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DIETARY CHIA MEAL AND HYDROXYTYROSOL OUTCOMES IN BROILER PERFORMANCE, LIPID BLOOD SERUM PARAMETERS AND MEAT QUALITY

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ABSTRACT

In poultry production, diet is an essential factor that must be considered to achieve optimal animal growth, directly influencing carcass characteristics and broiler health. The present research evaluated the effect of the addition in the diet of two by-products, chia meal and/or hydroxytyrosol, on broiler growth performance, serum metabolites, technological meat quality and color of the breast. Ninety-six broiler chicks were randomly assigned to 16 groups of 6 animals, randomly placed in 1 x 1 m pens, which were distributed in 4 blocks. In each block, experimental treatments were randomly assigned:1) C: control; 2) CM: 10% chia meal; 3) CM+HT: 10% chia meal + hydroxytyrosol; and 4) HT: hydroxytyrosol. The inclusion of hydroxytyrosol improved carcass and breast yield (P<0.05). Dietary chia meal addition may induce growth depression and higher organ weight (P<0.05). Broilers fed

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CM diet recorded lower serum total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL) concentrations, whereas birds fed HT diet increased high-density lipoprotein (HDL) levels (P<0.05) compared to the control. The CM diet resulted in negative effects on pH, water holding capacity (WHC), drip loss (DL) and color of the breast meat (P<0.05). However, the CM+HT diet improved animal blood health and technological meat quality. The results indicate that chia meal and hydroxytyrosol are beneficial dietary components in poultry meat production.

Keywords: Additives, productive traits, biochemical substances, functional properties, color, poultry meat.

INTRODUCTION

The composition of the diet is a critical factor that contributes to efficient sustainable animal production and meat quality. Broiler feeding in intensive systems accounts for up to 70% of total production costs (Thirumalaisamy et al., 2016). The adoption of new feed alternatives that reduce feed costs and meet animal nutritional requirements is essential. Therefore, there is a growing interest in the search for new low-cost feed ingredients that could improve the growth performance of broilers and enable the production of higher quality products that are hygienically safe for public health.

In this sense, several authors have described that diets enriched with different sources of omega-3 polyunsaturated fatty acids (omega-3 PUFAs) have beneficial effects on the immune system, enhancing growth and resistance to poultry diseases (Mendonça et al., 2022; Abdul-Rahman et al., 2023), as well as modifying the fatty acid profile of the meat (Fernández et al., 2022). A feed ingredient that provides high levels of omega-3 PUFAs in Latin America is chia (*Salvia hispanica* L.), which has been used for different purposes throughout history, standing out as a food product of high versatility since it can be used as whole seed, oil or derived products (Capitani, 2013). In Argentina, chia meal is a low-cost agribusiness by-product of oil seed extraction. However, the PUFAs in chia meal are susceptible to oxidation, which reduces their activity and leads to the production of free radicals, which have a deleterious effect on animal health and, consequently, on meat quality (Priscilla and Prince, 2009; Abdell-Moneim et al., 2021). Secondary products of lipid oxidation activate anaerobic glycolysis chain reactions at slaughter, resulting in lower pH, which directly affects meat bleeding and color (Jin et al., 2021). Meat color is a critical parameter that significantly influences consumer acceptability and purchasing decisions (Wideman et al., 2016). Meat paleness associated with PSE (pale, soft, exudative) meats is a problem in the modern poultry industry since it causes substantial economic losses (Chen et al., 2017).

This research hypothesizes that adding an antioxidant would protect these fatty acids from oxidation and improve their function. The use of antioxidants represents an effective method to prevent oxidative damage to cells by neutralizing free radicals (Surai et al., 2019; Vlaicu et al., 2023). Hydroxytyrosol (HT; 2-(3,4-dihydroxyphenylethanol)) is a novel organic antioxidant that could be extracted from different by-products of the olive oil production process (Chashmi et al., 2017). This compound has one of the highest antioxidant activities among polyphenols, exerting a metal ion chelating action and free radical scavenging effects (Rietjens et al., 2007) associated with reduced lipid oxidation and improved oxidative status of broiler meat (Branciari et al., 2017; Fernández et al., 2022). Furthermore, at the intestinal level, HT has an anti-inflammatory, immune-enhancing and bacteriostatic effect, reducing pathogenic bacteria, thus favoring intestinal health, promoting good gut function (Shan and Miao, 2022; Shehata et al., 2022). Most of the studies on the effects of hydroxytyrosol in broilers have been conducted with the addition of olive by-products to the diet. To the best of the authors' knowledge, the potential effect of hydroxytyrosol in broiler diets has been little explored, while there is even more scarce information regarding the synergistic effect of this compound combined with chia meal on broilers. Thus, the objective of this research was to determine the effect of the addition of two novel dietary compounds in poultry diets, hydroxytyrosol (GENOSA I+D, Spain) and chia meal (DESUS S.A., Argentina), on performance, carcass indices, lipid serum parameters and technological meat quality of broilers.

MATERIALS AND METHODS

The study was conducted at the Experimental Broiler Unit of the Department of Agronomy of the Universidad Nacional del Sur (UNS), located in Bahía Blanca (Lat. 38°47' Lo 62°37'), Argentina.

