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EFFECT OF NATURAL AND SYNTHETIC PIGMENTS ON GROWTH PERFORMANCE, RELATIVE WEIGHT OF SOME ORGANS AND CECAL CHARACTERISTICS OF YOUNG BROILERS

EFECTO DE LOS PIGMENTOS NATURALES Y SINTÉTICOS EN EL CRECIMIENTO, PESO RELATIVO DE ALGUNOS ÓRGANOS Y CARACTERÍSTICAS CECALES DE POLLOS DE ENGORDE JÓVENES

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To evaluate whether dietary supplementation with pigments (natural and synthetic) had a growth-promoting effect in broilers at the early stage (0-10 days), 480 one-day-old Ross 308® male chickens were randomly assigned to four treatments (four replicates and 30 birds). The experimental treatment consisted of a control diet and the supplementation with 1 % of *Bixa orellana*, 1 % of *Murraya koenigii* and 0.03 % of canthaxanthin. The experimental diets did not affect (P>0.05) the viability, however, *Bixa orellana* diet increased (P<0.05) the body weight compared to the other experimental groups, although this treatment did not change (P>0.05) the feed intake and feed conversion ratio in relation to the control diet. The treatments did not modify the relative weight of the gizzard, pancreas, and heart (P>0.05). However, the *Bixa orellana* and *Murraya koenigii* groups decreased (P<0.05) the relative weight of the proventriculus and cecum and the liver and abdominal fat, respectively. Furthermore, the canthaxanthin group increased (P<0.05) the relative weight of lymphoid organs and small intestine (P<0.05). Moreover, *Bixa orellana* diet decreased (P<0.05) the pH, total coliforms, *Enterobacteriaceae* and *Escherichia coli* in the cecum compared to the other diets, and the *Murraya koenigii* group increased (P<0.05) the population of lactic acid bacteria. Also, in this organ (cecum), yeast quantification decreased (P<0.05) due to canthaxanthin and the fungal population did not change among treatments (P>0.05). Dietary supplementation with *Bixa orellana* has a natural growth-promoting effect, as well as a marked antimicrobial effect against common cecal *Enterobacteriaceae* of broilers.

Keywords: *broiler, cecal microbiology, feed pigment, growth promoter, organ*

Para evaluar si la suplementación dietética con pigmentos (naturales y sintéticos) tenía un efecto promotor del crecimiento en pollos de engorde en la etapa temprana (0-10 días), se asignaron aleatoriamente 480 pollos machos Ross 308® de un día de edad a cuatro tratamientos (cuatro replicas y 30 aves). Los tratamientos experimentales consistieron en una dieta control y la suplementación con 1 % de *Bixa orellana*, 1 % de *Murraya koenigii* y 0.03 % de cantaxantina. Las dietas experimentales no afectaron (P>0.05) la viabilidad, sin embargo, la dieta con *Bixa orellana* incrementó (P<0.05) el peso vivo en comparación con los otros grupos experimentales, aunque este tratamiento no cambió (P>0.05) el consumo de alimento y el índice de conversión alimenticia en relación con la dieta control. Los tratamientos experimentales no modificaron el peso relativo de molleja, páncreas y corazón (P>0.05). Sin embargo, los grupos *Bixa orellana* y *Murraya koenigii* disminuyeron (P<0.05) el peso relativo del proventrículo y ciego y del hígado y la grasa abdominal, respectivamente. Además, el grupo de cantaxantina aumentó (P<0.05) el peso relativo de los órganos linfoides e intestino delgado (P<0.05). También, la dieta *Bixa orellana* disminuyó (P<0.05) el pH, coliformes totales, *Enterobacteriaceae* y *Escherichia coli* en el ciego en comparación con las otras dietas, y el grupo *Murraya koenigii* aumentó (P<0.05) la población de bacterias ácido-lácticas. Además, en este órgano (ciego), la cuantificación de levaduras disminuyó (P<0.05) debido a la cantaxantina y la población de hongos no cambiaron entre tratamientos (P>0.05). La suplementación dietética con *Bixa orellana* tiene un efecto promotor de crecimiento natural, así como un marcado efecto antimicrobiano contra las enterobacterias cecales comunes en pollos de engorde.

