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Dietary supplementation with natural antioxidants: assessment of growth performance and meat quality in broiler chickens

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ABSTRACT Exposure to air and light lowers the meat quality of chickens, which is mainly determined by the color alteration and accumulation of detrimental products from lipid oxidation. This study tests the effects of 2 supplements rich in polyphenols, Oxilem (**OX**) and OxiGem (**OG**), on broiler Ross 308 performance and meat quality in comparison to a control group (**C**). A total of 105 one-day-old Ross 308 male chicks were allocated to 21 pens and randomly assigned to 1 of the 3 treatments. The trial lasted 42 d. Individual live weight and feed intake per pen were recorded. Proximate analysis, color, cholesterol content and cholesterol oxidation products (**COPs**), fatty acids (**FAs**), volatile aldehydes profile, and secondary oxidation products of meat FAs were determined, with analyses for detecting oxidative alterations conducted on breast burgers preserved for 7 d at 4° C. Birds fed OG grew 7 g/d more than those receiving OX, reaching a higher final weight. After slaughtering, meat from the OX group had a higher yellow index compared to C. After 7 d of air and light exposure, the influence on the color parameters of the OG and OX burgers was significantly less pronounced than that of C. Secondary oxidation products of the FAs of the burgers were not significantly affected by the diet regimens. In addition, OX and OG burgers exhibited lower amounts of volatile aldehydes, triol and COPs. These results confirm the effectiveness of OX and OG supplementation against lipid oxidation at the inclusion level used in this trial.

Key words: TBARS, COPs, oxidation, polyphenol, additive

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INTRODUCTION

From 2011 to 2021, consumption of poultry meat increased in Europe by 2% per yr. In 2022 it increased a further 1.4%, and this trend is expected to continue for the next 8 yr (European Commission, 2021). The reasons are numerous. The production cycles are short (35 −42 d), the end-products are cheap, and consumers consider their nutritional value high, given that meat is rich in essential amino acids, low in fat, and highly digestible (Bordoni and Danesi, 2017). In addition, there are no religious constraints for chicken meat, thus making the consumption of poultry products suitable for most of the world's meat-eating population (European Commission, 2021; FAO, 2023).

Meat foods generally contain cholesterol the content of which varies depending on the animal species and on the feeding strategies used during rearing. In poultry meat, cholesterol ranges from 47 to 59 mg/100 g in chicken breast to 84 mg/100 g in chicken leg, and thigh (Dinh et al., 2011).

Although cholesterol is a lipid component of the cell membrane and is involved in several biological processes, a high cholesterol consumption is linked with disease onset, above all hypercholesterolemia, obesity, diabetes, brain and cardiovascular diseases (López-Fernández et al., 2022). More detrimental are the cholesterol oxidation products (**COPs**) or fatty acid peroxidation products whose synthesis is favored by air and light exposure of the meat during storage on market counters or during the processing chain and cooking. Moreover, fat oxidation may contribute to limit the shelf-life of the meat and, thus, decrease the profitability of the whole supply chain (Fellenberg and Speisky, 2006).

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The raw color of chicken meat is probably one of the most important indicators of spoilage and wholesomeness, factors that strongly influence consumers' purchasing choices (Font-i-Furnols and Guerrero, 2014). Hence, from a technological point of view, controlling against meat lipid oxidation is key to product treatment.

Polyphenols are bioactive molecules produced by plants in the secondary metabolism as a defense against pathogens and herbivores. This class of compounds is very heterogeneous, and the chemical or microbiological activity is strongly linked to their molecular structure and water solubility. The main properties of polyphenols are antimicrobial and antioxidant (Fellenberg and Speisky, 2006; Luciano et al., 2013; Scicutella et al., 2021). Their transfer to muscular mass has been hypothesized to enhance the antioxidant properties of meat, thereby improving its nutritional and technological quality (Luciano et al., 2013).

Focusing on lipid quality and meat oxidative stress, this study aimed to evaluate the effects of 2 commercial supplements rich in polyphenols, Oxilem and OxiGem, on the performance and meat quality of Ross 308 broilers, with particular interest in the oxidative status of meat.

