Research Article

Effects of Supplementation with a Novel Organic Chromium Product on Metabolic and Physiological Indicators of Broilers

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Abstract | The objective of this study was to evaluate the effect of supplementation with a novel organic chromium product on metabolic and physiological indicators of broilers. Using a completely randomized design, 45 male Ross 308 broilers (1-day old) were divided into 3 treatments (15 repetitions each), in which every animal constituted an experimental unit. One group was fed on basal diet and served as control (T1), while other two groups were supplemented Cr at the rate of 200 ppb/kg diet (T2) and 400 ppb/kg diet (T3) respectively. Metabolic indicators such as glucose, cholesterol, triglycerides and cortisol concentrations in blood serum were analyzed. Relative weights of liver and pancreas, as well as lymphoid organs (thymus, bursa of Fabricius and spleen), were also analyzed and compared. In addition, the abdominal fat percentage in broilers was considered. Concentrations of metabolic analytes viz., glucose, cholesterol and triglycerides were decreased ($p<0.05$) in all Cr treated groups (T2 and T3) as compared to control. Whereas, there was an increase ($p<0.05$) in the relative weight of all studied organs (viz., liver, spleen, thymus, bursa and spleen), and a decrease ($p<0.05$) in abdominal fat in Cr supplemented broilers as compared to broilers of control group. Results confirmed that supplementation of this organic Cr product had a positive effect on the health of broilers.

Keywords | Broiler, Organic chromium, Glucose, Lymphoid organs, Metabolites

Received | August 13, 2020; **Accepted** | November 27, 2020; **Published** | December 01, 2020
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Citation | Valera M, Casasola R, Gutiérrez O, Sánchez-Chiprés DR, Mireles S (2021). Effects of supplementation with a novel organic chromium product on metabolic and physiological indicators of broilers. J. Anim. Health Prod. 9(1): 13-21.
DOI | http://dx.doi.org/10.17582/journal.jahp/2021/9.1.13.21
ISSN | 2308-2801

INTRODUCTION

Beneficial effects of supplementation with organic chromium (Cr) products on productive and reproductive performance of broilers are considerable, as well as on certain physiological variables, and its importance has been demonstrated in numerous studies (Kani, 2015; Zheng et al., 2016; Mir et al., 2017; Li et al., 2018). Cr in its most stable oxidation state (Cr^{3+}), fulfills several important functions in broilers. Among them, its participation in the metabolism of carbohydrates, proteins and lipids, its enhancement of insulin action, as an active component of glucose tolerance factor (GTF), its anti-stress role, by decreasing cortisol concentration in blood, its fundamental function in the formation and expression of genetic information, its inhibitory effects on lipogenic activity and its improvement in the absorption of amino acids in muscle cells for protein synthesis (Herran et al., 2011).

There is a positive relationship between Cr supplementation in the diet and growth, increase and improvement of physiological and immune functions of commercially reared fowls, such as broilers and laying hens. Cr supplementation for broilers has been related to a high growth rate and antioxidant capacity, while there is a decrease of lipidic peroxidation, cholesterol content and abdominal fat of these animals (Dalólio et al., 2018).

Heat stress increases circulating concentrations of corticosterone in broilers and it is well documented that corticosterone reduces insulin sensitivity in these animals (Zhao et al., 2009). Since cooling of poultry houses (environment control) is very expensive, the main methods are focused on nutritional modifications (Attia et al., 2015), like search of new feed additives to increase the performance of birds naturally.

The use of native resources and biotechnological methods to obtain organic products that allow to reach animal productions with positive health indicators, is a vitally important task. The novel Cr product was obtained using a Cuban Cr source, from a Cr_2O_3 deposit in the province of Camagüey, by a biotechnological method.

In order to evaluate the effect of supplementation with this organic product on metabolic indicators of broilers, viz., concentration of glucose, cholesterol, triglycerides and cortisol in blood serum of broilers was analyzed. The evaluation of the effect of supplementation with this product on certain physiological indicators of broilers was also an objective of the current research.