Palabras clave: *microbiología cecal, órgano, pigmento alimenticio, pollo de engorde, promotor de crecimiento*

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Introduction

In many regions, the skin pigmentation of chickens defines their commercialization, since it is associated with a chicken raised in grazing or free range. Thus, the poultry industry is focused on pigmenting chickens according to consumer characteristics, considering intensity and color (Barbut and Leishmanm 2022). Many strategies have been used, such as the individual or mixed dietary use of yellow and red-orange pigments, whether natural or synthetic, that from the first stages of life can pigment the tarsus or the beak (Xue *et al.* 2021). One strategy is the dietary supplementation with canthaxanthin, which is a carotenoid synthetic pigment from the xanthophyll category (Bera 2020). This pigment has a marked antioxidant effect *in vivo* (Elia *et al.* 2019); studies have related this effect to the development of the embryo and its subsequent postnatal growth. The use of this synthetic pigment had positive results in the hatchability and vitality of the hatched chick (Araújo *et al*. 2020).

The European Union and other countries have limited the use of synthetic pigments; thus, nutritionists are looking for natural pigments for animal production (Pertiwi *et al*. 2022). Likewise, some natural pigments also have a phytobiotic function, since they are rich in secondary metabolites that, in low concentrations, have a positive productive response. Among the natural pigments initially proposed for laying hens are *Bixa orellana* and *Murraya koenigii* (Franco and Gomez 2022). Previous experiments indicated that the use of up to 1.5% *Bixa orellana* in hen diets markedly pigmented yolk color and improved albumen height and Haugh unit (Martínez *et al*. 2021a), as well as a study in broilers, indicated improvements in feed efficiency and edible portions (Moncayo and Ramírez 2020), related to the high concentration of bixin and norbixin in the seed powder of this pigment. Also, *Murraya koenigii* has been used daily in human food as a pigment/seasoning for its flavor and aroma (Tabashiri *et al*. 2022); however, few studies have been carried out to verify its effect on skin pigmentation in poultry.

On the other hand, the phytobiotic use of 1 % of *Murraya koenigii* in diets improved body weight and feed conversion ratio, without affecting edible portions (Karnani *et al*. 2018). Other studies demonstrated that dietary supplementation with 1.0 % *Murraya koenigii* reduced serum harmful lipids and abdominal fat, and increased feed efficiency and breast yield in broilers (Sharma *et al*. 2021). Considering the above results, it was hypothesized whether the use of natural and synthetic pigments can have a non-antibiotic growth-promoting effect in young broilers, associated with immune activity mediated by lymphoid organs and cecal microbial activity, especially at this critical stage of life (0-10 days) (Martínez *et al*. 2021b). Therefore, the present study was conducted to evaluate the effects of dietary supplementation of natural and synthetic pigments on growth, relative weight of some organs, and cecal traits of young broilers.

Materials and Methods

Ethics Statement: This research work followed the Guidelines for Experimental Animals (Reference number: 7926) of the Department of Agricultural Sciences and Production of the Zamorano University, San Antonio de Oriente, Francisco Morazan, Honduras.

Animals, housing, treatments, and diets: A total of 480 Ross 308® male chickens from birth to 10 days old, were randomly assigned to metabolic cages (0.70 m wide x 1.0 m long and 14.29 birds/m^2) in four treatments (four replicates and 30 birds per replicate). The experimental diets consisted in a control diet (CD), CD+1 % achiote (*Bixa orellana*), CD+1 % curry (*Murraya koenigii*) and CD+0.03 % canthaxanthin. Temperature and lighting were controlled daily. Previous studies (Moncayo and Ramirez 2020, Sharma *et al*. 2021, Bonamigo *et al*. 2022 and Franco and Gomez 2022) were taken as references for the dietary inclusion levels of pigments. Diets were formulated following Ross 308 requirements (table 1 and photo 1). The pigments used were acquired in the company "Alimento", Honduras. Feed and water were offered *ad libitum* in linear feeders and nipple drinkers, respectively. Initial and final body weights (IBW and FBW), as well as feed intake (FI) were measured to determine the feed conversion ratio (FCR).