MATERIALS AND METHODS

Ethics Statement

Animal handling was in accordance with Italian Government guidelines (D.lgs 26/2014, protocol number 232/2016PR).

Animal Handling and Experimental Design

The trial was carried out at the Department of Agriculture, Food, Environment and Forestry, University of Florence, Italy.

A total of 105 one-day-old Ross 308 male chicks were provided by a local hatchery (Incubatoio Settecrociari, Forlì-Cesena, Italy). Immediately after birth, the chicks were vaccinated against Marek's disease, infectious bronchitis, and Newcastle disease. The chicks were randomly allocated to 21 pens with a dimension of 1 m × 1 m, on coconut fiber litter (5 chicks per pen individually identified by a leg ring) and randomly assigned to 1 of the 3 treatments. The sample size and the power analysis were computed by G*Power 3.1 (Faul et al., 2007). The experimental design was conceived following Bello et al. (2016) considering the pen as the experimental unit (7 pens per treatment).

Diets

Isoproteic and isoenergetic diets were formulated to meet NRC animal requirements (NRC, 1994) and checked with Aviagen ones; the periods of growth were: starter (0−12 d), grower (13−21 d), and finisher (22−42 d). The trial lasted 42 d.

The feeding groups consisted of the control (**C**), Oxilem (**OX**), and OxiGem (**OG**). Each group consisted of 35 birds. The diet composition is shown in Table 1. The control group received no supplementation, while, throughout the entire test period, the experimental

Table 1. Ingredients, proximate composition, and fatty acid composition of the control diet during the 3 growth periods.

Ingredients	Unit of measurement	Starter (0−12 d)	Grower (13−21 d)	Finisher (22−42 d)
Corn meal	g/100 g of diet	52.05	57.00	58.00
Soybean meal	g/100 g of diet	35.50	33.10	31.05
Soybean oil	g/100 g of diet	5.50	6.25	7.30
Gluten	g/100 g of diet	3.00	-	-
Calcium phosphate	g/100 g of diet	1.90	1.90	1.90
Calcium carbonate	g/100 g of diet	1.50	1.20	1.20
Sodium bicarbonate	g/100 g of diet	0.25	0.25	0.25
Sodium chloride	g/100 g of diet	0.25	0.25	0.25
DL-methionine	g/100 g of diet	0.25	0.25	0.25
L-Lysine HCl	g/100 g of diet	0.15	0.15	0.15
Choline chloride	g/100 g of diet	0.15	0.15	0.15
Mineral-vitamin supplement	g/100 g of diet	0.50	0.50	0.50
Proximate composition				
Dry matter	g/100 g of diet	88.59	88.96	88.78
Crude proteins	g/100 g of diet	22.00	20.00	19.03
Ether extract	g/100 g of diet	7.43	7.99	8.24
Ash	g/100 g of diet	7.06	7.09	7.01
NDF	g/100 g of diet	10.13	13.84	13.01
ADF	g/100 g of diet	7.71	7.56	7.05
ADL	g/100 g of diet	2.12	2.05	1.96
ME	Kcal/kg of diet	3,000	3,100	3,200
Fatty acid composition				
C14:0	g/100 of FAs	0,3	0,3	0,3
C16:0	g/100 of FAs	11,4	11,4	11,4
C16:1 *9c*	g/100 of FAs	0,2	0,2	0,2
C18:0	g/100 of FAs	3,5	3,5	3,6
C18:1 *9c*	g/100 of FAs	24,8	24,8	24,6
C18:2 n-6	g/100 of FAs	53,6	53,6	53,7
C18:3 n-3	g/100 of FAs	5,9	5,9	6,0

NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; ME, diet metabolizable energy; FAs, fatty acids.

groups received: 2 g/kg on dry matter basis (**DM**) of Oxilem (OX) or 2 g/kg on DM basis of OxiGem (OG).