Experimental design, broilers and treatments

A total of ninety-six, one-day-old Cobb-500 broiler chicks were randomly divided into 16 groups of six animals (three male and three female), randomly placed in 1x1 m pens, which were distributed in four blocks. In each block, experimental treatments were randomly assigned: 1) C: control (without chia meal; without hydroxytyrosol); 2) CM: diet with 10% chia meal; 3) CM+HT: diet with 10% chia meal + hydroxytyrosol (7 mg kg BW^{-1} day^{-1}); and 4) HT: diet with hydroxytyrosol (7 mg kg BW^{-1} day^{-1}). The feeding program included two experimental feeding phases: a starter diet (day 1 to day 21) that was the same for all treatments (Table 1), followed by a second phase (day 22 to day 46) consisting of experimental treatments formulated to be isocaloric and isonitrogenous (Table 1). The compound feeds for all treatments were manufactured and stored under ambient conditions weekly. The chia meal presented 59.7% of linolenic acid/total fat (18%) and 4% of soluble fiber/total dietary fiber (40%). The additive was weighed and daily added to the diet. The dose was adjusted on weekly basis according to animal weight gain. Broilers had *ad libitum* feed and freshwater access throughout the study.

Ventilation was controlled and the lighting program consisted of 23 h for the first two days, followed by continuous lighting for the rest of the experiment. During the first week, the temperature was 31 °C, then decreased by 3 °C per week until it reached 23 °C. Handling of animals and experimental protocols in the present study met the code of practice for the care and handling of farm animals recommended by the Servicio Nacional de Sanidad y Calidad Agroalimentaria (SENASA, 2015), following international animal ethics guidelines.

Growth performance, carcass measurements, commercial cuts and organ development

Chicks (male and female) were weighed at 21 days (initial body weight; BWi) and 46 days (final body weight; BWf). Weight gain (WG, g $chick^{-1}$), feed intake (FI, g $chick^{-1}$), and feed conversion ratio (FCR, FI WG^{-1}) were calculated from 22 to 46 days. On day forty-six, thirty-two chicks (two male/block/treatment) were randomly selected,

Table 1. Composition of the starter and experimental diets.

Items (%)	Starter diet (1-21 days)	Experimental diets (22-46 days)			
		C	CM	CM+HT	HT
Ground corn	62.40	69.00	66.00	66.00	69.00
Soybean meal (41% CP)	30.00	23.50	16.66	16.66	23.50
Meat meal (41% CP)	5.75	6.20	6.20	6.20	6.20
Chia meal	-	-	10.00	10.00	-
Ground shell	0.75	0.30	0.30	0.30	0.30
L – Lysine - HCl	0.20	0.13	-	-	0.13
Salt	0.25	0.25	0.25	0.25	0.25
Vitamin-mineral premix[1]	0.50	0.50	0.50	0.50	0.50
DL-methionine	0.15	0.12	0.09	0.09	0.12
Hydroxytyrosol	-	-	-	+	+
Total ingredients					
Chemical composition					
Crude Protein (%)*	20.39	18.48	18.18	18.18	18.48
ME (kcal kg^{-1})**	3048.00	3141.14	3127.87	3127.87	3141.14
Ether Extract (%)*	4.10	4.33	5.86	5.86	4.33
Crude Fiber (%)*	2.85	2.59	4.73	4.73	2.59
Calcium (%)**	1.05	0.91	0.99	0.99	0.91
Total Phosphorus (%)**	0.69	0.70	0.73	0.73	0.70
Methionine+Cystine (%)**	0.83	0.74	0.80	0.80	0.74
Lysine (%)**	1.26	1.04	1.17	1.17	1.04

C: control; CM: 10% chia meal; CM+HT: 10% chia meal and hydroxytyrosol; and HT: hydroxytyrosol. [1]Vitamin A: 8,000,000 UI; vitamin D3: 1,500,000 UI; vitamin E: 30,000 UI; vitamin B2: 3,800 mg; vitamin B6: 1,800 mg; vitamin B1: 1,200 mg; vitamin K3: 1,500 mg; nicotinic acid: 26,000 mg; pantothenic acid: 9,000 mg; folic acid: 600 mg; biotin: 40 mg; choline: 180 g; vitamin B12: 10,000 μg; copper 8,500 mg; iron: 50,000 mg; iodine: 1000 mg; manganese: 70,000 mg; selenium: 250 mg; cobalt: 200 mg; zinc: 60,000 mg; antioxidant: 125 mg: Excipient C.S.P.: 1000 g. ME: metabolizable energy. *Analyzed values. **Calculated values.

weighted (slaughter body weight, BWs), held without feed for 12 h (overnight), slaughtered humanly by cervical dislocation, followed by the cutting of jugular veins at the Agriculture and Livestock abattoir of the UNS. Subsequently, the birds were processed and eviscerated.

Internal organs, small intestine, cecum, liver, pancreas, gizzard, proventriculus and were removed and weighed. In addition, the length of the small intestine was recorded from the pyloric sphincter up to the ileo-ceco-colic junction (full organ). Abdominal fat was weighed, including fat from proventriculus and gizzard. The relative weight of the different organs, length of the intestine and abdominal fat was calculated as the percentages of BWs.

