cr, complementary method, dosage of Cr, age and type of trial animals, and type of stress or duration caused by it.

The results for the cost of feed intake per kg of live weight in units of dollars and Iranian Rials, chicken weight on day 42 (gr per chicken), and the European production index indicated in Table 3 did not show a significant effect of Cr supplementation, but the best European production index and the lowest feed cost were observed in Cr700 treatment.

Table 3. Economic performance broilers fed diets containing different levels of Chromium

Treatment	Feed cost per kg live weight (Rial/kg)	Feed cost per kg live weight ($/kg)	European production index
Chromium (0 µg/kg)	59580.35	0.23	220
Chromium (700 µg/kg)	54363.14	0.21	246
Chromium (1400 µg/kg)	58515.84	0.22	212
SEM	1577.589	0.006	9.788
P-value	0.097	0.118	0.081

SEM= Standard Error of Means.

Carcass characteristics, carcass fat, and digestive organs

The percentage of the eviscerated carcass, the relative weight of the breast, and drumsticks were not affected by experimental treatments (Table 4). Increasing the amount of supplement consumption from 700 to 1400 µg/kg did not affect other relative weights of the carcass, including the relative weights of abdominal fat, pancreas, gizzard, and heart. In accordance with the findings of the present study, there was no significant effect of Cr supplementation on carcass characteristics (liver, carcass, pancreas, gizzard, and heart) of quails compared to the control group (El-Kholy *et al*., 2017). In addition, Anandhi *et al*. (2006) showed that the carcass performance was not affected by adding organic Cr supplement to the broiler diets at different levels of 250, 500, and 750 µg/kg. In confirmation of these results, Uyanik *et al*. (2002) stated that using different levels of Cr supplementation at 20, 40, 80, and 100 ppm in Japanese quail diets had no significant effect on carcass performance, which is in agreement with the results of the present experiment.

Table 4. Economically relevant carcass characteristics of broilers fed diets containing the different levels of Chromium (% of live body weight)

Treatment	Pancreas	Gizzard	Heart	Eviscerated carcass	Breast	Drumsticks	Abdominal fat
Chromium (0 µg/kg)	0.39	2.31	0.62	82.31	26.84	25.55	1.22
Chromium (700 µg/kg)	0.36	2.49	0.67	82.64	28.69	25.62	1.70
Chromium (1400 µg/kg)	0.34	2.22	0.58	82.48	28.64	24.62	2.05
SEM	0.030	0.110	0.066	2.257	1.179	0.801	0.311
P-value	0.487	0.266	0.687	0.994	0.479	0.628	0.216

SEM= Standard Error of Mean.

The results indicated that numerically the lowest amount of abdominal fat was observed in the control and Cr700 treatments. Since the abdominal fat content has more growth versus other fat content of tissues in poultry bodies in growing and terminal periods, it can be a reliable index for judging the fat content of the total body, because it is directly related to the whole fat content in birds (Chen *et al*., 2018). The researchers found that different levels of Cr-picolinate led to a decrease in abdominal fat in 21-day-old broilers. These researchers reported that Cr supplementation increases insulin activity and, as a result, optimal use of glucose, which leads to a decrease in the serum concentration of non-esterified fatty acids and lipolysis (Chen *et al*., 2018). In other words, Cr can lead to a decrease in abdominal fat content in broilers by rapidly hydrolyzing fats and reducing the activity of enzymes used in metabolism. Furthermore, Cr supplementation inhibited lipogenesis in adipocytes by inhibiting the production of tumor necrosis factor (TNF-alpha) which leads to a decrease in the amounts of glucose transporter type 4 (GLUT-4), lipoprotein lipase (LPL), and acetyl coenzyme A synthetase (Jain *et al*., 2003). Therefore, by reducing in fat content it leads to an increase in the deposition of protein in broiler carcasses (Safwat *et al*., 2020). The contradiction of these results with the findings of the present research can be related to the rearing conditions and the small size of the trial sample.