Oxilem (OX) is a natural biopolymer of hydrolyzed lignin extracted from *Pinus taeda* spp. (loblolly pine tree). OxiGem (OG) is a combined additive, composed of Oxilem (80%) plus Sanitres (20%), which contains gum rosin from the pine tree (mainly abietic acid, dehydroabietic acid, palustric acid, neoabietic acid, pimaric acid, isopimaric acid, and sandaropimaric acid. In total approximately 9% of OxiGem). Oxilem and OxiGem were obtained from Green Innovation GmbH, Innsbruck (Austria).

Oxilem is a powder composed of medium-small phenyl-propanic aggregates (10−12 monomers with a molecular weight of 12,000−13,000 g/mol). The most abundant monomers are the p-coumaryl alcohol polymers. A detailed description of the phenolic components and the antioxidant activity of Oxilem was reported by (Maggiolino et al., 2019). The total polyphenol content of Oxilem was 31.43 g/100 g of additive dry matter on a gallic acid equivalent (**GAE**) basis, thus resulting in a dietary concentration of 0.623 g/kg of diet dry matter. OxiGem contained 25.16 g/100 g of additive dry matter on a GAE basis, thus resulting in a dietary concentration of 0.503 g/kg of diet dry matter.

Diet Proximate Analysis

Diets were sampled at the start and end of each feeding period (starter, grower, finisher) and analyzed in terms of the proximate profile: crude protein (**CP**), ether extract (**EE**), crude fiber (**CF**), and ash. Analyses were performed according to the AOAC methods (976.06, 920.39, 962.09, and 942.05, respectively; AOAC, 1995). Neutral detergent fiber (**NDF**), acid detergent fiber (**ADF**) and acid detergent lignin (**ADL**) were determined according to Van Soest et al. (1991), using heat-stable amylase and sodium sulfite, and expressed inclusive of residual ash.

Diet metabolizable energy (**ME**) was estimated from feed tables according to Sauvant et al. (2002). Table 1 reports the chemical and nutritional profile of the basal diets.

In Vivo and Postmortem Data Collection

The individual body weight (**BW**) of the birds was measured weekly, and the feed intake (**FI**) was evaluated daily per pen. From these measurements, the average body weight gain (**BWG** g/d) was calculated as the gravimetric difference between 2 subsequent weeks (divided per the numbers of days), and the feed conversion ratio (**FCR**, feed kg/BWG kg) was calculated for each pen. Individual BWGs and FCRs were estimated by dividing the calculated values by the daily number of birds in the pen.

At the end of the experimental period, birds were individually weighed and slaughtered in a public abattoir located less than 10 km from the experimental farm. After slaughtering, the carcass of each bird was weighed (carcass weight, **CW**), and the dressing yield (**DY**) of each subject was calculated. Immediately after the slaughtering, the entire breast of each bird was collected, wrapped in aluminum foil, vacuum stored, and immediately transferred to the lab using a portable refrigerator under dry ice before being stored in a freezer at −20°C until the analysis.

Each breast (105 in total, namely 35 per treatment) was divided into 2 subsamples. Half of the breast was used to perform analysis of the nutritional and physicochemical quality of the meat. A subsample of 3 breasts for each pen (randomly chosen) was used to prepare burgers (63 burgers, 21 per treatment) each about 150 g, which were analyzed for lipid oxidation. Burgers were stored for 7 d at 4°C in a temperature-controlled cold room in the dark on a polystyrene platform for foodstuffs and wrapped in a transparent film until analysis. No modified atmosphere or other strategy intended to limit lipid oxidation, except for darkness, was employed.

Meat Analysis

Proximate Composition The moisture content was analyzed maintaining the samples at 105°C for 24 h in a laboratory ventilated oven. Crude fat was determined according to AOCS official method AM 5-04 (AOCS-American Oil Chemists' Society, 2004) using an Ankom XT10 Extractor (Astori Tecnica, Brescia, Italy). Protein content was analyzed according to AOAC official procedure 981.10 with a Kjeldahl apparatus (Macedon NY). The mineral level by weight difference was assessed by placing samples into a high-temperature muffle furnace where the temperature was maintained at 550°C for 3 h. The carbohydrate percentage was calculated as the complement to 100 of the sum of the fat, protein and minerals.