Table 1. Ingredients and nutritional contributions of broilers (0-10 days)

Ingredients (%)	Control diet
Corn meal	59.16
Soymeal	32.29
Mineral and vitamin premix[1]	0.35
Sodium chloride	0.25
Sodium bicarbonate	0.25
African palm oil	3.44
Choline	0.05
DL-Methionine	0.34
L-Threonine	0.16
L-Lysine	0.32
Calcium carbonate	1.60
Monocalcium phosphate	1.54
Mycotoxin binder	0.20
Coccidiostat	0.05
Calculated nutritional contributions (%)	
Metabolizable energy (MJ/kg)	12.44
Crude protein	22.00
Ca	0.90
Available P	0.45
Digestible Lysine	1.22
Digestible Methionine+Cistine	0.91
Digestible Threonine	0.83
Digestible Tryptophan	0.20
Na	0.18
Cl	0.21

[1]Each kg contains: vitamin A 11,550 IU, vitamin D3 4,300 IU, vitamin E 27.5 IU, vitamin K3 3.85 mg, vitamin B1 2.75 mg, vitamin B2 9.9 mg, vitamin B6 3.85 mg, vitamin B12 22.0 Mcg, niacin 49.5 mg, pantothenic acid 15.4 mg, folic acid 1.38 mg, biotin 166 Mcg; selenium 0.09 mg, iodine 0.18 mg, copper 3.00 mg, iron 36.0 mg, manganese 54.0 mg, zinc 48.0 mg, cobalt 0.12 mg.

Photo 1. Finished feed after the inclusion of pigments

Relative weight of digestive, visceral, and immune organs: At 10 days old 80 broilers (20 chickens per replicate) per treatment were randomly selected to determine relative organ weights. From each chicken, the viscera (liver and heart), the immune organs (thymus, spleen, and bursa of Fabricius), the digestive organs (proventriculus and gizzard), and the intestines (small and caecum) were extracted. The relative weight was determined according to the body weight at the slaughter of broilers (Aguilar *et al.* 2013).

Cecal traits: The left cecum of 20 broilers per treatment (five samples per replicate) was taken, and the pH was determined was determined using an Oakton®model 700 digital pH meter (Oakton Instruments, Vermon Hills, IL, USA). Before testing, the potentiometer was calibrated with pH buffers at 1.68, 4.01, 7.00, 10.01, and 12.45 according to the manufacturer's recommendations.

Also, the right cecum of five birds per treatment was taken, and the mucosa with a scalpel was scraped for microbiological culture. Each sample's cecal content was placed in a sterile tube; weight was recorded and diluted with Buffered Peptone Water (BPW, Liofilchem, Italy) to a 1:9 ratio (w:v). Diluted cecal contents were homogenized, and serial dilutions (1/10) were made from it until dilution 10^5. Aliquots of 0.1 mL of each dilution were spread-plated on the surface of selective and differential media for microbiological analysis. Violet Red Bile Glucose agar plates for *Enterocateriaceae* and Violet Red Bile Lactose MUG Agar for coliform and *E. coli* counts (Liofilchem, Italy), were incubated at 35 °C for 24 h. Yeast and molds were determined with Rose-Bengal Chloramphenicol Agar (Liofilchem, Italy) incubated at 25 °C for five days. Lactic acid bacteria were enumerated on Man Rogosa Sharpe agar (Liofilchem, Italy) supplemented with methylene blue (0.016 g/1000mL) at 37 °C with a pH of 5.6 for 48 h in anaerobiosis (Gas Pak system, BBL, Cockeysville, USA) (Lan *et al*. 2017).