Using two various levels of Cr had no significant effect on some parts of the intestine (Table 5) such as the relative weight and length of the duodenum, jejunum, and ileum ($P > 0.05$), but in terms of numerically, the weight and relative length of the intestinal parts in the Cr1400 group were lowest compared to the Cr700 group and the control group.

Cr absorption is influenced by various factors (Sirirat *et al*., 2013), it is well known that organic sources of Cr are more absorbable and then mineral sources. Organic Cr is usually found in the form of Cr -L-methionine (Sahin *et al*., 2010; 2017). However, the mechanisms of Cr absorption are not yet well-defined, it is supposed that chelated Cr is absorbed in the first section of the jejunum (Khan *et al*., 2012). With a relative increase in the length and weight of the small intestine in the present study, we can expect more Cr absorption in the intestine of broilers in this study.

Table 5. Intestinal segments of broilers fed diets containing different levels of Chromium

Treatment	Duodenum	Jejunum	Ileum
	Relative weight (% defeather body weight)		
Chromium (0 µg/kg)	1.08	1.83	1.41
Chromium (700 µg/ kg)	0.95	1.91	1.41
Chromium (1400 µg/ kg)	0.90	1.62	1.51
SEM	0.056	0.146	0.122
P-value	0.111	0.390	0.808
	Length (mm)		
Chromium (0 µg/kg)	375.0	967.5	977.5
Chromium (700 µg/kg)	322.5	892.5	937.5
Chromium (1400 µg/kg)	322.5	847.5	890.0
SEM	17.680	41.370	43.120
P-value	0.104	0.173	0.395

SEM= Standard Error of Means.

Blood constitutes and liver enzymes

The results of blood parameters are summarized in Table 6. The result showed that various levels of Cr had no significant effect on the amount of VLDL-c, total protein, and the number of triglycerides, total cholesterol, albumin, hemoglobin, and HTC. Also, these results are not in agreement with our study hypothesis. HDL-c and LDL-c levels were affected by experimental treatments ($P < 0.05$). The lowest level of HDL-c was observed in the Cr1400 treatment and the highest in the control treatment. In addition, the highest level of LDL-c was found in the blood of the broilers receiving the Cr1400 treatment and the lowest in the birds of the control group. In other words, Cr700 treatment showed the level of HDL-c and LDL-c at the average level of the other two groups.

Table 6. Blood constitutes of broilers fed diets containing the different levels of Chromium

Treatment	VLDL-c (mg/dL)	LDL-c (mg/dL)	HDL-c (mg/dL)	Total Protein (g/dL)	Triglycerides (mg/dL)	Total Cholesterol (mg/dL)	Albumin (g/dL)	Hemoglobin (g/dL)	HCT (%)
Chromium (0 µg/ kg)	18.7	25.7 [c]	68.2 [a]	3.5	95.0	117.2	1.81	12.09	32.75
Chromium (700 µg/ kg)	22.0	34.7 [b]	56.5[b]	3.3	110.0	117.0	1.84	11.24	30.25
Chromium (1400 µg/kg)	21.5	47.7 [a]	40.0 [c]	3.4	108.0	114.2	1.88	12.06	32.50
SEM	0.939	2.562	1.412	0.098	4.282	2.913	0.048	0.257	0.717
P-value	0.076	0.001	<0.001	0.434	0.070	0.730	0.566	0.074	0.068

[*] Means within each column of dietary treatments with no common superscript differ significantly at $P < 0.05$.
SEM= Standard Error of Means; VLDL-c = very low-density lipoprotein cholesterol; LDL-c = low density lipoproteins cholesterol; HDL-c = high- density lipoproteins cholesterol; HCT = hematocrit

parameters such as cholesterol and triglycerides. In broilers, Uyanik *et al*. (2002) reported that total serum protein increased at levels of 20, 40, and 80 ppm of Cr, while serum albumin was increased only at the 20-ppm level, and led to a significant decrease in serum cholesterol level, which is contrary to the results of the present study. Cr-methionine supplementation at 500 and 1000 ppb levels in the diet reduced levels of plasma glucose and cholesterol, while triglycerides were not affected (Mirfendereski and Jahanian, 2015).