MATERIALS AND METHODS

Location

The study was conducted in the Broiler Experimental Facility, of the University Center of Biological and Agricultural Sciences (CUCBA, initials in Spanish) of the University of Guadalajara, Mexico.

Organic Cr product characterization

Indicators of humidity and volatile matter, crude protein, ether extract, ashes, crude fiber, nitrogen free extract and dry matter were determined, according to methodologies described in AOAC (2016). In order to establish the concentration of organic Cr, the obtained product was ground to a particle size of 1 mm. Later, three samples of 0.5 g were taken and placed in tubes of CEM Corporation MARS 5 (Microwave Accelerated Reaction System) Digestion Microwave System. Then, 10 ml of HNO_3 (70%) were added per tube, to perform an acid digestion according to the Ramp to Temperature and to Pressure programming cycle. Maximum power was 600 W (50%), with a ramp time of 20 min, reaching 170 psi and 210 °C of pressure and temperature, respectively, and hold lasted 7 min. The method for obtaining this product is under intellectual property registration procedures.

Samples were extracted, after total cooling and transferred to 100 ml volumetric flasks. Immediately, they were made up to the mark with deionized water and determinations were made in a Thermo Solar AA Series Atomic absorption spectrometer, from Thermo Electron Corporation. For the final calculation of Cr concentration in samples, the following formula was used:

$$ppm = (Abs*100) / g\ sample$$

where,

ppm: Concentration in parts per million; Abs: Absorbance determined by atomic absorption spectrometer; g sample: quantity (in grams) of weighed sample.

Animals, experimental treatments and diets

A total of 45 Ross 308 male broilers were used, starting from 1 d of age, coming from a productive performance experiment with 300 animals, distributed in 12 pens of 2.5 m long and 1 m wide, at the rate of 10 broilers per m^2. Treatments were: (T1) Control without organic Cr, (T2) Treatment with Cr, at a rate of 200 ppb/kg diet; and (T3) Treatment with Cr at a rate of 400 ppb/kg diet. Broilers feed was supplemented with organic Cr product from day 1. Diets were formulated and produced in the Marketing Company of Animal Food "Nutrimentos Ramírez (S.A de CV)", in Jalisco, Mexico. Two diets, starter and finisher were formulated (Table 1) and used in the study. Broilers were fed a starter diet up to 21 days and a finisher diet from 22 to 42 days. Intake estimates were made according to "Ross 308 AP Broiler Chicken Manual: Performance Objectives" by Aviagen (Aviagen, 2017).

Table 1: Composition of diets used in the experiment according to the different stages.

Ingredients (%, except those mentioned)	Starter feed			Finisher feed		
	T1	T2	T3	T1	T2	T3
Sorghum meal	48.18	48.18	48.18	59.28	59.28	59.28
Soybean paste	35.92	35.92	35.92	26.34	26.34	26.34
Vegetable oil	6.00	6.00	6.00	6.00	6.00	6.00
Organic Cr (ppb/kg)	0	200	400	0	200	400
$CaCO_3$	1.21	1.21	1.21	1.10	1.10	1.10
$CaPO_4H,2H_2O$	0.68	0.68	0.68	0.28	0.28	0.28
Premix (vit./min.)[1,2]	8.00	8.00	8.00	7.00	7.00	7.00
Calculated contribution (%, except those mentioned)						
ME (MJ/kg)	13.22	13.22	13.22	12.43	12.43	12.43
Crude Protein	21.91	21.91	21.91	18.60	18.60	18.60
Ca	0.98	0.98	0.98	0.74	0.74	0.74
P	0.48	0.48	0.48	0.40	0.40	0.40

[1]Vitamin supplement: Vit. A 13000 IU; Vit D_3 5000 IU; Vit E 80 mg; Vit K 3.2 mg/kg; Vit B_1 3.2 mg/kg; Vit B_2 8.6 mg/kg; niacin 60 mg/kg; pantothenic acid 17 mg/kg; Vit B_6 5.4 mg/kg; biotin 0.30 mg/kg; folic acid 2.20 mg/kg; Vit B_{12} 0.017 mg/kg.
[2]Mineral supplement: selenium 0.30 mg/kg; iron 20 mg/kg; copper 16 mg/kg; zinc 110 mg/kg; magnesium 0.50 mg/kg; iodine 1.25 mg/kg; cobalt 0.75 mg/kg.