The pH of the intestinal content was measured on a 0.8 g homogenate of jejunum intestinal contents with 10 mL of distilled water, with a thermo-pH-meter (Altronix®) used with a combined electrode, calibrated with pH 4.0 and 7.0 standards. After evisceration, the carcasses were cooled and kept at 4 °C for 24 h. After that, carcasses and commercial cut weights (breast, thigh, drumstick, skin) were obtained. Carcass yield was calculated based on BWs and carcass weights, while commercial cut yield was determined using BWs and commercial cut weights.

Blood serum metabolites

At 41 days of age, blood samples were collected by puncture of the alar vein in two males/block/treatment. Serum was separated by centrifugation (2,300 g x 15′ at 4 °C) and stored at -20 °C until further analyses. Subsequently, serum total cholesterol (TC), triglycerides (TG), and high-density lipoprotein (HDL) concentrations were determined using commercial kits (WIENER LAB, Argentina) following the manufacturer's instructions. The LDL fraction in the serum was determined according to Friedewald et al. (1972) using the following formula: LDL (mg dL^{-1}) = TC - (HDL + TG/5).

Meat quality parameters

After 24 h at 4°C refrigeration of the carcass, pH was determined in the breast muscle *(Pectoralis major)* muscle using an Altronix pH meter by inserting a specific electrode for meat measurements at a depth of 2.5 cm. The pH of each side of the breast (left and right) was read.

Poultry meat color was determined in breast muscle using a Minolta CR-400 colorimeter (Konica Minolta Co., Japan) with standard illuminant D65, following the Commission Internationale de l'Éclairage method (CIE, 1976). The principle of the method is based on the measurement of three parameters: lightness (L*), redness (a*) and yellowness (b*). The variable L* is defined on a scale from 0 (no light, black) to 100 (white). Variables a* and b* define coloration, where a* is a function of the red-green difference ranging from green if negative to red if positive, and b* defines the blue component in negative values and the yellow component in positive values. In addition, Hue angle (*h**, tone) and Chroma (*C**, saturation) were calculated as: $h^* = \tan^{-1} x\ (b^*/a^*)$ and $C^* = [(a^*)^2 + (b^*)^2]$

Quality parameters of water holding capacity (WHC), drip loss (DL) and cooking yield (CY) were performed in duplicate on the breast muscle. Water holding capacity was measured according to the method of Braña Varela et al. (2011), using a breast sample of approximately 0.3 g introduced inside a folded filter paper, previously weighed (initial weight: iw) and placed between two glass plates and subjected to compression with a 2.25 kg weight for 5 min. Subsequently, the meat sample was removed, and the filter paper was weighed (final weight: fw). WHC % was calculated as the percentage of juice weight obtained (g) per g of meat sample using the following formula: WHC (%) = (fw-iw/sample weight) . 100.

The DL was estimated following the methodology of Honikel (1998), where breast pieces of approximately 20 g of fresh breast were cut longitudinally to the muscle fiber. Samples were weighed (iw), kept inside a plastic bag hanging with a wire hook and stored refrigerated at 4°C until final weighing at 48 h (fw). The DL was obtained using the following equation: DL (%) = (iw - fw)/iw . 100.

Cooking yield was determined according to the technique suggested by Honikel (1998) with the following modifications. Samples of approximately 5 g (iw) were placed in plastic zip lock bags and placed in a water bath for 10 min to reach 80 °C internal temperature. Subsequently, the samples were brought to room temperature in a recipient with water, dried without pressing, and weighed (fw). CY was expressed as a percentage of the initial weight of the sample using the following formula: CY (%) = (fw /iw) . 100.

Statistical Analyses

Performance data were analyzed by randomized block ANOVA. BWi was used as a covariate. The experimental unit was the pen, and the unit of measurement was the animal. Commercial cuts, organ weight, abdominal fat weight, serum metabolites, and technological parameters of meat were analyzed as a randomized complete block ANOVA. BWs was used as a covariate for commercial cuts, organ weight, and abdominal fat weight. Analyses were performed using Infostat (Di Renzo et al., 2008) with a significant level of $P<0.05$, and mean values were compared using the Tukey's test.

RESULTS AND DISCUSSION

Growth performance, carcass and commercial cut yield

In the present study, no significant differences ($P>0.05$) were detected in terms of BWi and FI among diets (Table 2). Covariate to BWi was not significant ($P>0.05$) in all variables measured. Broilers fed HT diet gained more weight, presented higher BWf, and converted their feed more efficiently than those receiving chia meal, but no differences were observed with respect to the control diet (Table 2). Similar results were reported by Agah et al. (2019), who used olive leaf extract (OLE) as a component of broiler diet. However, another study conducted by Erener et al. (2020) using OLE at higher doses obtained a significant increase in weight gain in broiler chickens. Similarly, Chila Covachina (2014) observed an increase in weight gain by adding 150 g t^{-1} of syrup of hydroxytyrosol in feed for broilers. Higher doses and different administration forms could explain the differences observed with respect to the present study.