Cr-chloride supplementation at 30 mg/L of water decreased the serum cholesterol concentration, VLDL-c, LDL-c, glucose, and triglyceride, but enhanced the concentration of HDL-c and total lipids (Taha *et al*., 2013). The results of the effect of Cr consumption on enzymes of the liver are presented in Table 7. Accordingly, the various levels of Cr in the diet of broilers receiving experimental treatments did not affect creatine phosphokinase and lactate dehydrogenase ($P > 0.05$). Creatine phosphokinase level in the Cr700 group was lower than other

The type of Cr in the diet also affects the level of LDL-c in the blood. Contrary to the findings of the present study, organic Cr reduced serum LDL-c, whereas inorganic Cr in broiler diets can enhance serum LDL-c levels (Kani, 2015). Studies have shown that dietary supplementation of Cr-picolinate (800, 1600 or 3200 micrograms of Cr per kg of diet) decreased LDL-c and VLDL-c while enhanced HDL-c in broilers (Lien *et al*., 1999). Similarly, another study reported that, although Cr supplementation led to a decrease in total cholesterol, total lipids, LDL-c and VLDL-c, it increased HDL-c and serum triglyceride (Taha *et al*., 2013), which conflicts with the results of this research.

Sahin *et al*. (2010) reported that with increasing Cr in the broiler diets, the concentration of serum cholesterol was significantly reduced, while the serum total protein concentration increased linearly. In a study by Ebrahimnazhad and Ghanbari (2014), broilers fed with Cr at different levels (800, 1200, 1600, and 2000 µg/kg of diet) reduced some lipid

concentration of liver enzymes (Huang *et al*., 2016). In the Horváth and Babinszky (2018) study, compounds with antioxidant properties prevent damage to the liver and other internal organs by preventing oxidative stress and protecting cell membranes from oxidative stress, which is consistent with the findings in the present experiment.

Damage to the liver tissue can lead to the increase of various enzymes of this organ in the blood plasma (Ajayi and Odutuga, 2004). Also, several studies have reported liver tissue damage and increased experimental treatments, and the level of lactate dehydrogenase in the Cr1400 group was lowest which indicates the normal mechanism of the liver with Cr consumption in fed broilers.

Table 7. Liver enzymes of broilers fed diets containing the different levels of Chromium

Treatment	Creatine phosphokinase (U/L)	Lactate dehydrogenase (U/L)
Chromium (0 µg/kg)	3988.750	472.500
Chromium (700 µg/kg)	3738.750	462.500
Chromium (1400 µg/kg)	4227.250	457.250
SEM	271.642	30.281
P value	0.475	0.937

SEM= Standard Error of Means.

Immune system

The results of blood cells related to the immune response are presented in Table 8, comparing the mean white and red blood cell results affected by the experimental treatments ($P < 0.05$). Broiler chickens receiving control treatment showed the highest amount compared to chickens receiving Cr1400 and Cr700 treatments. The results demonstrated that the percentage of monocytes and eosinophils was not affected by the experimental treatments ($P > 0.05$). However, numerically, the percentage of monocytes and eosinophils in the Cr700 and Cr1400 groups was lower than the control group. The percentage of lymphocytes and heterophils was affected by different Cr levels ($P < 0.05$), and dietary Cr decreased the percentage of heterophil cells. Cr1400 and Cr700 treatments had the highest percentage of lymphocytes compared to the control treatment ($P < 0.05$).

Table 8. The immune response of broilers fed diets containing different levels of Chromium

Treatment	White blood cells ($n \times 10^3$/mL)	Red blood cells ($n \times 10^6$/mL)	Heterophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
Chromium (0 µg/kg)	10475.000 [a]	2366250.000 [a]	41.750 [a]	54.250 [b]	3.000	1.000
Chromium (700 µg/kg)	8575.000 [b]	2211250.000 [b]	33.000 [b]	63.750 [a]	2.250	1.000
Chromium (1400 µg/kg)	7775.000 [b]	2302500.000 [ab]	31.000 [b]	66.750 [a]	1.750	0.500
SEM	316.338	37495.370	1.498	1.493	0.391	0.441
P-value	0.001	0.048	0.002	0.001	0.129	0.664

Means within each column of dietary treatments with no common superscript differ significantly at $P < 0.05$.
SEM= Standard Error of Means.