Conditions for experimentation and animal management

The experiment was developed according to rearing ethical principles for experimental broilers. Before the beginning of evaluation, a health control was applied, with total disinfection of the unit and the equipment to be used, with Bioddá (3%) (Viatormex S.A de C.V). A rice husk bed was uniformly distributed in pens, as well as initiation chicken trays (1 per pen), and 4 L chicken fountain (1 per pen) were used during the first 3 weeks. Six JAT brand P1000 gas brooders were used during the first 10 days of age. After 21 days of age, 11 kg capacity hanging cone feeders were used, and a food restriction program was implemented, with only 8 hours a day, to avoid ascites syndrome. Free access to water was guaranteed through automatic drinking troughs for laying hens (2 per pen). Vaccination system for animals consisted of a dose of Trovac® against smallpox and avian influenza, Newxxitek® against Newcastle disease and a dose of Triacef® antibiotic, mixed with Marek vaccine, against Gram (+) and Gram (-) bacterial strains producing ß-lactamases resistant to other ß-lactam antibiotics, was given at the day of birth of broilers. Later, a vaccination schedule was followed against Gumboro, avian influenza and Newcastle disease according to routine practice.

Sample collection and analyses

At 42 days, a total of 45 broilers were used (15 per treatment), out of a productive performance with 300 animals (100 animals each), for sample collection. Blood was collected with disposable syringes from the jugular vein. Samples were collected in plastic tubes and properly preserved until their use. Concentrations of glucose, cholesterol, triglycerides and cortisol in blood serum were determined in the Clinical Analysis Laboratory "Núcleo Veterinario: Mundo Vivo" in Guadalajara, Mexico. Glucose, cholesterol and triglycerides were determined by spectrophotometry in a semi auto analyzer Spectrumlab 23A®, using commercial kits supplied by Pointe Scientific®. Cortisol was determined by ELISA. These same animals were sacrificed by bleeding of jugular vein (Sánchez, 1990). They were dissected, and accessory (liver and pancreas) and lymphoid (thymus, bursa of Fabricius and spleen) organs were removed with precision and weighed in a digital balance. Likewise, abdominal fat received the same procedure. Weights were expressed as animal live weight percentage.

Statistical analysis

A completely randomized design was used, with 3 treatments and 15 repetitions each. Every animal constituted an experimental unit. For the analysis of results, INFOSTAT (Di Rienzo et al., 2012) statistical package was used. Average values were compared using Duncan (1955) test, in the necessary cases.

RESULTS AND DISCUSSION

Organic Cr product characterization

Table 2 shows the results obtained by bromatological analysis of the organic Cr product. Results demonstrates that the product has good bromatological and organoleptic characteristics. It has a sweet smell and a brownish yellow color. Its protein content is around 10.24%. Likewise, its mineral content is generally low, which is reflected in the result of ash contents. Apparent digestibility of dry matter, crude protein and fiber fractions significantly decrease with the increase of fiber content in diet in monogastric animals (Sklan et al., 2003). Therefore, the low fiber and fat content of the product presuppose that this is an easily digestible product and will not show resistance to absorption in the gut.

Table 2: Results of the bromatological analysis carried out on the Cr organic product.

Item	Method	Results (%)
Humidity and volatile matter	AOAC 934.01	8.14
Protein (%N * 6.25)	AOAC 655.04	10.24
Ether extract	AOAC 920.39	3.04
Total ash	AOAC 942.05	2.46
Crude fiber	AOAC 962.09	1.33
Nitrogen free extract	By difference	74.79
Dry matter	By difference	91.86

Table 3 shows results regarding Cr content of the product, after performing a triplicate analysis.