The pH value was determined using a HI98190 portable pH-meter (Hanna Instruments, Padua, Italy) equipped with XS sensor 2-pore S7 (XS Instruments, Modena, Italy).

Color The color was assessed all the chicken breasts. The assessment of color on the 63 burger (21 per treatment), was performed immediately after burger manufacturing and repeated after 7 d of storing. The color was measured in the CIE $L^*a^*b^*$ space (CIE, 19876) with a measured area diameter of 8 mm, specular component included, and 0% UV, D65 standard illuminant, observer angle 10°, and zero and white calibration by a Konica Minolta CR-600 portable apparatus (Holdings, Inc., Osaka, Japan). Lightness (L^*),$a*$ index, and b^* index, were recorded. Hue angle (h°) and chroma (C*) indexes were calculated as $h° = \tan^{-1}(b/a^*)$ expressed as an angle between 0° and 360°, and $C^* = \sqrt{a^2 + b^2}$ expressed as a numerical value. Measurements were taken at 4 randomly selected locations for each sample. Total color evaluation used the following formula: $\Delta E* = [(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2]^{1/2}$. According to Sanz et al. (2009) the color differences are not visible to

the human eye when $\Delta E* < 3$. Values were expressed as mean ± standard deviation.

Fatty Acid Composition The fatty acid composition was evaluated for both diets and the half chicken breast used for the nutritional and physicochemical quality evaluations (105 samples in total, 35 per treatment). Total lipids were extracted from 5 g of sample using a chloroform/methanol mixture (2:1, v/v) according to Serra et al. (2009). Fatty acid (**FA**) composition was determined by cold base-catalyzed transesterification according to Christie (1982). The methyl ester of nonadecanoic acid (Sigma Aldrich, St. Louis, MO) was used as an internal standard. Fatty acid methyl esters (**FAME**) were separated and identified using a GC-FID apparatus (GC 2000 plus, Shimadzu, Columbia, MD) according to Serra et al. (2009).

Cholesterol and COPs The analysis of the lipid oxidation was conducted on breast burgers (63 samples in total, 21 per treatment). To quantify the cholesterol and cholesterol oxidation products (**COPs**), 250 mg of total lipids were saponified according to Hulshof et al. (2006). Dehydrocholesterol and 19-hydroxycholesterol (Steraloids, Newport, RI) were used as internal standards for cholesterol and COPs, respectively. An aliquot (about one-tenth) of the unsaponifiable matter was used for the analysis of total cholesterol, and the rest was used for the analysis of COPs after purification and concentration using an NH2-SPE cartridge (Sigma-Aldrich, St. Louis, MO). A sequence of 3 eluents was used: n-hexane: ethylacetate (95:5, v/v), n-hexane: ethylacetate (9:1, v/v), acetone. The last fraction, containing COPs, was saved. Cholesterol and COPs were silylated by a solution of pyridine, hexamethyldisilazane and trimethylchlorosilane (5:2:1, v/v/v). The silylated derivatives were injected into a GC-FID apparatus (GC 2000 plus, Shimadzu, Columbia, MD) equipped with a VF 1-ms apolar capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; Varian, Palo Alto, CA). The COPs were identified by direct comparison with commercial standards (Sigma Aldrich, St. Louis, MO) and quantified using internal standards. Data were expressed as mg/kg of sample.

Secondary Oxidation Products of Fatty Acid Analysis (TBARS Test) Breast burgers (63 in total) were analyzed in terms of lipid oxidation. The secondary oxidation products of FAs were evaluated by the thiobarbituric acid reactive substance (**TBARS**) test in accordance with Salih et al. (1987). Briefly, malondialdehyde was extracted with a solution of trichloroacetic acid (5%) from 5 g of the sample; a 40 mM solution of thiobarbituric acid (**TBA**) was added, and the sample was put into a water bath at 93°C for 20 min until complete color development. Lastly, malonaldehyde (**MDA**) was quantified using a spectrophotometer (Cary 50, Varian, Palo Alto, CA) comparing the absorbance at 532 nm, with a calibration curve using a solution of tetraethoxypropane.

Volatile Aldehydes The volatile aldehydes were determined on 63 burgers by SPME-GC/MS. Five grams of each sample were put into a 20-mL glass. Headspace conditioning was achieved in 15 min, by keeping the sample in a water bath at 35°C. A SPME fiber (50/30 μm; 2-cm long, DVB/Carboxen/PDMS; Supelco, Bellefonte, PA), conditioned for 30 min at 270°C in a GC injector, was exposed to the headspace for 30 min. Lastly, the sample was injected into a single quadrupole GC/MS apparatus (TRACE GC/MS, Thermo-Finnigan, Waltham, MA) coupled with a Varian CP-WAX-52 capillary column (60 m × 0.32 mm; coating thickness 0.5 μm). The injector was set at 250°C and the complete volatile organic compounds (**VOCs**) desorption was achieved in 30 min. The oven GC program temperature was set according to Serra et al. (2009). The temperature of the transfer-line and the ion source was 250°C, while the filament emission current was set at 70 eV. The acquisition was performed in full scan mode (TIC) using a mass range from 35 to 270 m/z (1.6 amu/s).

Statistical Analysis

Data were statistically analyzed using the following linear mixed model with the JMP software, v. 17.0 (SAS Institute Inc., Cary. NC):

$$y_{ij} = \mu + D_i + B_j[D_i]random + e_{ij}$$

where:

y_{ij} = variable related to ith diet and to jth box.
μ = mean.
D_i = fixed effect of ith diet with i ranging from 1 to 3 (CON, OX, OG).
B_j = variable effect of jth pen with j ranging from 1 to 21, nested into ith diet (7 pens per group).
e_{ij} = residual error related to ith diet and to jth box.

Differences were declared significant at $P < 0.05$. When the treatment effect was significant, the post hoc analysis was conducted with a Tukey test.

RESULTS AND DISCUSSION

Animal Performance and Meat Yield

Throughout the whole period of the trial, no mortality events or diarrhea episodes occurred, and the birds showed behaviors consistent with their ethology.

All diets were consumed by the birds, indicating that the 2 supplements tested in this trial did not affect the palatability of the feeds. In fact, no statistical differences in FI and FCR were found between groups throughout the trial, except for the FI at wk 6 and the average FCR (Table 2). Despite these limited differences, several other modifications were found, some of which seemed to be due to the additives fed (or not fed) to the birds.

Table 3 lists the body weight (**BW**) and the average body weight gain (**BWG**) as affected by the diet supplementation. The BW of the 3 groups was similar over the first 4 experimental weeks. Differences became statistically significant between groups at the fifth and sixth weeks.

Table 2. Feed intake and Feed conversion ratio.

	Feed intake (kg/wk)					Feed conversion ratio (kg feed/kg LW/wk)				
Wk	C	OX	OG	SE	S	C	OX	OG	SE	S
1	0.16	0.16	0.16	0.01	ns	1.20	1.25	1.21	0.05	ns
2	0.43	0.43	0.43	0.01	ns	2.23	2.23	2.23	0.07	ns
3	0.74	0.74	0.77	0.01	ns	2.70	2.77	2.74	0.04	ns
4	0.91	0.85	0.82	0.03	ns	2.58	2.60	2.52	0.05	ns
5	1.11	1.18	1.20	0.04	ns	3.00	3.43	3.36	0.13	ns
6	1.03[a]	0.89[b]	1.06[a]	0.03	**	2.65	3.05	3.00	0.27	ns
Total	4.39	4.26	4.34	0.08	ns	1.85[b]	1.99[a]	1.85[b]	0.03	*

C, control diet; LW, live weight; OX, Oxilem; OG, OxiGem; SE, standard error; S, significance; ns, not significant.
*$0.01 < P < 0.05$.
**$0.01 < P < 0.001$.
different superscript letters indicate significant differences ($P < 0.05$) between treatments

The OX group had a significantly lower BW and BWG than the other 2 groups. These differences were also confirmed by the final BW and by the BWG throughout the experimental period. