Effect of a commercial product containing canthaxanthin for in ovo feeding to broiler embryos on hatchability, chick quality, oxidation status, and performance

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ABSTRACT In ovo feeding has been indicated to improve hatchability, newly hatched chick quality, and broiler performance. The aim of this study was to investigate the effect of in ovo feeding of a commercial canthaxanthin product (**CCX**) containing lignosulphonate, corn starch, canthaxanthin, dextrin (yellow), and ethoxyquin through assessing incubation results, newly hatched quality and oxidation status and broiler performance at 1 to 14 d of age. A total of 780 egg were distributed in a randomized complete block design with 5 treatments (levels of CCX: 0.0, 0.35, 0.45, 0.55, and 0.65 mg/0.5 mL of sterilized and distilled water) and 156 eggs per treatment. The blocking factor was setters. At 17.5 d of embryo development, in ovo injected treatments were applied, using a manual needle. The in ovo feeding of CCX resulted in lower hatching rates ($P < 0.05$) and a longer hatching window ($P < 0.05$) as compared with noninjected CCX treatment. The CCX injection did not affect the bursa and spleen percentage of newly hatched chick ($P > 0.05$). In addition, a higher percentage of chicks with poor physical quality score (<71.0 points) was obtained among the chicks from eggs injected with 0.55 and 0.65 mg of CCX ($P < 0.05$). There were higher total proteins and catalase activity in the livers of the chicks injected with CCX. Broiler chicks in the control group (0.0 mg of CCX) presented higher BW and BW gain during 1 to 7 and 7 to 14 d of after hatch ($P < 0.05$). The viability (%) of chicks at 1 to 14 d of after hatch decreased with inoculation greater than 0.45 mg of CCX in ovo ($P < 0.05$). Although the CCX shown an improvement in oxidation status of chicks, the hatchability and performance of broilers decreased. We concluded that a commercial CCX is not recommended for injection in ovo, and furthers studies should carried out to elucidate the use of pure canthaxanthin.

Key words: antioxidant, carotenoid, in ovo feeding, lignosulphonate, newly hatched chick

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INTRODUCTION

The artificial incubation process accounts for about 33% of the total productive life of broilers, which are slaughtered on average at 42 d. Thus, the embryo period is very important for obtaining better production rates among broiler chickens on farms. According to Cardeal et al. (2015) and Peebles (2018), studies on artificial incubation of broiler eggs are fundamental. These authors pointed out the need to study in ovo feeding (**IOF**), a technique that has been gaining ground in industrial hatcheries around the world.

The productivity and efficiency of hatcheries are measured mainly in terms of number of hatched chicks and the quality of neonatal chicks. In addition, their incubation process also influences the initial performance of the broiler chicks. Maximizing the oxidation status of newborn chicks is a way of making broiler production feasible and improving performance. According to Surai et al. (2016), there is a positive correlation between increased oxidative protection for embryos and the hatching of chicks.

Antioxidants have been used in diets of broiler breeders to improve fertility rates, chick hatching, and broiler performance. Products such as vitamin E, vitamin C, canthaxanthin, and essential oils with

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antioxidant properties have been used (Lin et al. 2005; Murakami et al., 2007; Zhang et al. 2011; Rosa et al. 2012). Canthaxanthin is a carotenoid with antioxidant properties and other relevant biological functions (Surai et al., 2003). Zhang et al. (2011), verified that broiler breeders fed a diet supplemented with 6 mg of canthaxanthin for 24 wk, produced a progeny with better oxidation status. In addition, administration of canthaxanthin can also be related to improvement of initial oxidation status of newly hatched chicks and development of the immune system because canthaxanthin can act as a system for removal of free radicals and absorption of excess energy from highly reactive oxygen reactive species (Zhang et al., 2011; Böhm et al., 2012).

Eggs contain antioxidant agents that are deposited in the yolk consequent to the diet of breeders, and chicks use these antioxidants during embryo development (**DE**) (Surai et al., 1997). The use of antioxidant agents may lead to reduction of the levels of free radicals available for newly hatched chicks. However, it is possible to improve the oxidation status of neonatal broiler chicks to also improve characteristics such as the quality of their physical and immunologic state and their performance, especially in the prestarter phase.

The IOF with antioxidant agents can improve the initial embryos oxidative status, supporting antioxidant protection during the hatching process, and improving their starter performance (Selim et al., 2012; Saki and Salary, 2015; Yair et al., 2015; Neves et al., 2017; Zhang et al. 2018; Araújo et al. 2019; Zhu et al. 2019). In addition, these studies have shown improvements in DE, incubation, and performance results.