Table 3: Cr concentration in the Cr organic product.

Mineral	Mean (%)	CV	SD
Cr	2.89	0.76	0.89

As observed, the result demonstrated an average concentration of 2.89 mgCr/kg of product. Due to the procedure used, all determined Cr is guaranteed to be organic. Because there is no literature on the use of this biotechnological method for obtaining organic complexes with microelements, it is difficult to establish a comparison with previously obtained results. However, bromatological characteristics and Cr concentration in the product are considered to be acceptable for the intended purpose.

Effects of the organic Cr product on metabolic indicators

After the diagnosis of analytes, results of metabolic indicators determined in the experiment are shown in Table 4. The organic Cr product demonstrated its effectiveness in reducing the metabolic variables.

Table 4: Metabolic indicators determined in animals according to different treatments.

Indicator	Treatments			SE (±)	p
	T1	T2	T3		
Glucose (mg/dL)	172.85[b]	125.39a	133.67a	7.06	0.0001
Cholesterol (mg/dL)	186.65[b]	155.08[a]	167.25[a]	6.04	0.0037
Triglycerides (mg/dL)	124.20[b]	87.09[a]	81.87[a]	3.69	0.0001
Cortisol (nmol/L)	9.45	8.91	7.66	1.66	0.7370

Different letters in a row indicate significant differences ($p \leq 0.05$).

T1 group (172.85 mg/dL) exhibited significantly ($p<0.05$) high level of glucose as compared to Cr treated groups (T2 and T3). T2 treatment (125.39 mg/dL) showed lower values than T3 (133.67 mg/dL), although they had no statistical differences among them. Despite not observing differences between these two treatments, the best results were demonstrated by T2.

Results of this study were similar to those found by Al-Bandr et al. (2010). They evaluated the effects of supplementation with $CrCl_3$, Cr picolinate (CrPic) and Cr yeast (CrY) (1,000 ppb) in broilers and obtained a significant decrease in blood glucose in the groups supplemented with CrY. Ibrahim et al. (2010) supplemented broilers with increasing levels of CrY (500, 1,000, 1,500 and 2,000 ppb) and had a significant reduction of blood glucose levels in these animals. It is important to highlight that, in these two studies, levels of supplemented organic Cr were higher than in the present research, in order to reach the same results. Noori et al. (2011) studied the effects of Cr-methionine (CrMet) on broiler blood serum metabolites, and also obtained a decrease of glucose concentration, coinciding with the results of this present research.

Decrease of blood glucose concentration of broilers supplemented with organic Cr, for both treatments studied in this study, could be attributed to the organic Cr product effect on insulin. This microelement constitutes an integral component of GTF, enhancing insulin action. This way, glucose transportation into cell increases, due to the increase of insulin activity. The mechanism through which this phenomenon occurs includes the increase of the number of insulin-sensitive cell receptors, the consequent increase of sensitivity to this hormone, the regulation of its binding capacity and the increase of sensitivity of β pancreatic cells (Malathi, 2015). This results in an increment of absorption and utilization of glucose by cells, reducing their concentration in serum (Akbari and Torki, 2014).

Other authors also reported a decrease in serum glucose levels after supplementation with other organic sources of Cr (Herran et al., 2011; Talbott et al., 2013; Rajalekshmi et al., 2014; Zhang et al., 2018; Orhan et al., 2018). Also, Haq et al. (2018) found similar results to those obtained in this study, evaluating CrY supplementation alone, or in combination with ascorbic acid or vitamin E. However, these decreases of serum glucose levels were achieved like the previously analyzed cases, with higher supplementation levels than those used in T3 of this research. This result differs from that obtained by Malathi (2015), who found lower glucose concentrations in the treatment with 400 ppb of CrY.