All bacterial counts were presented as Log CFU/g. Presence of *Salmonella* on each cecum was determined by pre-enrichment of the diluted cecum in BPW at 35 °C for 24 h, followed by selective enrichment for with tetrathionate broth (Liofilchem, Italy) and Rappaport-Vassiliadis broth (Neogen Acumedia, Mich) at 35 and 42 °C, respectively. After selective enrichment, one loop of each selective media was streaked onto the surface of XLD agar, Hektoen Enteric Agar, and Bismuth Sulphite Agar (Neogen Acumedia, Mich). All selective plates were incubated at 35 °C for 24-48 h. Typical colonies were picked and confirmed by biochemical test on Triple Sugar Iron Agar and Lysine Iron Agar (Liofilchem, Italy). Serological confirmation was made with *Salmonella* O antiserum poly A-I & Vi Antiserum (BD Difco, MD) (Martínez *et al.* 2021b).

Statistical analysis: The data was processed using the one-way ANOVA test of SPSS 23.0 (SPSS Inc., IBM Corporation, New York, NY, USA) in a completely randomized design, before carrying out the analysis of variance, the Kolmogorov-Smirnov test was performed, and the Bartlett's test was used to evaluate the uniformity of the variance, where necessary. Finally, Duncan range test was used to determine the differences between means (P<0.05).

Results and Discussion

The experimental diets did not change (P>0.05) the viability in the first 10 days old of broilers (table 2). However, the dietary supplementation with *Bixa orellana* increased (P<0.05) the body weight compared to the other treatments. Furthermore, diets with *Murraya koenigii* and canthaxanthin decreased feed intake, which provoked the highest feed conversion ratio (table 2, P<0.05) in relation to the control and *Bixa orellana* groups.

Table 2. Effect of the dietary supplementation of natural and synthetic pigments on growth performance of young broilers

Items	Experimental treatments				SEM±	P-value
	Control	*Bixa orellana*	*Murraya koenigii*	Canthaxanthin		
IBW (g)	47.52	47.54	47.58	47.57	0.095	0.091
FBW (g)	258.48^{b}	270.00^{a}	203.16^{c}	206.08^{c}	6.314	0.001
FI (g)	198.15^{ab}	210.25^{a}	175.85^{b}	176.5^{b}	5.180	0.038
FCR	0.94^{b}	0.95^{b}	1.13^{a}	1.11^{a}	0.051	0.001
Viability (%)	100.00	100.00	100.00	100.00		

^{a,b,c}Means within the same row with different superscript differ significantly (P<0.05).

IBW: initial body weight; FBW: final body weight; FI: feed intake; FCR: feed conversion ratio

In this study, only the *Bixa orellana* seed powder promoted the body weight, the other ingredients decreased this productive indicator (table 2). In this sense, Moncayo and Ramírez (2020) reported that the use of 1.5 % achiote in the diet improved the feed efficiency of broilers up to 28 days old, due to the presence of bixin and norbixin in achiote, which has an antioxidant effect *in vitro* and *in vivo* (Ashraf *et al.* 2023). Likewise, Handayani *et al*. (2024) reported that when testing different carotenoids as an antioxidant, norbixin was the only one that inhibited the oxidative deterioration of lipids. Also, Garcia *et al.* (2012) mentioned that bixin and norbixin in achiote decrease lipid peroxidation in meat. In a study by Nathan *et al.* (2019) reported that bixin and norbixin, as the main chemical components of achiote, do not present carcinogenic and mutagenic effects by silico methods, in addition, the authors found a marked bactericidal effect against enterobacteria. Apparently, the antioxidant and antimicrobial effect of the achiote benefited the productivity of young birds (up to 10 days old), considering that the first days old is the most critical moment for these animals, which present low immunological, enzymatic and antioxidant activity (Martínez *et al*. 2021b).