Currently, there are several commercial products containing canthaxanthin on the market that aim to provide broilers and breeders dietary needs. However, these have never been tested in relation to embryo supplementation. The objective was to study the effect of injection of a commercial product containing canthaxanthin (10%) and other compounds, inject in ovo at 17.5 d of DE on hatch measurements, oxidation status of newly hatched chick, hatchling quality, and chick performance.

MATERIAL AND METHODS

All procedures used in the experiments of this study received prior approval from the Animal Ethics Committee of the Federal University of Goiás, Brazil (protocol 023/2015).

Eggs

Freshly laid eggs (819 eggs) from a 39-wk-old flock (Cobb 500 breed) were collected from a commercial supplier. The eggs were weighed, labeled, processed, and stored (2 d), in accordance with the experimental design, without delay (at 16°C). The breeders were fed with diets that were prepared following the recommendations for this breed.

Incubation and in Ovo Feeding

The eggs were incubated after storage. They were weighed and allowed to warm up to the ambient temperature (24.5°C ± 2.0). They were then loaded into a rotating single-stage incubator (Gaiolas Almeida Commercial, Goiânia, Brazil) that was set to 37.8°C and 56.0% RH, with 45° rotation every hour, until 17.5 d of DE.

The experiment began at 17.5 DE, when in ovo injection was performed as per treatments. This injection included various levels of a commercial product containing canthaxanthin (0.0, 0.35, 0.45, 0.55, and 0.65 mg of the commercial product). The levels used were based on the previous study by Rocha et al. (2013) who studied the inclusion of a commercial product containing canthaxanthin in the breeders feed. The solutions were prepared from a commercial product contain 62.8% lignosulphonate, 15.0% corn starch, 10.0% canthaxanthin, 10.0% dextrin (yellow), and 2.2% ethoxyquin (**CCX**). Solutions were prepared by the suspension of CCX into 0.5 mL of distilled and sterilized water. The solutions were injected in ovo, via the amniotic cavity, using an adapted version of the methodology proposed by Gonzales et al. (2013). At 17.5 DE, all the eggs were candled, and 780 eggs with embryo lives were injected (mean weight and SD of 55.5 ± 2.9 g), as per the treatment group. These eggs were then placed individually in air-permeable fabric bags for treatment control. Next, 780 eggs were distributed in 12 hatching trays with a capacity of 65 eggs each, being randomly placed 13 eggs from each treatment in each. After that, each setter received 4 hatching trays containing the experimental eggs.

The experimental design consisted of randomized blocks (setters), with 5 treatments and 12 repetitions (trays), consisting of 13 eggs each, thus resulting in 156 eggs per treatment. The temperature and RH of the hatchers were set at 36.7°C and 65.0%, respectively, and the incubation trial was halted when it reached 504 h of incubation.

For the hatching rates (ratio between the number of fertile incubated eggs and the number of hatched chicks), the fertility rate was taken to be 90.0% (which was informed by the company that donated the eggs). The hatching window studied began when the first egg hatched, and each window had duration of 6 h. The incubation trays were the experimental unit, with 12 repetitions per treatment.

At the end of the hatching window, the unhatched eggs underwent a residual analysis in which 4 stages of embryonic mortality were considered: infertile eggs; phase I, including the period between zero and 4 d of DE (**MI**); phase II, between 5 and 10 d of DE (**MII**); phase III, between 11 and 18 d of DE (**MIII**); and phase IV, between 19 and 21 d of DE (**MIV**). The numbers of

pipped eggs with dead and live chicks, eggs with dead and live chicks presenting any abnormalities, unpipped eggs with live chicks, and eggs with evidence of bacterial or fungal contamination were counted.

Newly Hatched Chick Quality

In evaluating newborn chick physical quality, the analysis was conducted at the end of the hatching window. All newly hatched chicks were individually weighed and evaluated by using the score proposed by Tona et al. (2003). Newly hatched chick length was obtained as described by Wolanski et al. (2007) in which chicks were the experimental unit.

For all remaining quality variables, the chicks were firstly decapitated for organ analyses: 3 chicks per repetition (12), resulting in 36 birds per treatment. To obtain the yolk sac weight, the yolk sacs were weighed on a precision scale (0.0001 g), and then, the yolk-free chick weight was calculated (g). To evaluate chick lymphoid organ and intestinal development, the percentage of organ weight relative to the yolk-free chick weight was calculated.