In the case of total cholesterol, there was also a significant decrease among treatments where organic Cr was supplemented compared, to control treatment. Values determined in T2 (155.08 mg/dL) and T3 (167.25 mg/dL) were significantly lower ($p \leq 0.05$) than those obtained in T1 (186.65 mg/dL). Despite not observing differences between T2 and T3, the best results were observed for T2.

Likewise, and coinciding with these results, Dębski *et al*. (2004) stated that CrY supplementation in broilers decreased the concentration of total cholesterol in blood, with significantly lower levels. Ibrahim et al. (2010) also found a decrease of total cholesterol levels in blood, coinciding with the results obtained in this research. Navidshad et al. (2010) supplemented broilers with 250, 500, 750, 1,000 and 1,250 ppb of CrPic and found decreased blood cholesterol levels using 500 and 1,000 ppb. Noori et al. (2011) also referred a decrease of serum cholesterol levels in animals supplemented with CrMet.

This marked decrease of serum cholesterol could be explained through the stimulating effect of the organic Cr product on the islets of Langerhans in the pancreas, which increases insulin secretion. Supplementation with this product, and this increase of secretion of this blood glucose regulatory hormone, intensifies its own activity, inhibiting lipid catabolism. This, in turn, would result in a decrease of lipolysis in adipocytes, lowering serum cholesterol concentration (Akbari and Torki, 2014).

Low blood cholesterol levels recorded in this research coincided with those obtained by Haq et al. (2018), who supplied CrY with ascorbic acid and vitamin E to broilers. The combination of CrY with ascorbic acid reported the lowest total cholesterol values, suggesting that regulatory role of Cr is enhanced in the presence of vitamin C. Other authors, such as Habibian et al. (2013), have also demonstrated that animals had low concentrations of total cholesterol in blood serum, after supplementation with CrMet. Similarly, Malathi (2015) also found a significant decrease of serum concentration of total cholesterol in broilers supplemented with CrY, compared to control animals.

Other authors also explained a decrease of total blood cholesterol values in broilers (Habibian et al., 2013). However, Zheng et al. (2016) reported that Cr supplementation, regardless of the organic source or not, did not affect total serum cholesterol concentration. Similarly, Xiao et al. (2017) showed no decrease in blood cholesterol concentration of Cobb 500 broilers, despite using four supplementation levels (200, 400, 800 and 1,600 ppb) of Cr propionate (CrPro).

These authors demonstrated the effects of CrPro supplementation on blood serum lipids of supplemented broilers, among other analyzed indicators. Results showed that concentration of serum triglycerides and LDL linearly decreased as Cr dose increased. However, this effect was only confirmed when higher organic Cr doses were used in the diet (800 or 1,600 ppb). It is important to emphasize that the decrease of this analyte in this research was achieved with lower levels of CrY than those of CrPro referred by this author, which represents an advantage from metabolic and economical points of view.

Treatments supplemented in this investigation with 200 and 400 ppb of organic Cr product showed concentrations of 87.09 and 81.87 mg/dL of serum triglycerides respectively, showing a significant decrease of this indicator respect to control treatment (124.20 mg/dL).

Similarly, results of the present research coincide with Haq et al. (2018), who referred a decrease of triglyceride content in broilers. In the treatment with CrY alone, concentration of this indicator did not decrease, which enhances the importance of the organic product obtained in this study, because a significant decrease of this indicator was achieved with it. According to Malathi (2015), CrY supplementation tends to decrease blood triglyceride concentration in broilers, among other indicators. Results of this research evidenced this statement, since a significant decrease ($p \leq 0.05$) was found in blood serum triglyceride concentration of animals under experimentation.

The decrease in this analyte is closely related to cholesterol decrease and could also be explained through the stimulating effect of the organic Cr product on the islets of Langerhans in the pancreas, which increases insulin secretion, resulting in a decrease in lipolysis in adipocytes (Akbari and Torki, 2014), also decreasing serum triglyceride concentration.