El-Kholy *et al*. (2017) noted that the parameters of Hb, PCV, or H/L ratio were not affected by Cr addition to Japanese quail diets. According to these results, Toghyani *et al*. (2007) stated that PCV and hemoglobin values were not affected by Cr addition (0, 500, 1000, and 1500 ppb). These findings compared with Kani (2015), who reported that hemoglobin (HB) was not influenced by dietary Cr. In broiler chickens, the addition of Cr propionate to drinking water or feed intake improves the immune response by regulating the expression of interferon-gamma (IFN-γ) after vaccination against the R2B strain of Newcastle disease (ND) (Ghazi *et al*., 2012).

Rama Rao *et al*. (2012) hypothesized that organic Cr supplementation in the broiler diets does not affect the ratio of heterophils and lymphocytes and the relative weight of immune system organs including the spleen, thymus, and bursa, as well as the production of antibodies against Newcastle disease (ND) vaccination. Nevertheless, the proliferation ratio of lymphocytes was affected by the addition of Cr in the diet. In addition, Rajalekshmi *et al*. (2014) reported that the lymphoid organ weight was not significantly affected by the Cr-propionate supplementation in the diet of male broilers during the whole experiment (42 days of age). Increasing Cr levels increased the antibody responses in contrast to the lymphocyte proliferation ratio response with ND vaccination. The addition of Cr methionine supplement to the diet of broiler chickens increased the level of antibodies against IBV and NDV, also increased lymphocytes, and decreased the ratio of heterophils to lymphocytes at 800 ppb levels of the diet (Ebrahimzadeh *et al*., 2012; Rouhalamini *et al*., 2014).

Bahrami *et al*. (2012) used 250 broiler chickens to investigate the effect of Cr-methionine

supplementation on the immune response of broiler chickens under heat stress conditions and showed that the antibody titer against Newcastle disease in broilers at the age of 18 and 30 days was increased with organic Cr supplements. Heterophil to lymphocyte ratio was decreased in birds receiving Cr-methionine supplementation compared to other experimental treatments. The albumin-to-globulin ratio was not affected by treatments at 28 days, but increased at 1200 ppb CrCl3 treatment at 42 days, while lymphoid organ weights were not affected by experimental treatments (Bahrami *et al*., 2012).

The results of the immune system indicated that the antibody titer against Newcastle virus in the initial response was affected by the experimental treatments, and the Cr700 treatment showed the highest level compared to the Cr1400 treatment and the control group, although the Cr700 and Cr1400 groups could not be separated. However, the antibody titer against Newcastle virus in the secondary response was not affected by the experimental treatments. Also, the amount of antibody titer against influenza in the initial response was affected by experimental treatments, and the Cr1400 treatment showed the highest amount of antibody titer ($P < 0.05$). The investigation of SRBC antibody titer in the initial response was not affected by the experimental groups ($P > 0.05$), while in the second injection, chickens receiving Cr700 produced the lowest amount compared to the control group and Cr1400 (Table 9).

Table 9. Antibody titer of broilers fed diets containing different levels of Chromium

Treatment	Antibody titer against first injection of Newcastle (28th day of age) (lg2)	Antibody titer against first injection of Newcastle (42nd day of age) (lg2)	Antibody titer against Influenza (28th day of age) (lg2)	Antibody titer against Influenza (42nd day of age) (lg2)	Total antibody against Sheep Red Blood Cell (35th day of age) (IgT)	Total antibody against Sheep Red Blood Cell (42nd day of age) (IgT)
Chromium (0 µg/kg)	1.000 [b]	1.500	2.003 [b]	2.500	2.000	2.500 [a]
Chromium (700 µg/kg)	2.000 [a]	1.500	2.003 [b]	2.500	1.500	1.000 [b]
Chromium (1400 µg/kg)	1.500 [ab]	1.500	4.025 [a]	1.500	1.500	2.500 [a]
SEM	0.167	0.289	0.015	0.441	0.236	0.408
P value	0.007	1.000	<0.0001	0.234	0.274	0.044

Means within each column of dietary treatments with no common superscript differ significantly at $P < 0.05$.
SEM= Standard Error of Means.