To investigate small intestine histomorphometry, histologic slides were made from the small intestine (duodenum and jejunum), as described by Luna (1968). For intestinal histomorphometry (villus height, crypt depth, and villus/crypt ratio), 10 measurements were made on each intestinal segment, per bird, resulting in 360 measurements per treatment. These measurements were made by using an optical microscope (5×) coupled to an image analyzer system (AxioVision 3.0; Zeiss).

Newly Hatched Chick Oxidation Status

To investigate the oxidation status of the hatched chicks, total protein levels and the catalase (**CAT**) enzyme activity present in the liver and breast muscles were measured. A total of 45 1-day-old chicks from the incubation experiment were used, 9 chicks per treatment. After the chicks were removed from the incubators, 2 g of the liver and 2 g of the breast skeletal muscle (pectoralis major) were sampled to quantify total protein and CAT enzyme activity. The liver and skeletal muscle samples were stored in a TSX Thermo Scientific freezer (−80°C). After storage, the samples were macerated in the plastic microtube itself by using a glass rod. Next, they were individually homogenized using 1 mL of distilled water and then centrifuged (6,000 rpm for 10 min at 5°C). The supernatant material was sampled in duplicate, totaling 18 samples per treatment, and the concentration of plasmatic protein was determined by spectrophotometry.

To determine CAT, the decomposition velocity of hydrogen peroxide (H_2O_2) at 230 nm was quantified from the enzymes present in the samples, using a method adapted from Higashi and Shibata (1964). An aliquot of the supernatant material (50 µL) was added to 950 µL of buffer solution of Tris base (0.25 mol) with EDTA (5 mmol/L) (pH 8.0), with addition of Triton (0.01%). A solution of H_2O_2 (0.1%) was also prepared. A 1-mL volume of the H_2O_2 solution was put into a quartz cuvette, and an absorbance reading was made on this. After this, 10 µL of the sample was added to the H_2O_2 solution. After homogenization, the H_2O_2 decomposition velocity was determined over a period of 1 min and 30 s by means of spectrophotometry. The samples were analyzed in duplicate, totaling 18 samples per treatment, and the values were expressed in (U), that is in mmol of H_2O_2 consumed (1 micromole consumed per minute).

Broiler Chicken Performance

A total of 300 1-day-old chicks from the incubation experiment were distributed into 5 treatments (CCX injection) with 5 replicates, totaling 25 experimental units with 12 birds each (6 males and 6 females). The birds were housed in pens of 5.25 m^2, individually supplied with tubular feeders and pendulum drinkers. Water and feed were provided ad libitum throughout the experimental period (1–14 d). The pen floor was covered with wood shaving litter. A lighting program of 23L:1D was adopted. The birds were fed diets based on corn and soybean meal formulated to supply their nutritional requirements during the prestarter (1–7 d) and starter phases (1–14 d), as described by Rostagno et al. (2011). The broilers remained under constant ventilation (natural and artificial). The average registered room temperature during the experimental period was of 27.5°C ± 4.2 with a minimum of 20.0 and maximum of 33.0°C.

At the end of each week, feed consumption, weight gain, and mortality were recorded. The performance indexes used were final weight, feed consumption, feed conversion, weight gain, and viability during the 2 phases: 1–7 d and 1–14 d.

Statistical Analysis

All the data analyzed were firstly evaluated for normality using the Shapiro-Wilk test. Next, the data underwent ANOVA, and the means were compared using Tukey's test (quantitative) and Kruskal-Wallis test (qualitative). A significance level of 0.05 was used. Only the residual analysis data were submitted to Fisher's exact test ($P < 0.05$). All analyses were conducted using the R software, version 3.4.4 (R Development Core Team, 2016).

RESULTS

Incubation

In ovo feeding of CCX negatively influenced the hatchability of broiler chicks ($P < 0.05$). Injection of 0.45, 0.55, and 0.65 mg of CCX significantly reduced the hatching/total (%) and the hatching/fertile (%) compared with the control group. In addition, the CCX injection in ovo resulted in longer hatch windows ($P < 0.05$) (Table 1).

Table 1. Effect of in ovo feeding of a commercial product containing canthaxanthin at 17.5 d of embryo development on hatchability and hatch window[1] of broiler chicks.

	Commercial product containing canthaxanthin (mg/0.5 mL/egg)						
Items	0.00	0.35	0.45	0.55	0.65	SEM	*P*-value
Hatching/total (%)	83.44^{a}	59.15^{a,b}	50.00^{b,c}	32.14^{c}	30.35^{c}	1.22	<0.0001
Hatching/fertile (%)	89.82^{a}	64.30^{a,b}	54.32^{b,c}	34.93^{c}	32.99^{c}	1.34	<0.0001
Hatch window (h)	20.00^{b}	26.50^{a}	28.50^{a}	24.00^{a}	26.50^{a}	1.25	0.0334

$^{a–c}$Means within a row with different superscript letters are different by Tukey test ($P < 0.05$).
[1]Hatching window: period (h) between the first and the last chick that hatched in the basket.

The residual analysis is presented in Table 2. There were no differences in the numbers of infertile eggs, MI, MII, MIII, abnormal alive, abnormal dead, and contaminated eggs between the treatments ($P > 0.05$). Injection of 0.45, 0.55, and 0.65 mg of CCX resulted in higher mortality in the MIV and pipped dead groups. A higher number of pipped eggs with live chicks were observed among the eggs injected with CCX, independent of level of injection ($P < 0.05$). It was observed intense orange coloration in the gastrointestinal tract of the embryos that proves the absorption of CCX. In addition, a black compacted mass was present in the ceca of the embryos.

Newly Hatched Chick Quality

There were no significant differences in average newly hatched chick weight, yolk-free chick weight, the chick length, percentage of intestine, and percentage of yolk sac among the treatments ($P > 0.05$) (Table 3). There were no significant effects of injection of CCX on percentage of the bursa and spleen of chicks, suggesting similar immunologic quality of the chicks ($P > 0.05$).

The physical quality scores of the newborn chicks were divided into categories, as shown in Table 4. In ovo injection with CCX influenced the quality scores of newly hatched chicks ($P < 0.05$). Chicks that received 0.65 mg of CCX exhibited the higher scores <70.0 points than all other treatments, also those injected with 0.65 mg of CCX had greater 71.0–80.0 points in comparison with the embryos injected with CCX ranged from 0 to 0.45 mg. Chicks that received 0.35 and 0.45 mg of CCX presented higher scores between 81.0 and 90.0 points than embryos injected with 0.55 and 0.65 mg of CCX ($P < 0.05$). There were a higher number of chicks with scores between 91.0 and 100.0 points produced from eggs that did not receive injection of CCX ($P < 0.05$).

The analysis on the intestinal histomorphometry of the duodenum and jejunum of the chicks is presented in Table 5. Injection greater than 0.45 mg of CCX decreased ($P < 0.05$) the size of the villi of the duodenum. The crypt depth or the villus–crypt ratio of duodenum was not affected by treatments ($P > 0.05$). In contrast to the duodenum, the villus height, crypt depth, or villus–crypt ratio of the jejunum was not affected by injection of CCX ($P > 0.05$).

Newly Hatched Chick Oxidation Status

The chick oxidation status, as measured through quantification of total protein and CAT in the liver and breast musculature of newly hatched chicks, is shown in Table 6. Birds that received 0.65 mg of CCX presented higher concentration of total proteins and higher CAT activity in the liver in comparison with other treatments ($P < 0.05$). However, there were no significant effects of injection of CCX on values of total protein and catalase activity of the breast muscles of the birds ($P > 0.05$).

Table 2. Effect of in ovo feeding of a commercial product containing canthaxanthin at 17.5 d of embryo development on residual analysis (%) among unhatched eggs relative to the total number of incubated eggs.

	Commercial product containing canthaxanthin (mg/0.5 mL/egg)					
Residual analysis (%)	0.00	0.35	0.45	0.55	0.65	*P*-value
Infertile eggs	9.50	8.50	7.90	8.10	9.25	0.5946
MI	2.60	2.24	2.23	3.16	2.99	0.9874
MII	2.20	1.00	2.51	1.35	2.90	0.5587
MIII	1.80	1.77	3.34	1.64	1.85	0.0910
MIV	1.27^{b}	3.90^{b}	10.31^{a}	19.14^{a}	19.62^{a}	>0.0001
Pipped dead	1.10^{c}	6.33^{b}	9.20^{a}	15.90^{a}	12.91^{a}	>0.0001
Pipped alive	0.80^{b}	6.33^{a}	6.51^{a}	9.90^{a}	11.1^{a}	>0.0001
Abnormal alive	0.15	0.00	0.52	0.00	0.00	1.0000
Abnormal dead	0.00	0.00	0.00	0.00	0.00	0.9211
Unpipped alive	0.50^{b}	4.81^{a}	3.11^{a}	5.12^{a}	6.55^{a}	0.0153
Contaminated eggs	1.10	0.50	0.00	0.87	0.00	0.124