Despite this evidence, there are reports of previous studies in which the same effects were not observed, even with chemically synthetized organic sources. For instance, Navidshad et al. (2010) used supplementation levels up to 1,250 ppb of CrPic, in different treatments, and found no significant decrease of this indicator in any of them. Likewise, Habibian et al. (2013), despite using CrMet supplementation levels up to 1,200 ppb, did not observe any effect on serum triglyceride levels.

In the case of cortisol, there was also a decrease of it in the serum of broilers supplemented with the organic Cr product, but it was not statistically significant (p = 0.7370). In the case of control treatment, concentration was 9.45 nmol/L. Cortisol concentration in the serum of T2 and T3 animals was 8.91 nmol/L and 7.66 nmol/L, respectively.

These results coincide with Samanta et al. (2008), who supplemented broilers under caloric stress with 500 and 1,000 ppb of CrPic, and observed a cortisol reduction, among other indicators. Bahrami et al. (2012) supplemented 800 and 1,200 ppb of CrMet in broilers and found, among other results, a decrease of serum cortisol concentration. Ebrahimzadeh et al. (2012) also reported results coinciding with those found in this research, by supplementing broilers with 200, 400 and 800 ppb of CrMet, with a reduction of cortisol in blood serum of experimental animals.

Caloric stress induces a cascade of neuronal and hormonal events, beginning with hypothalamic stimulation and the production of corticotropin releasing factor, which stimulates the anterior pituitary, releasing ACTH. This hormone, in turn, stimulates the adrenal cortex, increasing production and release of corticosteroids (corticosterone and cortisol). Corticosterone decreases antibody production. This could be related to an increase of anti-inflammatory cytokines, which stimulate the hypothalamic production of corticotropin-releasing factor.

The Cr supplementation increases the immune response and reduces stress, caloric in this case, either through a direct effect on cytokines, or through an indirect effect on the decrease in glucocorticoid levels. Decrease of serum cortisol levels after supplying organic Cr is one of the main mechanisms through which this microelement contributes to caloric stress reduction and immune system improvement, related to this type of stress in broilers (Hamidi et al., 2017).

This decrease on cortisol concentration, although not statistically significant, has an important biological implication. It demonstrates the effectiveness of the organic product, because it reduces the stress resulting from high temperatures in broiler rearing. Despite not observing differences between these two treatments, the best results were shown by the treatment with 400 ppb.

Effects of the organic Cr product on physiological indicators

Once the animals were sacrificed, weight of the previously mentioned organs as well as abdominal fat were determined and used to calculate their relative weights. Table 5 shows these results, according to the different treatments.

Table 5: Relative weight of accessory and lymphoid organs and abdominal fat percentage determined in animals, according to the different treatments.

Indicator	Treatments/relative weights (g/kg live weight)			SE (±)	p
	T1	T2	T3		
Liver	1.86[a]	2.74[b]	2.60[b]	0.09	0.0001
Pancreas	0.18[a]	0.20[b]	0.21[b]	0.005	0.0008
Thymus	0.46[a]	0.70[b]	0.59[b]	0.04	0.0006
Spleen	0.09[a]	0.16[b]	0.15[b]	0.01	0.0001
Bursa	0.18[a]	0.21[b]	0.21[b]	0.005	0.0002
Abdominal fat (%)	2.32[b]	1.63[a]	1.65[a]	0.09	0.0001

Different letters in a row indicate significant differences ($p \leq 0.05$).

After analyzing mean values obtained per treatment, there was a significant difference ($p \leq 0.05$) in relative weight of the liver in the treatments with organic Cr supplementation. The increase of relative weight of this organ in T2 (2.74 g/kg Live Weight (LW)) and T3 (2.60 g/kg LW), compared to T1 (1.86 g/kg LW), demonstrates the significant efficiency of this organic Cr product in the increase of this indicator and, consequently, in the improvement of the functions of this organ. Even though there were no differences between T2 and T3, there was a tendency to better results in the treatment of 200 ppbCr/kg of diet.

In the case of pancreas, there was a similar performance, in which mean values for T2 (0.20 g/kg LW) and T3 (0.21 g/kg LW) significantly differ from those obtained with T1 (0.18 g/kg LW). There were no differences between T2 and T3 either.