In the present experiment, the dose of 7 mg kg BW^{-1} day^{-1} of hydroxytyrosol could have been insufficient to trigger differences in body weight and FCR of broilers fed HT diet compared to the control. Nevertheless, when the carcass and commercial cut yields were analyzed, male broilers receiving the HT diet recorded higher values in terms of BWs and carcass and breast yield compared to the other treatments (Table 3). In all cases, covariate to BWs was not significant ($P>0.05$). These results could be associated with the presumed health effects of the antioxidant, anticarcinogenic, anti-inflammatory, and antimicrobial activities exerted by hydroxytyrosol (Viveros et al., 2011). Dietary polyphenols can interact with the intestinal microbiota by modifying its composition. For example, lactobacilli can metabolize phenolic compounds supplying energy to cells and positively affecting bacterial metabolism and growth (García-Ruiz et al., 2008). Furthermore, polyphenols would prevent the growth of pathogenic bacteria but promote the presence of non-pathogenic

Table 2. Growth performance of broilers (21 to 46 days of age) fed chia meal and/or hydroxytyrosol diets.

	C	CM	CM+HT	HT	SEM	*p*-value
BWi (g)	772.13	843.07	831.95	813.50	25.20	NS
BWf (g)	2863.56 a	2686.48 b	2673.36 b	2917.77 a	52.04	0.020
WG (g $chick^{-1}$)	2091.43 a	1843.41 b	1841.41 b	2104.27 a	41.51	0.001
FI (g $chick^{-1}$)	4165.54	4134.83	4039.96	4128.80	54.89	NS
FCR (FI WG^{-1})	1.99 b	2.25 a	2.20 a	1.96 b	0.05	0.003

C: control; CM: 10% chia meal; CM+HT: 10% chia meal and hydroxytyrosol; and HT: hydroxytyrosol. BWi: initial body weight; BWf: final body weight; WG: weight gain; FI: feed intake; FCR: feed conversion ratio. SEM: standard error of the mean. [a-b] Means in the same row with different letters differ significantly ($P<0.05$). NS: not significant ($P>0.05$).

Table 3. Carcass and commercial cut yield of broilers (21 to 46 days of age) fed chia meal and/or hydroxytyrosol diets.

	C	CM	CM+HT	HT	SEM	*p*-value
BWs (g)	2947.63 b	2880.00 b	2792.25 b	3241.00 a	84.91	0.02
Carcass (% BWs)	74.60 b	74.48 b	74.25 b	76.06 a	0.41	0.04
Breast (% BWs)	22.35 ab	20.63 b	20.24 b	24.64 a	0.91	0.03
Thigh (% BWs)	10.97	11.54	11.09	10.72	0.37	NS
Drumstick (% BWs)	8.30	8.92	8.82	8.44	0.23	NS
Skin (% BWs)	7.50	7.32	7.34	6.87	0.28	NS

C: control; CM: 10% chia meal; CM+HT: 10% chia meal and hydroxytyrosol; and HT: hydroxytyrosol. BWs: slaughter body weight. SEM: standard error of the mean. [a-b] Means in the same row with different letters differ significantly ($P<0.05$). NS: not significant ($P>0.05$).

microorganisms, improving the health and functioning of the intestinal tract, which might lead to better absorption of nutrients that would be reflected in weight gain and slaughter weight (Viveros et al., 2011).

Modern genotypes of broilers respond to augmented amino acid availability with increased body weight and breast yield (Murray et al., 2014). Polyphenols added to broiler diets have raised the production of amino acids such as serine and L-ornithine (Xiong et al., 2016). L-ornithine could stimulate insulin secretion and pituitary growth hormone (GH) release, promoting muscle growth (protein deposition) and fat catabolism (Wang et al., 2008). In addition, via its receptor, GH stimulates more satellite cells to proliferate, which is associated with postnatal skeletal muscle growth in animals by increasing muscle fiber size and adding more nuclei to the growing muscle (Kim et al., 2005; Pallafacchina et al., 2013). This effect is vital in increasing fiber size, muscle mass and meat production. The number of satellite cells is higher in fast-growing muscles, such as the breast (Al-Musawi et al., 2011; Gao et al., 2016). In treatment HT, the improvement in breast yield could be explained by the more significant response to muscle growth factors (Qiao et al., 2013) and the augmented proliferation of satellite cells in this muscle. The effect of hydroxytyrosol on muscle tissues in broilers is worthy of further study. In contrast to the results obtained in breast yield, no effects on thigh and drumstick muscle yields were observed among treatments (Table 3), which could be explained by differences in fiber-type composition and chemical composition (Chen et al., 2016) between breast and thigh-drumstick muscles.

Regarding chia meal addition, birds fed diets containing omega-3 PUFAs (CM and CM+HT) recorded a lower (P<0.05) performance (BWf, WG and FCR) compared to those fed HT and C diets (Table 2). In addition, values of BWs and carcass and breast yields were lower (P<0.05) compared to the HT diet, showing no differences with respect to the control diet (Table 3). These findings agree with previous reports indicating a lower or no effect with the addition of different sources of omega-3 PUFAs (Azcona et al., 2008). In contrast, Ayerza and Coates (2005) reported greater body weight and feed efficiency in rats fed chia seed or chia oil diets, while Mendonça et al. (2022) evidenced similar results by adding chia oil to the broiler diet but not with chia seed. The differing results between these studies could be associated with different sources of omega-3 PUFAs, the form of supply (flour, oil or seed) and the dietary level of inclusion.