$^{a–c}$Means within a row with different superscript letters are different by Fisher Exact test ($P < 0.05$).
Abbreviations: MI, mortality from 0 to 4 d of embryo development; MII, mortality from 5 to 10 d of embryo development; MIII, mortality from 11 to 17 d of embryo development; MIV, mortality from 18 to 21 d of embryo development.

Table 3. Effect of in ovo feeding of a commercial product containing canthaxanthin at 17.5 d of embryo development on chick weight, free-yolk chick weight, chick length, and percentage of organ weight relative to the free yolk chick weight.

	Commercial product containing canthaxanthin (mg/0.5 mL/egg)						
Items	0.00	0.35	0.45	0.55	0.65	SEM	*P*-value
Chick weight (g)	44.51	43.91	43.25	43.41	43.36	3.56	0.6580
FYCW (g)	37.08	39.50	37.51	36.61	38.74	5.98	0.1646
Chick length (cm)	17.08	16.93	16.91	16.97	16.92	1.25	0.8732
Bursa (%)	0.091	0.093	0.094	0.091	0.105	0.07	0.9019
Spleen (%)	0.053	0.052	0.053	0.053	0.050	0.03	0.9320
Small intestine (%)	3.95	4.49	4.38	4.72	4.08	0.39	0.0981
Yolk sac (%)	16.04	15.34	13.99	1.85	15.12	1.10	0.1233

Abbreviation: FYCW, free yolk chick weight.

Broiler Chicken Performance

The chicks that received 0.65 mg of CCX presented the lowest BW and the lower BW gain at 7 d of age, whereas the chicks that did not received CCX shown the better BW and higher BW gain ($P < 0.05$). The injection of 0.35 to 0.55 mg CCX resulted in intermediate values of BW and the BW gain in comparision with 0.0 or 0.65 mg CCX. The feed intake of broilers at 7 d of age decreased with injection of CCX higher than 0.55 mg ($P < 0.05$). In addition, the feed conversion ratio and the viability were negatively affected by use of 0.65 mg of CCX ($P < 0.05$) (Table 7).

The BW, BW gain, and feed intake were higher in broilers from 1 to 14 d of age that did not received CCX ($P < 0.05$). There was a difference in the feed conversion ratio, where the chicks that did not received CCX yielded greater feed conversion ratio than the chicks that received CCX. Furthermore, the viability of chicks decreased when received greater than 0.45 mg of CCX (Table 7).

DISCUSSION

There have been no reports of IOF of commercial canthaxanthin-based products to broiler embryos or on their effects when provided directly to embryos. In our study, interesting results from such usage were observed.

The hatching results were worse when CCX was injected with CCX higher level was, the worse the hatching rate was ($P < 0.05$). In a study on supplementation of broiler breeders' feed using a commercial product containing canthaxanthin, Johnson-Dahl et al. (2016) found out that there were improvements to the incubation results, with higher hatching rates among chicks from breeders that received canthaxanthin in the diet. In ovo feeding of canthaxanthin may reduce the cost of using this product because the amount used in breeders' feed is much higher than that used for in ovo injection. However, it was observed that there was no improvement in hatching results. One possible explanation for this is that the commercial product contained other chemical compounds in addition to canthaxanthin, such as 62.0% lignosulphonate, which may have hindered chick hatching.

Lignosulphonate is an organic polymer complex derived from lignin. This product is considered to be fibrous and has agglomerating and emulsifying properties. It is applied as part of animal nutrition, mainly for formation of feed pellets (Waldroup and Desai, 2002). Prestarter diets for broiler chickens containing 5% lignosulphonate that was studied by Acar et al. (1991) have been shown to give rise to compaction of the cecum and reduction of the digestibility of the diet. Thus, it can be inferred that the embryos of the broiler chickens died as a result of injection performed using an agglutinative product. According to Stringhini et al. (2003), broiler chickens are hatched with not fully developed gastrointestinal tract, with low rates of formation of digestive enzymes and mature enterocytes during the first day of life and thus have limited digestive capacity. Thus, supplying a product with a high concentration of lignosulphonate may have caused death among the chicks and reduction of hatching.

Table 4. Effect of in ovo feeding of a commercial product containing canthaxanthin at 17.5 d of embryo development on the quality scores[1] (0–100 points) of newly hatched chicks.

	Commercial product containing canthaxanthin (mg/0.5 mL/egg)						
Quality score[1]	0.00	0.35	0.45	0.55	0.65	SEM	*P*-value
<70.0 points (%)	1.68^{c}	6.58^{c}	6.63^{c}	35.76^{b}	49.00^{a}	12.59	<0.0001
71.0–80.0 points (%)	1.30^{c}	7.55^{c}	9.13^{b}	33.62^{b,a}	39.31^{a}	3.58	<0.0001
81.0–90.0 points (%)	6.00^{c}	33.56^{a}	45.16^{a}	17.48^{b}	4.98^{c}	3.89	<0.0001
91.0–100.0 points (%)	91.01^{a}	53.30^{b}	39.06^{c}	13.37^{d}	6.70^{d}	16.31	<0.0001

$^{a–d}$Means within a row with different superscript letters at different by Kruskal-Wallis test ($P < 0.05$).

[1]Observed items: chick activity, dry feathers, opens eyes, navel, abdominal distention, legs and red hocks. Score adapted from Tona et al. (2003).

Table 5. Effect of in ovo feeding of a commercial product containing canthaxanthin at 17.5 d of embryo development on the small intestine histomorphometry of newly-hatched chicks at pulling.

	Commercial product containing canthaxanthin (mg/0.5 mL/egg)						
Items	0.00	0.35	0.45	0.55	0.65	SEM	*P*-value
Duodenum							
Villus (µm)	335.87[a]	336.73[a,b]	309.97[b]	317.79[b]	319.40[b]	7.87	0.0012
Crypt (µm)	62.99	64.14	54.87	59.61	54.81	4.24	0.5310
Villus-crypt ratio	6.87	6.35	6.51	6.13	7.53	1.98	0.1201
Jejunum							
Villus (µm)	308.67	326.77	298.62	302.92	287.08	6.22	0.2130
Crypt (µm)	71.90	71.22	56.38	73.73	55.71	13.56	5,671
Villus-crypt ratio	55.71	6.19	6.01	5.32	5.85	13.56	0.3609

[a-b]Means within a row with different superscript letters are different by Tukey test ($P < 0.05$).

From necropsies that were performed to collect organs and conduct residual analysis, a very evident orange coloration in the digestive tract and yolk sac of the chicks from the eggs injected with CCX was verified (Figure 1). This proved that the absorption of the product occurred. However, possibly due to the presence of lignosulphonate-agglutinating agents, there was no improvement in egg hatchability results and there was mortality among the chicks that received high doses of the product.

The length of hatching window was 20 h to the chicks that did not received CCX. This duration agrees with that recommended by Araújo et al. (2016), which would ensure better physical quality among the hatched chicks. However, in the treatments with IOF of CCX, the hatching windows were greater than 24 h. According to Calil (2013), chicks that are hatched at the beginning of the window present dehydration at the end of the window because they remain inside the hatcher for an excessive length of time. On the other hand, chicks that are hatched at the end of the window do not have time to develop a healthy navel and are classified in the pulling room as unmarketable chicks. Results relating to chicks quality scores (Tona et al. 2003) have confirmed that birds from longer hatching windows are classified as nonsalable because they have worse physical quality.

The IOF of CCX resulted in higher embryo mortality at the prehatching time (MIV) than noninjected CCX treatment. Residual analysis variables such as infertile eggs, MI, MII, MIII, abnormal alive, abnormal dead, and contaminated eggs did not present any differences between the treatments, these results ensured that incubation occurred homogeneously and without changes until the time of IOF with CCX. On the other hand, the high final level of mortality observed among the chicks was caused by CCX. Chicks that received only distilled water presented residual analysis results compatible with those in the literature relating to normal incubations from breeders of the same age (Araújo et al., 2016). The levels of 0.45, 0.55, and 0.65 mg of CCX provided not only canthaxanthin but also other compounds such as lignosulphonate. The high rates of dead chicks with pecked eggshell may have indicated that the chicks died after injection of the product. There may have been difficulty in digesting CCX, and this may have caused embryos to die.

Higher final mortality usually relates to problems of setter, such as inadequate temperature, humidity, egg turning or ventilation, or wrong positioning of the eggs in the tray (Noiva et al. 2014; Van Emous et al. 2015; Araújo et al. 2016). All of these may negatively affect the hatching. However, given that the eggs injected with distilled water only presented acceptable hatchability, it can be concluded that the in ovo injection of CCX was determinant for embryo mortality during the final phase because the physical aspects of incubation were achieved.

Chick weight, free-yolk chick weight, and chick length were not influenced by injection with CCX. Chick quality aspects relating to size at the time of hatching indicate that larger chicks result in better broiler performance at 42 d (Leandro et al., 2006). The results from our experiment were in agreement with the findings of Bhanja et al. (2012), Salary et al. (2014), and

Table 6. Effect of in ovo feeding of a commercial product containing canthaxanthin at 17.5 d of embryo development on total protein and catalase activity in the livers and breast muscles of newly-hatched chicks at pulling.

	Commercial product containing canthaxanthin (mg/0.5 mL/egg)						
Items	0.00	0.35	0.45	0.55	0.65	SEM	*P*-value
Liver							
Protein (mg/mL)	1,161.1[b]	1,147.1[b]	1,158.0[b]	1,187.0[b]	1,666.1[a]	118.12	<0.0001
Catalase (U/mg protein)	32.3[b]	31.5[b]	29.5[b]	30.9[b]	39.1[a]	3.91	<0.0001
Breast muscle							
Protein (mg/mL)	1,228.3	1,431.1	1,333.1	1,431.2	1,498.1	142.03	0.099
Catalase (U/mg protein)	3.00	3.10	2.98	2.78	3.10	1.5	0.763

[a-b]Means within a row with different superscript letters are different by Tukey test ($P < 0.05$).

Table 7. Effect of in ovo feeding of a commercial product containing canthaxanthin at 17.5 d of embryo development on the growth performance of broiler chicks.

	Commercial product containing canthaxanthin (mg/0.5 mL/egg)						
Items	0.00	0.35	0.45	0.55	0.65	SEM	*P*-value
1–7 d of age							
Feed intake (g)	147.5[a]	142.5[a]	143.2[a]	130.2[b]	128.6[b]	9.91	<0.0001
BW (g)	186.4[a]	157.8[b]	153.8[b]	152.0[b]	139.7[c]	8.65	0.0174
BW gain (g)	142.4[a]	113.8[b]	110.9[b]	111.0[b]	95.2[b]	6.66	0.0012
Feed conversion ratio	1.025[b]	1.261[b]	1.280[b]	1.199[b]	1.397[a]	0.98	<0.0001
Viability (%)	100.0[a]	100.0[a]	98.0[a]	98.0[a]	75.0[b]	2.10	<0.0001
1–14 d of age							
Feed intake (g)	512.9[a]	465.9[b]	499.9[b]	349.7[c]	353.3[c]	7.33	0.0310
BW (g)	411.5[a]	360.5[b]	368.3[b]	365.3[b]	243.6[c]	3.54	0.0205
BW gain (g)	400.1[a]	286.8[b]	307.1[b]	220.2[b]	172.3[c]	8.97	0.0333
Feed conversion ratio	1.281[c]	1.635[b]	1.629[b]	1.586[b]	2.029[a]	0.82	<0.0001
Viability (%)	100.0[a]	100.0[a]	75.0[b]	75.0[b]	60.0[b]	6.65	<0.0001

[a–c]Means within a row with different superscript letters are different by Tukey test ($P < 0.05$).

Rajkumar et al. (2015), who also did not find any improvement in the weight of newly hatched chicks from eggs supplemented in ovo with vitamin E, which was used as an in ovo antioxidant during DE. Thus, canthaxanthin does not seem to have influenced the BW of chicks at hatching. In addition, in a study on antioxidants based on grape seed used in ovo, Hajati et al. (2014) found that there was no difference between chicks that received the antioxidants and a group that did not receive the additive.

The lymphoid organ weight (%) in neonatal chicks may reflect greater development of the immune system of broilers in the initial phase (Pope, 1991). Development of the bursa and the spleen are of fundamental importance for these birds. Within the bursa, lymphocyte B maturation already occurred at time of hatching, whereas the spleen is considered to be a secondary organ of the immune system with the function of eliminating old erythrocytes and storing cells of the immune system (Abbas et al., 2014). Our results showed that the lymphoid organ weights (%) were similar among the treatments. The mean spleen and bursa weights found in all treatments were similar to those found by Goel et al. (2017) and were considered normal for all the treatments.

The small intestine weight (%) was also not affected by injection of CCX in ovo. This weight may be indicative of greater gastrointestinal development in neonate chicks. These results indicate that the injection of CCX at the levels studied did not improve the development of the small intestine of the chicks, and the hypothesis that this might have led to improvement was therefore rejected. It was observed that the cecum of the birds injected with CCX was dark and fairly swollen, and when it was opened, its contents were very thick. It is possible that the binding capacity of the lignosulphonate compound caused adherence to this specific region of the gastrointestinal tract and thus caused compaction of the cecum. This could have disrupted the hatching process of the chicks and may have led to mortality among them at the prepiping stage.

The yolk sac weight (%) was not influenced by the treatments. More consumption of the yolk is indicative of greater utilization of the nutritional contribution provided by the breeders. However, for levels tested, there was no difference in these results. In a study on the relationship between yolk sac weight and chick weight, Araújo et al. (2016) found values that were lower than the findings in this experiment. Consumption of the residue may be indicative of greater use of the nutrients provided in the egg for development of the chick.

Total protein amount and CAT activity in the livers of neonatal chicks were higher when 0.65 mg of CCX was injected in ovo. Although this increased the oxidative protection in the tissues of chicks, there was no positive response to the quality of the newly hatched chicks. According to Surai et al. (2016), higher levels of antioxidants in the tissues of embryos can result in better newly

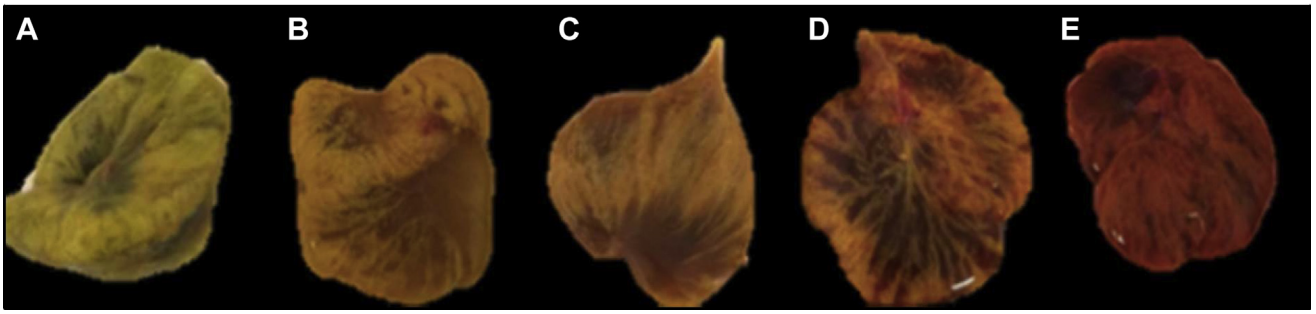

Figure 1. Examination of yolk sac of newly hatched chick injected in ovo with 0.00, 0.35, 0.45, 0.55, and 0.65 mg of commercial product contended canthaxanthin (A, B, C, D, and E, respectively).

hatched chicks. Using vitamin E for IOF, Araújo et al. (2019) found high levels of CAT in the liver and muscle tissues of chicks. They correlated this result with high hatchability, chick quality, and performance. In our present study, in spite of better oxidation status among the chicks, there was high mortality among those that received the highest level of CCX. This made in ovo use of this product unviable because its action was possibly hampered by presence of binders in the commercial compound.

The performance of the broiler chickens evaluated in the preinitial and initial phases indicated that there was low growth among the chicks that received the CCX in the egg. Moreover, there was high mortality among the birds that received the highest doses. It could be seen from the necropsies performed on the dead birds that they died because of omphalitis and dehydration. These problems occurred mainly because of the poor physical quality of the chicks, which had a poorly healed navel and distended abdomen. According to Tona et al. (2003), good initial quality among newly hatched chicks is fundamental for good performance during the breeding phases. It should be pointed out that the villi were longer in the chicks that received CCX. However, despite their greater length, it may be assumed that some components of the product disrupted the digestion and absorption of feed consumed in the initial stages.

CONCLUSION

Despite improving the oxidation status of newly hatched chicks, CCX should not be recommended in ovo to embryos at 17.5 DE because it causes embryo mortality and decrease the broiler performance at initial phase.

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