Results regarding the increase of liver relative weight coincide with those obtained by Hamidi et al. (2017), who evaluated (for 21-42 days) two forms of organic Cr supplementation (CrPic and nanoparticles of this compound) in Ross 308 broilers, observing increases in the weight of this organ. However, most of the experiments developed in this regard show results contrary to those obtained in this research.

Ibrahim et al. (2010) supplemented broilers with different levels of CrY (500, 1,000, 1,500 and 2,000 ppb) and determined that supplementation had no influence on liver and pancreas relative weights. Noori et al. (2012) also found no effect on liver relative weight when they supplemented broilers with 200 and 800 ppb of CrMet. Ebrahimzadeh et al. (2013) and Mohammed et al. (2014) had similar results when evaluating CrMet (0, 100, 200, 300, 400ppb and 800 ppb) and CrY (500 ppb) respectively, without verifying any influence of Cr sources on liver relative weight. Haq et al. (2018) found no significant differences either in liver relative weight, when they supplemented broilers with CrY alone, or combined with vitamin E or ascorbic acid.

Later, relative weight of lymphoid organs (thymus, bursa of Fabricius and spleen) was evaluated. As they are important organs for immune function in animals, they were assessed to determine the effect of the organic Cr product on them. In the case of thymus, there was a significant difference ($p \leq 0.05$) for the treatments supplemented with organic Cr, when comparing T2 (0.70 g/kg LW) and T3 (0.59 g/kg LW) with values belonging to T1 (0.46 g/kg LW). There were no statistical differences between T2 and T3, although there were numerical differences, in which T2 showed higher relative weight of this organ, and, therefore, a higher incidence on the immune system of the animals under experimentation. Regarding the spleen, the performance was similar to the previously analyzed. There was a significant difference ($p \leq 0.05$) between the treatments supplemented with organic Cr (T2= 0.16 g/kg LW and T3= 0.15 g/kg LW) as compared to T1 (0.09 g/kg LW). In the case of the bursa of Fabricius, the performance observed in sampled animals of this research was similar to that of the previously analyzed organs. Mean weight of this organ of animals from T2 and T3 (0.21 g/kg LW) significantly differed ($p \leq 0.05$) from the weight of T1 (0.18 g/kg LW).

As reported by Lu et al. (2018), spleen, thymus and bursa of Fabricius are very important organs for the immune response, and their relative weights are useful for evaluating the immune status of animals. Increased relative weight of these organs means stronger cellular and immune functions in the body. In this study, significant increases were found in the weight of thymus and spleen, showing the impact of this microelement on animal immune response. Results regarding relative weight of these organs in the current research also coincides with the findings of Jahanian and Rasouli (2015). These authors determined that, under heat stress conditions, relative weight of thymus and bursa of Fabricius of broilers decreased. These animals, once supplemented with CrMet, regained the normal relative weight of these organs, specifically with the use of 1,000-ppb dose. Farag et al. (2017) also evidenced that dietary supplementation with Cr has effects on the immune system of avian species, with the increase of the relative weights of lymphoid organs, improving cell-mediated immune response and increasing antibody response to infectious diseases.

According to the results of the current study, this difference in spleen weight is due to supplementation with this organic Cr product. Once the animals are supplemented, they are able to increase their metabolic rate, with the consequent increase of their relative weight, and, therefore, of the activity of this organ. Probably this same condition is the one that influences on the performance of the weight of thymus and bursa of Fabricius. Their relative weights increase depending on the supplementation with Cr, which suggests a significant improvement of their functions.

Another indicator under study was abdominal fat percentage. The organic Cr product under evaluation significantly reduced abdominal fat percentage of animals under experimentation. Values obtained in T2 (1.63 g/kg LW) and T3 (1.65 g/kg LW) differ significantly ($p \leq 0,05$) from T1 (2.32 g/kg LW), although the first two treatments showed no differences between them. Nevertheless, there was a tendency to better results in the treatment of 200 ppbCr/kg of diet.