Immunological functions are increased with trivalent Cr and its effect also seems to be greater under stress conditions. It has been reported that broiler chickens under heat stress conditions have lower levels of total circulating antibodies, specific levels of IgG and IgM during primary and secondary humoral responses, as well as weight loss of thymus, bursa, spleen, and liver (Bartlett and Smith, 2003). The use of Cr methionine supplement at the level of 800 ppb can increase the immune responses against infectious bronchitis (IB) and Newcastle disease in broilers under heat-stress conditions (Ebrahimzadeh *et al*., 2012). Cr-picolinate (CrPic) supplementation in drinking water or feed increases immune response by increasing interferon-gamma expression after ND vaccination in broilers (Bhagat *et al*., 2008).

Investigating the immune response of broilers receiving diets containing different levels of Cr-methionine (CrMet) under heat stress conditions showed that antibody titers against NDV and IBV at the ages of 21 and 42 days were higher in broilers fed with CrMet supplement than in broilers receiving control treatment ($P < 0.05$). An increase in the number of lymphocytes and as a result a decrease in the ratio of heterophil to lymphocyte was observed in broilers fed with the 800 ppb Cr in 21 and 42 days. The lymphatic organs of broilers were not significantly affected by CrMet supplementation. The findings show that a Cr-methionine (CrMet) feed supplement at the level of 800 ppb can improve the immune system of broilers under heat-stress conditions (Ebrahimzadeh *et al*., 2012). The exact mechanism by which the immune system is improved by Cr is not known. However, one finding that has been shown is that Cr reduces serum cortisol levels. A reduction in serum cortisol levels is an- important mechanism leading to decreased immunity. Cortisol, the most important glucocorticoid, is known to suppressor the immune system, inhibiting the production and function of antibodies, the function of lymphocytes, and the leukocytes population (Roth and Kaeberle, 1982; Munck *et al*., 1984).

Meat sensory taste evaluation

The effect on meat taste evaluation showed that fat content, juiciness, color, chewing ability, elasticity, oral sensation, and general acceptance were influenced by experimental groups ($P < 0.05$; Table 10). However, the various levels of Cr did not lead to significant differences in odor and tenderness attributes. The fat content in the Cr1400 treatment is the highest compared to the Cr700 treatment and the control groups.

Table 10. Evaluation traits of breast meat in broilers fed diets containing the different levels of Chromium

Treatment	Fat content	Juiciness	Tenderness	Color	Odor	Chewing ability	Elasticity	Oral Sensation	General acceptance
Chromium (0 µg/kg)	3.25 [b]	4.00 [a]	3.25	3.25 [b]	3.00	4.75 [a]	4.50 [a]	4.50 [a]	4.25 [a]
Chromium (700 µg/kg)	2.75 [b]	2.25 [c]	2.25	2.50 [c]	2.75	2.00 [c]	2.75 [b]	3.00 [b]	2.25 [c]
Chromium (1400 µg/kg)	4.25 [a]	3.25 [b]	2.75	4.00 [a]	4.00	3.75 [b]	3.50 [b]	3.00 [b]	3.00 [b]
SEM	0.250	0.204	0.250	0.220	0.363	0.204	0.276	0.289	0.204
P value	0.006	0.001	0.057	0.003	0.083	<0.0001	0.005	0.007	< 0.0001

Means within each column of dietary treatments with no common superscript differ significantly at $P < 0.05$.
SEM= Standard Error of Means.