Both canthaxanthin and *Murraya koenigii* decreased feed intake, which had a negative influence on body weight, although without affecting the viability of young broilers (table 2). Several studies have demonstrated the antioxidant role of canthaxanthin in animals (Mathimaran *et al.* 2020). In this sense, von Lintig *et al*. (2020) stated that canthaxanthin has shown antioxidant activity in various *in vitro* and *in vivo* model systems, which may enhance endogenous antioxidant activity and reduce cellular oxidative stress. Bonamigo *et al*. (2022) showed that diets containing canthaxanthin associated with 25-OH-D3 improved productivity and bone development in broilers. However, Araújo *et al* (2020) did not recommend the dietary use of canthaxanthin *in ovo* because it decreased the feed efficiency in broilers, even though this synthetic product increased hatch indicators and oxidative status. Further studies are needed to understand the role of canthaxanthin in gastrointestinal health and endogenous antioxidant capacity in young broilers.

The antioxidant effect of *Murraya koenigii* is known because it captures the free radicals formed in the lipid peroxidation process (Bharathi *et al*. 2011). Das *et al.* (2011) verified the antioxidant effect of this natural product when they evaluated the formation of lipid peroxides, free fatty acids and thiobarbituric acid substances (TBARS) in meat. Although studies in broilers are scarce, Bharathi *et al*. (2011) reported that the use of 0.1 % of *Murraya koenigii* could reduce the toxicity of chlorpyrifos (organophosphate insecticide) in broilers. Apparently, the use of *Murraya koenigii* at 1.0 % had an opposite effect on the response of the animals (table 2), future studies should consider lower levels of inclusion in the diet of broilers.

Table 3 shows the effect of diets with natural and synthetic pigments on changes in the relative weight of digestive, visceral, and immune organs of young broilers. The canthaxanthin group increased (P<0.05) the relative weight of the thymus and bursa of Fabricius compared to the other treatments. Furthermore, the relative weight of the spleen changed significantly (P<0.05) due to this treatment (canthaxanthin), which provoked differences with the control diet and the *Murraya koenigii* group.

In addition, the *Bixa orellana* group decreased (P<0.05) the relative weight of the proventriculus and cecum, although, the latter organ (cecum) without significant changes (P>0.05) with the *Murraya koenigii* group. Likewise, this natural pigment (*Murraya koenigii*) decreased (P<0.05) the relative weight of abdominal fat and liver in relation to the control, canthaxanthin and *Bixa orellana* treatments, respectively. The relative weight of the gizzard, pancreas, and heart did not change due to the experimental treatments (P>0.05).

On the other hand, the canthaxanthin diet increased the relative weight of the primary lymphoid organs (bursa of Fabricius and thymus) and secondary (spleen) in relation to the control (table 3), although the feed efficiency decreased in young broilers. Other studies that challenged lipopolysaccharide found an increase in bursal morphometric index in broilers when using lutein+canthaxanthin (Koutsos *et al*. 2003). Commonly, a greater relative weight of immune

Table 3. Effect of the dietary supplementation of natural and synthetic pigments on the relative weight of some organs of young broilers

Items (g/kg)	Experimental treatments				SEM±	P-value
	Control	*Bixa orellana*	*Murraya koenigii*	Canthaxanthin		
Proventriculus	1.04[a]	0.90[b]	0.97[a]	0.98[a]	0.035	0.048
Gizzard	5.71	5.78	5.90	5.73	0.075	0.091
Pancreas	0.47	0.49	0.49	0.53	0.054	0.068
Liver	3.03[b]	3.22[ab]	2.92[c]	3.26[a]	0.105	0.034
Spleen	0.06[b]	0.10[ab]	0.07[b]	0.16[a]	0.028	0.008
Thymus	0.22[b]	0.22[b]	0.24[b]	0.30[a]	0.015	0.050
Bursa of Fabricius	0.16[b]	0.18[b]	0.17[b]	0.24[a]	0.025	0.048
Heart	0.67	0.60	0.60	0.62	0.037	0.059
Cecum	1.65[a]	1.07[c]	1.15[bc]	1.44[b]	0.156	0.034
Small intestine	9.83[b]	10.10[ab]	9.87[b]	11.05[a]	0.384	0.050
Abdominal fat	0.36[a]	0.33[ab]	0.27[b]	0.36[a]	0.028	0.027

[a,b,c]Means within the same row with different superscript differ significantly (P<0.05)

organs such as the thymus, bursa of Fabricius, and spleen is related to greater immunological activity in the early stages of broilers (Guo *et al*. 2021), because the primary lymphoid organs produce B lymphocytes (bursa of Fabricius) and T (thymus) involved in memory and immunological defense, respectively (Aguilar *et al*. 2013). However, in this experiment, it appears that the relative weight of the thymus, bursa of Fabricius and spleen of broilers (10 days old) with the canthaxanthin group is abnormal. In a study by Martínez *et al.* (2021b) on the allometry of immune organs in broilers up to 10 days old, they reported mean relative weights of the thymus, bursa of Fabricius, and spleen of 0.25, 0.18, and 0.11 %, respectively. Furthermore, in apparently healthy birds, hyperimmunity leads to a higher energy cost to produce lymphocytes and antibodies and for the activity of macrophages and anti-inflammatory mechanisms (Pålsson-McDermott and O'Neill 2020). This could justify decreasing the FI and weight gain of the chickens with the canthaxanthin diet (table 2).

Several studies with natural products rich in beneficial secondary metabolites have reported that abnormal growth of lymphoid organs and other digestive organs results in a decrease in the productive response, with a higher emphasis on young broilers (Aguilar *et al*. 2013 and Moncayo and Ramírez 2020). In this sense, canthaxanthin as a synthetic pigment modified the relative weight of the small intestine (table 3), this organ has a statistical correlation with immune organs in birds (Martínez *et al*. 2021b), perhaps, this diet caused postprandial intestinal inflammatory processes, which increased the production of T and B lymphocytes and influenced the relative weight of these hematopoietic organs in young broiler chickens (Madej *et al*. 2024) as occurred in this study (table 3). Furthermore, the *Murraya koenigii* group decreased the relative weight of abdominal fat (table 3). The contribution of alkaloids due to the dietary inclusion of this natural pigment could reduce the incorporation of fat in this portion, similar to the results reported by Sharma *et al.* (2021).

The proposed additives in this study (higher emphasis on *Bixa orellana*) decreased the relative weight of the cecum (table 3), intestine portion that is responsible for intestinal health, the fermentation of nutrients and modulation of the intestinal microbiota (Yadav and Jha 2019). Some studies have found that some natural products have a more obvious effect on the small intestine than on the cecum (Liu *et al*. 2021). Is worth noting that the results of the effect of ingredients rich in secondary metabolites on this organ are not conclusive, because it depends on the concentration and type of secondary metabolites, intestinal health, diet, age, and experimental conditions. Vase-Khavari *et al.* (2019) reported that using 0.5 % of the powder of *Rhus coriaria*, *Heracleum persicum* and *Mentha piperita* increased the relative weight of the cecum. However, Liu *et al.* (2021) found no change in the relative weight of the cecum when they used up to 1 % of *Camellia sinensis* powder, apparently a decrease in cecal content in broilers because of these dietary compounds on the microflora influenced the results of this study (table 3). Other studies found that the use of natural products with antimicrobial properties decreased cecal content and, in turn, the load of enterobacteria such as *Salmonella* spp. and *Campylobacter* (Almuhayawi *et al*. 2023).

The effect of natural and synthetic pigments on cecal traits of young broilers is shown in table 4. The diets with the pigments decreased cecal pH, with a higher emphasis in the group with *Bixa orellana* which provoked a reduction

Table 4. Effect of the dietary supplementation of natural and synthetic pigments on cecal traits of young broilers.

Items (Log CFU/g)	Experimental treatments				SEM±	P-value
	Control	*Bixa orellana*	*Murraya koenigii*	Canthaxanthin		
pH	6.79[a]	5.99[c]	6.17[b]	6.22[b]	0.159	0.034
Total coliforms	6.14[c]	6.00[d]	6.63[a]	6.36[b]	0.333	0.050
Enterobacteriaceae	6.21[b]	6.05[c]	6.49[a]	6.24[b]	0.108	0.008
E. coli	6.14[c]	6.04[d]	6.45[a]	6.33[b]	0.127	0.043
Salmonella spp.	Presence	Absence	Absence	Absence		
Total LAB	6.64[b]	6.81[ab]	8.56[a]	8.43[ab]	0.449	0.044
Yeast	2.84[a]	2.49[ab]	2.54[ab]	2.26[b]	0.149	0.016
Fungus	3.02	3.49	3.10	3.62	0.489	0.786

[a,b,c,d]Means within the same row with different superscript differ significantly (P<0.05). LAB: Lactic acid bacteria

(P<0.05) of total coliforms, *Enterobacteriaceae* and *E. coli* in relation to the control diet and the other groups of pigments. Likewise, cecal *Salmonella* spp. of broilers fed with the proposed pigments was not found (table 4). On the other hand, the *Murraya koenigii* group increased (P<0.05) the population of lactic acid bacteria and the canthaxanthin group decreased (P<0.05) the quantification of cecal yeasts compared to the control diet. Also, the experimental diets did not change (P>0.05) the population of cecal fungus (table 4).

It is known that the bactericidal or bacteriostatic effect of natural and synthetic products used in the poultry industry has a direct impact on intestinal health and productive response of the host (Arena *et al.* 2020). A decrease in intestinal pH could be due to the product used having an acidic pH, the growth of cecal LAB or a decrease in the pathogenic bacterial population, since the final product of bacterial fermentation is volatile fatty acids that emit protons and reduce the pH (Scicutella *et al*. 2021). Thus, Al-Tarazi and Alshawabkeh (2003) found a decrease in cecal pH when including a phytobiotic pigment on broiler diets. In this sense, Nathan *et al.* (2019) demonstrated that *Bixa orellana* has an important bactericidal effect against opportunistic bacteria such as *Salmonella* spp. and *E. coli*. As it is known, this is the first study that demonstrates the bactericidal effect of *Bixa orellana* in broilers, since this experimental group notably reduced total coliforms, *Enterobacteriaceae*, *E. coli* and *Salmonella spp*. in the cecum, although without changes in the population of lactic acid bacteria.

On the other hand, *Murraya koenigii* has a high concentration of carbazole alkaloids (mainly mahanimbine, murrayanol and mahanine), which in small concentrations have a microbial effect against *Staphylococcus epidermidis* (Handral *et al.* 2012). Interestingly, the use of 1 % *Murraya koenigii* on broiler diets decreased the cecal pH due to the increase in the population of LAB in this intestinal portion, however, this experimental group exacerbated the growth of total coliforms, *Enterobacteriaceae* and *E. coli*. Apparently, excess of these secondary metabolites (alkaloids) caused intestinal disorders, confirmed by the decrease in productive efficiency, without cecal competitive exclusion, which affects the selective growth of some pathogenic and beneficial bacteria (table 4). Also, no studies were found that referred to the *in vitro* and *in vivo* bactericidal effect of *Murraya koenigii* against common enterobacteria such as *E. coli* and *Salmonella* spp. However, further research is necessary to confirm this hypothesis.

Other findings of the study are that the synthetic pigment (canthaxanthin) decreased the cecal pH and the population of cecal yeasts and increased the population of total coliforms and *E. coli* compared to the control group (table 4). Therefore, the greater population of cecal pathogenic bacteria could influence the decrease in the response of young birds in this experimental treatment (table 2). Furthermore, the lower cecal yeast population could provoke microbial dysbiosis, since the yeast cell wall serves as a nutritive substance for cecal LAB (Hernández-Ramírez *et al.* 2021), which stimulates the immune response when challenged by opportunistic *Enterobacteriaceae* (Bonato *et al.* 2020). This research demonstrates that the synthetic pigment (canthaxanthin) has no antimicrobial effect on common cecal *Enterobacteriaceae* in young broiler chickens.

Conclusions

Dietary supplementation with 1 % *Bixa orellana* as a natural pigment had a growth-promoting effect and a marked antimicrobial effect *in vivo* in young broiler chickens, without affecting the relative weight of digestive, visceral, and immune organs.

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