The decrease of abdominal fat content in supplemented animals could be due to the fact that Cr promotes rapid hydrolysis of fats, and a possible decrease of the activity of enzymes related to lipid metabolism in broilers. Fatty acid synthase, acetyl-CoA carboxylase, hormone-sensitive lipase and lipoprotein lipase are essential enzymes in the metabolism, transport and storage of fatty acids, whom activity decreases with organic Cr supplementation, according to a study developed by Chen et al. (2018).

According to a study developed by Brooks et al. (2016), it was demonstrated that Cr supplementation increased the insulin activity and consequently, the use of glucose was optimized. This resulted in a lower serum concentration of non-esterified fatty acids, which brought about a lipolysis reduction. The abdominal fatty tissue grows faster compared to other fatty tissues in fowls. Abdominal fat is a reliable indicator to judge total body fat content, since it is directly associated with total fat content in avian species (Chen et al., 2018). Therefore, considering the results, it is possible to affirm that the organic Cr product decreased total fat content of broilers under experimentation.

Noori et al. (2012) recorded a significant reduction of abdominal fat percentage, supplementing 200 and 800 ppb of CrMet in broilers. These results also coincide, although with a different organic Cr source, with Toghyani et al. (2012), who evaluated the effects of supplementing 500, 1,000, and 1,500 ppb of CrNic and $CrCl_3$, and observed a significant reduction of abdominal fat percentage at all supplementation levels with the organic source.

In addition, Ebrahimzadeh et al. (2013) reported results that coincide with those reported in the current study, when supplementing broilers with 200, 400 and 800 ppb of CrMet and achieving significant reductions of abdominal fat percentages, under heat stress conditions. Malathi (2015) stated the significant reduction of this indicator when supplementing broilers with 200 and 400 ppb of CrY and obtained the best results for the second level of supplementation. Results reported in this research also coincided with those obtained by Chen et al. (2018), when supplementing Ross broilers from one day of age up to 21, with CrPic and observing a significant decrease in the abdominal fat content of these animals.

However, not all the results of previous studies are consistent with those derived from this research. Xiao et al. (2017), when working with Cobb 500 broilers, supplemented from one day of age with 200, 400, 800 and 1600 ppb of CrPro, found no differences regarding the reduction of abdominal fat percentage.

CONCLUSIONS

Supplementation with this organic Cr product, obtained from a Cuban deposit, resulted a decrease in serum concentration of glucose, total cholesterol, triglycerides and cortisol in broilers, demonstrating its beneficial effects on the health of these animals. Moreover, Cr supplementation increased the organ relative weight and decreased abdominal fat percentage. The treatment with the best performance was that with 200 ppb Cr/kg diet, as it showed better results in terms of decreased glucose, cholesterol and abdominal fat levels, while increased the organ indices. In the case of triglycerides and cortisol, the best results were shown by the treatment with 400 ppb Cr/kg diet. Nevertheless, treatment with 200 ppb Cr/kg diet also exhibited significant decrease respect to control treatment in these indicators, which makes it the most suitable to be used, because using larger quantities for achieving better results will suppose other economic and environmental consequences.

ACKNOWLEDGMENTS

Thanks to Miguel Martínez, MIPPE Master Program student, from CUCBA, Guadalajara, for his expertise and collaboration during all the experiment. The authors would also like to thank Óscar Franco and Jorge Eng Gon, Veterinary Medicine students, for their help for preparing the experimental conditions of the study.

AUTHORS CONTRIBUTION

Moisés Valera Rojas: Conceptualization, Investigation, Data curation, Formal analysis, Writing – Original draft, Writing – Review & Editing, Software, Methodology, Ricardo Casasola Torres: Conceptualization, Investigation, Odilia Gutiérrez Borroto: Conceptualization, Methodology, Supervision, David R. Sánchez Chiprés: Conceptualization, Project administration, Funding acquisition, Supervision, Validation, Salvador Mireles Flores: Supervision

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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