In the present study, the control treatment showed the highest level of Juiciness compared to the Cr1400 and Cr700 treatments, respectively. Cr increases myosin and actin mRNA, decreases tumor necrosis factor (TNF) production, and enhances cellular signals for insulin recognition by binding to low molecular weight Cr substrates (LMWCr) with anabolic effects, leading to an increase in protein percentage in breast muscle, and inhibiting lipogenesis and increased expression of GLUT-2 (Vincent, 2010; Rama Rao *et al*., 2012; Pan *et al*., 2013; Sahin *et al*., 2017). Increasing the protein content by reducing the denaturation of sarcoplasmic proteins leads to an increase in water concentration in the meat of broiler chickens (Bowker and Zhuang, 2015). Dalólio *et al*. (2021), found that water retention capacity increased by 0.59 mg/kg with CrMet supplementation. This increased water retention by CrMet supplementation and improved performance in the production of frozen processed meat derivatives or in the form and commercialization of fresh meat of broiler chickens. Yang *et al*. (2016) showed higher water retention capacity in broiler chickens slaughtered at 28d which were fed diets containing CrCl3 and probiotic (*Bacillus subtilis*). Moreover, Mir *et al*. (2017) demonstrated that the water retention capacity of broilers fed for 42d with 1.50 mg/kg of Cr-Picolinate supplementation was affected compared to the control treatment. Also, they observed that the increase in sensitivity of insulin due to Cr supplementation enhanced glucose absorption and protein deposition by the cell, and decreased the fat content. This usually happens in the breast tissue that contains 50% of the total protein and approximately 30% of the edible protein in the carcass of newer breeds of broilers. Because of that, there is more water accumulation in the muscle of the breast, which helps to enhance hydration. Variables like color, elasticity, and chewing ability, are parameters of meat quality that can be affected by pH 24h after slaughter (Sterten *et al*., 2009). The findings of the present research demonstrated that the highest amount of color was observed in the Cr1400 treatment and the lowest in Cr700, which is due to pH 24h after slaughter.

Chewing ability was the highest in the control group, and the lowest in the Cr700 group. Also, the elasticity and oral sensation were the highest in the control group compared to the Cr1400 and Cr700 groups. In the study of Dalólio *et al*. (2021), there was no effect of CrMet supplementation on color, weight loss, and cooking in the broiler's meat. However, CrMet supplementation did not affect pH15min, pH24h, color, cooking weight loss, and elasticity (Dalólio *et al*., 2021). Finally, the level of general acceptance in the control group was the highest compared to the Cr1400 and Cr700 groups, and the Cr700 group showed the lowest level.

Fatty acid profile of breast meat

The fatty acid profile in breast meat is presented in Table 11. The amount of saturated C12 fatty acid in the three treatment groups was absent in the meat of all three treatments. Also, the amount of fatty acids C18:3, C20:0, C20:1, C22:0, and C22:1 was observed in the control group at 0%, but in other experimental groups, with the increase of Cr level, their amount increased. The amount of C18:3 fatty acid was not detected in the Cr700, and Cr1400 treatments compared to the control treatment. The amount of saturated fatty acids including C18:0, C18:1, C14:0, and C16:0 in the Cr1400 group showed the lowest amount compared to the Cr700 group and the control. Finally, other fatty acids such as C18:2, C15:0, C16:1, and C17:1 showed the lowest levels in the control group compared to the Cr700 and Cr1400 treatment.

The meat of broilers contains a relatively large amount of unsaturated fatty acids, which in the muscle membrane leads to the sensitivity of meat to fat oxidation and reduces the quality of meat products. Therefore, the use of natural antioxidants in poultry breeding is a method to achieve greater antioxidant stability, improve sensory properties (aroma and smell), and prolong meat storage (Li *et al*., 2013). In a study, adding Cr supplement in the form of Cr-nicotinate and Cr-chloride to the diet of broilers reduced the concentration of malondialdehyde in thigh and breast muscles during storage in the refrigerator (Toghyani *et al*., 2012). Studies have also shown that Cr reduces thiobarbituric acid, leading to an increase in meat quality. The mechanism of action is that the reactive substances of thiobarbituric acid and malondialdehyde are one of the final products of lipid

peroxidation, which reflects the amount of meat fat oxidation (Emami *et al*., 2015) on the other hand, trivalent Cr leads to the activation of insulin receptor kinase and acts as an indirect antioxidant (Untea *et al*., 2019). Cr supplementation helps control the oxidation process due to the production of more pro-oxidants such as myoglobin and other iron-containing proteins, reduces malondialdehyde, and improves the quality of meat preservation (Li *et al*., 2013).

Table 11. Profile of breast fatty acids in broilers fed diets containing different amounts of Chromium Profile of breast fatty acids (%)

Trait	Chromium (0 µg/kg)	Chromium (700 µg/kg)	Chromium (1400 µg/kg)
Lauric acid C12:0(%)	0	0	0
Myristic Acid Methyl Ester C14:0(%)	0.58	0.57	0.55
Silicic AcidC15:0(%)	0.11	0.14	0.15
Monosilicic Acid C15:1(%)	0.03	0.5	0.04
Palmitic Acid C16:0(%)	28.41	27.4	26.32
Palmitoleic Acid Methyl Ester C16:1(%)	6.68	7.42	7.15
Margaric acid C17:0 (%)	0.02	0.04	0.03
Heptadecanoic acid C17:1(%)	0.01	0.02	0.05
Stearic Acid Methyl Ester C18:0(%)	6.15	5.36	5.41
Oleic Acid Methyl Ester C18:1(%)	45.46	44.5	44.12
Linoleic Acid Methyl Ester C18:2(%)	12.43	13.47	15.21
Linolenic Acid Methyl Ester C18:3(%)	0	0.04	0.38
trans Linolenic Acid Methyl Ester C18:3t (%)	0.007	0	0
Arachidic acid C20:0(%)	0	0.05	0.12
cis-11,14- Eicosadienoic Acid Methyl Ester C20:1(%)	0	0.35	0.36
Behenic acid C22:0(%)	0	0.03	0.03
Erucic acid. C22:1(%)	0	0.1	0.03
Others (%)	0.05	0.1	0.05

Cecal microbial flora

Intestinal bacteria populations of *E. coli*, *Coliform*, and *Lactobacillus* are shown in Table 12. The Cr1400 group had the lowest amount of these bacteria. However, the population of *Clostridium* bacteria was not observed in the three treatment groups. Since minerals in organic forms act as antioxidants, they lead to a reduction in intestinal disorders and improved functional characteristics (Wang *et al*., 2008). Considering the benefits of low-consumption minerals, the discovery of the possible functional methods of Cr such as various nutritional biochemistry, medicinal and biological activities, and molecular mechanisms of action is of great importance for the success of poultry and farm animal management, which may result in from a greater understanding of performance and health consequences. Oxidation and stability of different forms of Cr varies and hence added forms of Cr must be carefully controlled to the diet since excess Cr can cause toxicity.

Table 12. Cecal microflora of broilers fed diets containing different amounts of Chromium

Treatment	*E. coli* (log10 CFU/gr)	*Coliform* (log10 CFU/gr)	*Lactobacillus* (log10 CFU/gr)	*Clostridium* (log10 CFU/gr)
Chromium (0 µg/kg)	1.204	2.064	2.524	0
Chromium (700 µg/kg)	1.982	2.097	2.387	0
Chromium (1400 µg/kg)	1.544	1.944	1.580	0

Conclusion

Cr feeding in broilers at the level of 700 µg/kg may lead to improvement in feed intake, weight gain and feed conversion ratio, as well as the lowest price per kg of live chicken, and the best European production index. Although the Cr effect was not significant on the parameters of the carcass, intestinal characteristics, and blood parameters, it led to the improvement of the immune system and increased meat quality, as well as the cecal microbial flora and led to a reduction in saturated fatty acid and an increase in unsaturated fatty acid in Arbor Acres commercial broilers. Thus, the findings of this research recommended adding 700 µg/kg of Cr in the diet, as an antioxidant and cheap growth promoter.

Ethical Approval

The usage of broilers in this research was confirmed by the Rasht Branch, Islamic Azad University, Rasht, Iran. Also, all the trial methods explained here were confirmed by this university. Confirmation number is 117482593767650139814234 5. The study was approved by the research committee of the authors' institution.

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