Regarding the level of chia in poultry feed, a limiting factor is its soluble fiber or mucilage. Chia seed contains between 36 and 40 g of dietary fiber per 100 g. During oil extraction, chia flour is obtained with 40% fiber, with 4% corresponding to soluble fiber or mucilage (Muñoz et al., 2013). As indicated by Reyes-Caudillo et al. (2008), this mucilage is a branched, high-molecular weight non-starch polysaccharide (NSP) composed of residues of D-xylose, D-mannose, D-arabinose, D-glucose, glucuronic and galacturonic acid. In contact with water, these polysaccharides expand, forming a highly viscous mass capable of modifying the physiological functions and transit of the gut (Capitani, 2013), which could be reflected in the lower animal performance of broilers fed CM diet. In turn, it is noteworthy that soluble fiber activity could also be reflected in pancreas weight.

Internal organs, pH and small intestine length

Development and health of the gastrointestinal tract are key factors in farm animal productivity, particularly in poultry (Stanley et al., 2013). At 46 days, the gizzard, proventriculus, pancreas, small intestine, and cecum were heavier (P<0.05) in the chia meal diets compared to the C and HT diets (Table 4). In addition, birds fed chia meal diets had longer (P<0.05) intestines than those fed C and HT diets.

As discussed above, the soluble dietary fiber in chia meal appears to be responsible for altering intestinal viscosity by forming a sticky and viscous gel that reduces digesta passage rate and food contact with digestive enzymes, which interferes with nutrient utilization (Tejada and Kim, 2021). The animal compensates for this inefficiency in nutrient digestion and absorption by increasing the weight of the digestive organs, altering the intestinal surface morphology and enlarging the small intestine, thus increasing the maintenance requirements and reducing the energy available for growth (Navidshad, 2009; Sadeghi et al., 2015; Jha and Mishra, 2021). The increase in organ size could be considered as an adaptive response to favor the digestive system capacity and could be another reason for the poor performance in the CM diets. These results are consistent with those of Yaghobfar and Kalantar (2017), who found higher digesta viscosity and pancreatic enzyme activity but lower growth in chicks fed wheat diets, mainly due to the presence of soluble fiber. Similarly, Jimenez-Moreno et al. (2013) observed a higher weight of pancreas, proventriculus, small intestine, cecum, and greater intestinal length due to the effect of fiber from beet pulp.

Regarding liver weight, it is critical to consider that this organ is responsible for fatty acid synthesis in poultry. In this study, no effect was

Table 4. Internal organ weight, pH, and length of small intestine of broilers (46 days of age) fed chia meal and/or hydroxytyrosol diets.

	C	CM	CM+HT	HT	SEM	*p*-value
Intestinal pH	6.99	7.13	7.15	7.18	0.06	NS
Small intestine length (% BWs)	5.93 ab	6.56 a	6.51 a	5.65 b	0.21	0.03
Small intestine (% BWs)	2.29 b	2.70 a	2.69 a	2.11 b	0.11	0.01
Proventriculus (% BWs)	0.30 bc	0.35 ab	0.37 a	0.28 c	0.02	0.01
Gizzard (% BWs)	1.68 c	1.95 a	1.93 ab	1.76 bc	0.06	0.03
Caecum (% BWs)	0.78 ab	0.85 a	0.84 a	0.69 b	0.04	0.06
Pancreas (% BWs)	0.14 ab	0.16 a	0.16 a	0.12 b	0.01	0.05
Liver (% BWs)	1.89 a	1.73 a	1.55 b	1.77 a	0.06	0.01
Abdominal fat (% BWs)	1.57 a	1.35 b	1.36 b	1.21 b	0.06	0.02

C: control; CM: 10% chia meal; CM+HT: 10% chia meal and hydroxytyrosol; and HT: hydroxytyrosol. BWs: slaughter body weight. SEM: standard error of the mean. [a-c] Means in the same row with different letters differ significantly (P<0.05). NS: not significant (P>0.05).

observed (P>0.05) due to the addition of chia meal in the CM diet (Table 4). However, it is noteworthy that liver weight was reduced (P<0.05) when the antioxidant was added in combination with chia meal (CM+HT) (Table 4). This result could be due to an effect of hydroxytyrosol on PUFAs from chia meal, protecting them from lipid peroxidation.

Dublecz et al. (2008) observed that a greater supply of omega-3 PUFAs inhibits hepatic lipogenesis. In this regard, the three treatments containing dietary components showed a decrease in abdominal fat content compared to the control diet (Table 4). The lower abdominal fat content in animals on the chia meal diet could be due to the hepatoprotective effect of PUFAs, which induces lipid redistribution in the body (Poudyal et al., 2012), or the result of poor growth performance due to soluble fiber in the diet. On the other hand, a study conducted by Priore et al. (2017) demonstrated that hydroxytyrosol from extra-virgin olive oil inhibits de novo synthesis of fatty acids (lipogenesis) and cholesterol synthesis through a reduction in the activity of critical enzymes of fatty acid biosynthesis (acetyl-CoA carboxylase-ACC) and cholesterogenesis (3-hydroxy-3-methylglutaryl-CoA reductase-HMGCR), which could account for the reduction in abdominal fat content observed with the use of this compound in the present study. Furthermore, Dagla et al. (2018) found that adding hydroxytyrosol to rat diet has a beneficial effect on hyperlipidemia, by acting upon genes related with adipocyte maturation and differentiation, and inhibiting fat formation.

Blood serum metabolites

Cholesterol levels in CM and CM+HT diets were lower (P<0.05) than those in the C and HT treatments. In turn, serum TG and LDL concentrations decreased (P<0.05) in chia meal diets compared to the control diet (Table 5), with HT values being intermediate. These findings agree with Ibrahim et al. (2018), Long et al. (2020) and Qassim et al. (2022), regardless of the source of omega-3 PUFAs supplied. The reduced content of these compounds observed in chia meal diets could be attributed to the effect of omega-3 PUFAs, which would decrease cholesterol concentrations through the suppression of triglyceride synthesis, increase the removal of very low-density lipoproteins by peripheral tissues or the liver, and increase the excretion of bile in feces (Harris et al., 1990). Regarding HDL concentrations, no differences were observed with the addition of chia meal to the diet, but a better LDL/HDL ratio was obtained, which would be originated by the decrease in LDL levels.

In contrast, higher HDL levels (P<0.05) were observed in HT compared to those in C and CM, whereas CM+HT presented intermediate values (Table 5). The increased levels of HDL could be due to hydroxytyrosol, which would exert a protective effect by increasing HDL resistance to lipoperoxidation by eliminating reactive oxygen species (ROS) (Berrougui et al., 2015). Furthermore, Hernáez et al. (2014) showed that olive oil polyphenols increase HDL particle size, and improve stability of these metabolites and oxidative status by improving particle fluidity, thus enhancing HDL cholesterol efflux capacity. The results of this study align with the findings of Erener et al. (2020), who found higher HDL values by adding different doses of olive leaf extract to broiler chicken diets. Furthermore, González-Santiago et al. (2006) found similar results with the addition of pure hydroxytyrosol

Table 5. Blood serum parameters and LDL/HDL ratio of broilers (46 days of age) fed chia meal and/or hydroxytyrosol diets.

	C	CM	CM+HT	HT	SEM	*p*-value
TC (mg dL^{-1})	129.25 a	106.50 b	109.25 b	133.50 a	6.12	0.01
TG (mg dL^{-1})	91.00 a	74.25 b	68.25 b	80.50 ab	4.78	0.04
LDL (mg dL^{-1})	62.18 a	39.65 bc	34.33 c	49.77 ab	4.82	0.01
HDL (mg dL^{-1})	48.88 c	52.00 bc	61.27 ab	67.63 a	3.79	0.02
LDL/HDL (mg dL^{-1})	1.27 a	0.76 b	0.56 b	0.74 b	0.11	0.01

C: control; CM: 10% chia meal; CM+HT: 10% chia meal and hydroxytyrosol; and HT: hydroxytyrosol. TC: total cholesterol; TG: triglycerides; LDL: low density lipoprotein; HDL: high density lipoprotein. SEM: standard error of the mean. [a-c] Means in the same row with different letters differ significantly (P<0.05).

in the diet of rabbits and rats.

To the best of the authors' knowledge, there is scarce information in the literature regarding the use of pure hydroxytyrosol in the diet of broiler chickens since most of the studies are carried out in humans or laboratory animals. Therefore, the data obtained in the present study provide novel insights into the effects of the incorporation of this compound into broiler diets. Specifically, a noteworthy and novel contribution of the present research is related to the results obtained with the combination of hydroxytyrosol and chia meal in the diet, recording lower TC and TG values compared to those observed in the C and HT diets (Table 5). Likewise, the CM+HT diet presented higher HDL values and a better LDL ratio than the control (P<0.05). Additionally, LDL values were the lowest among all treatments (Table 5).

When analyzing the addition of each dietary component separately, it was observed that hydroxytyrosol did not significantly affect TC and TG levels, while chia meal incorporation did not influence HDL concentrations. However, a synergistic effect was observed when combining both dietary components, boosting the beneficial effects, and thus improving haematological parameters. It should be noted that HDL, LDL, TC, and TG levels are not direct markers of the health status of an animal since they are metabolites of hepatic metabolism (Erener et al., 2020). Nevertheless, alterations in these parameters reflect changes in the physiological and metabolic status and are frequently evaluated in experimental studies to determine and interpret results directly related to animal health (Ozturk et al., 2011; Toghyani et al., 2011).

Meat quality

In this experiment, the pH values at 24 h postmortem registered differences among treatments (Table 6). Animals fed CM diet showed lower values (P<0.05) compared to those fed C and HT diets, with intermediate values in CM+HT. This agrees with the findings of Mendonça et al. (2020), who reported that the addition of chia seeds (16.4%) or oil (2.5%) decreased breast pH at 24 h postmortem. Likewise, similar results were also observed in previous studies conducted by Betti et al. (2009) and Meineri et al. (2018) with the addition of other sources of omega-3 PUFAs (flax seeds or oil) in the broiler diet. It should be noted that although the pH values observed in the present study were lower in diets with chia meal, the values are within the range for standard meat as reported by several authors (Khatun et al., 2018; Mendonça et al., 2020). According to Droval et al. (2012), PSE meats present lower values than meats considered normal (5.9), with final pH estimates at 24 h postmortem of 5.61. Following Braden (2013), the pH of PSE meats decreased rapidly postmortem, reaching even lower values ranging from 5.2 to 5.4. Nevertheless, it is essential to mention that the lower pH observed in the chia meal treatments suggests that these diets must have slightly affected the rate of anaerobic glycogen degradation. Such an assumption is based on the fact that the breast is a cut constituted predominantly by muscle fibers highly specialized in storing and metabolizing glycogen, and the degradation rate of glycogen to lactic acid presents a close relationship with pH at 24 h postmortem (Braden, 2013).

The high concentration of PUFAs in chia meal would be responsible for the decreased pH, as these fatty acids tend to oxidize at a high rate with the consequent production of ROS (Temprado, 2005). Postmortem ROS production activates the enzyme AMPK (AMP-activated protein kinase), which initiates anaerobic glycolysis in muscle, resulting in lactic acid accumulation and a decrease in pH (Hwang et al., 2005; Shen et al., 2007). Under these circumstances, denaturation processes and loss of protein solubility occur, reducing the reactive groups available for water binding in protein muscle (Mir et al., 2018). As a result, the amount of water retained by the

Table 6. Technological parameters and color of *Pectoralis* major muscle of broilers (21 to 46 days of age) fed hydroxytyrosol and/or chia meal.

	C	CM	CM+HT	HT	SEM	*p*-value
pH (24 h)	5.98 a	5.88 b	5.92 ab	6.01 a	0.03	0.050
WHC	30.17 b	34.88 a	31.44 b	31.51 b	0.99	0.040
DL	2.56 c	4.69 a	3.48 b	3.16 bc	0.27	0.002
CY	71.38	69.45	69.76	68.88	0.76	NS
L*	52.43 b	58.32 a	55.94 ab	54.41 ab	1.25	0.050
a*	3.22 a	1.89 b	2.48 b	2.55 ab	0.22	0.010
b*	14.77	15.49	16.67	15.68	0.87	NS
*h**	77.77 b	83.04 a	81.54 ab	80.76 ab	0.90	0.010
*C**	15.12	15.60	16.85	15.89	0.86	NS

WHC: water holding capacity; DL: drip loss; CY: cooking yield. L: luminosity: a*: red intensity; b* yellow intensity; *h**: hue angle; *C**: color saturation index. C: control; CM: 10% chia meal; CM+HT: 10% chia meal and hydroxytyrosol; and HT: hydroxytyrosol. SEM: standard error of the mean. [a-c] Means in the same row with different letters differ significantly (P<0.05). NS: not significant (P>0.05).

muscle is altered, resulting in drier meat cuts and by-products (Jankowski et al., 2012). In the present experiment, WHC values are consistent with those found in pH, observing a smaller water retention capacity at lower pH. The CM diet presented a higher (P<0.05) amount of water loss (Table 6), which is in agreement with the findings of Betti et al. (2009) and Mir et al. (2017), who added flaxseed or flax meal as a source of omega-3 PUFAs. In turn, based on WHC and pH observations, the CM diet increased (P<0.05) the DL value with respect to the C and HT diets, with CM+HT recording intermediate values (Table 6). The higher DL values observed in the CM diet would be explained by the effect of PUFAs as indicated above and agree with the results of Mir et al. (2017), who used flax meal as a source of omega-3 PUFAs. Likewise, no differences (P>0.05) were found in terms of CY between treatments, with values averaging 70.61%. Although it could be argued that there is a certain level of methodological differences, the CY values obtained are similar to the those reported by Betti et al. (2009) and Mendonça et al. (2020).

It is interesting to note (Table 6) that the lower pH values and higher water losses observed in the CM diet were linked to a lighter color (L* values) and lower a* value in the breast. These results support the assumption that rapid postmortem glycolysis, resulting in low pH, leads to protein myoglobin denaturation (Suman and Joseph, 2013) with the consequent greater water exudation, increased light dispersion and, subsequently, lighter and less redness of meat. As mentioned above, PUFA chia meal oxidation would be responsible for decreased pH. In this sense, Qi et al. (2010) suggested that color changes could be related to variations in the oxidative capacity of broilers fed diets with different levels of n6/n3. These authors reported an increase in L* values when the n6/n3 ratio decreased from 10:1 to 5:1 and a lower a* value with increasing omega-3 PUFA (linseed oil) levels up to 5:1 and 2.5:1 in broiler diets. The negative correlation between pH and L* values has been shown by several authors (Petracci et al., 2004; Cori et al., 2014). Betti et al. (2009) observed higher L* and a* values with the addition of flaxseed in broiler diets. In the present study, however, there was there was a decrease in a* values, which could be attributed to the fact that chia meal has a higher content of omega-3 PUFAs compared to that of flax, so its effect on postmortem glycolysis would be more pronounced. In contrast, Terevinto et al. (2023) observed no differences in L* and a* values with the addition of increasing doses (2.5, 5, 10%) of chia seeds, whereas b*, *h** and *C** values in the 10% chia seed diet were lower with respect to the control. In the present research, b* and *C** values were not affected by treatments. Nevertheless, although values were higher in the treatments with respect to the control, all values were in the yellow scale (70 to 100 degrees) of the CieL*a*b* system (Mendonça et al. 2020; Terevinto et al., 2023). In this sense, Mendonça et al., (2020) found decreasing b* values in the breast of broilers fed diets with chia oil or seed, attributing it to greater diversity in the pigment content.

In the present research, it is relevant to highlight that adding the antioxidant hydroxytyrosol to chia flour improved all technological quality parameters, except for meat color (Table 6).

According to Amador (2013), antioxidants exert a protective effect on the integrity of cell membranes, reducing water losses through intercellular spaces. Hence, the CM+HT diet presented lower WHC and DL values ($P<0.05$) with respect to the CM diet, indicating that the incorporation of hydroxytyrosol reduced the effects of the addition of omega-3 PUFAs by preventing its oxidation, thus decreasing the formation of ROS and their effects on pH decrease and water losses. Similarly, Olivo et al. (2001) obtained lower water loss in the breast muscle with vitamin E supplementation, attributing this effect to improved stability of PUFAs and cholesterol due to the incorporation of the antioxidant in the subcellular membrane, where it maximizes its function. It should also be considered that antioxidants would inhibit the glycogen phosphorylase enzyme by suppressing the activation of the AMPK enzyme and would consequently slow down glycolysis in the first postmortem stages, preventing the decrease in pH and the formation of PSE meats (Shen et al., 2005; Betti et al., 2009). A study conducted by Mir et al. (2018) revealed that the supplementation of different doses of lysine and flax meal in poultry increased breast pH compared to the control, which may indicate that the antioxidant slows down postmortem glycolysis in the early stages.

The use of different antioxidants with promising results on broiler meat technological parameters has been widely studied. For instance, the addition of chromium (Mir et al., 2017) and turmeric powder (Kumar et al., 2020) to flaxseed in broiler diets recorded improvements in WHC and DL values. Furthermore, Cheah et al. (1995) suggested that antioxidants would inhibit the enzyme phosphorylase A2, responsible for the hydrolysis of long-chain fatty acids, thereby preventing fatty acid loss and maintaining membrane fluidity. The results obtained in the present study suggest that hydroxytyrosol may have prevented PUFA loss from the muscle membrane, improving its fluidity and integrity, as evidenced in the significant increase in ALA levels in the CM+HT breast, which agrees with the findings of Fernández et al. (2022).

Nevertheless, it should be noted that when hydroxytyrosol was added individually, no differences were observed in technological parameters or meat color. The HT and C diets showed no differences ($P>0.05$) in terms of pH, WHC, DL, L*, a*, and h*. Similar results were obtained by Al-Harthi and Attia (2016) and Branciari et al. (2017), who added olive pomace in broiler chicken diets. However, Marangoni et al. (2017) reported improvements in breast technological quality (pH, DL and WHC) by adding 10 g of olive leaves. In the same study, the authors found no differences in the treatment containing 5 g of leaves. This finding might indicate that the dose of 7 mg kg BW^{-1} day^{-1} of hydroxytyrosol used in the present study could have been insufficient, bearing in mind the difference between the sources of polyphenol supply, its composition and the concentration of active substances. Therefore, the discrepancy with the present work could be explained by differences in the types and doses of antioxidants added. Given the scarce information available in the literature, the results obtained provide the bases for future research on the effect of higher doses of hydroxytyrosol, alone or combined with chia meal, and its effect on the process of AMPK regulation and postmortem glycolysis.

CONCLUSIONS

The results obtained indicate that the single addition of 10% chia meal to the diet is not recommended to improve the growth performance in broilers. However, the combined use of hydroxytyrosol (7 mg kg BW^{-1} day^{-1}) and 10% chia meal in the diet showed a synergistic effect on animal health and the technological quality of the meat, resulting in benefits in shelf-life refrigeration time and cooking process, with no appreciable loss of product yield up to 2 days. Further research is needed to determine the effect of removing chia mucilage or using enzymes to hydrolyze its soluble fiber, as well as the effect of higher doses of hydroxytyrosol on broiler growth performance and meat quality.

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Author Contributions

The authors declare active participation in the bibliographic review by Hebe Fernández and Victoria Fernández Etchegaray; in the development of the methodology: Hebe Fernández, and Victoria Fernández Etchegaray; in the discussion of the results: Hebe Fernández, Claudia de Abreu Rosas, and María Cristina Cabrera; in review and approval of the final version of the article: Hebe Fernández, Victoria Fernández Etchegaray, Rocío Torraca Argüelles, Alí Saadoun, Claudia de Abreu Rosas, and María Cristina Cabrera.

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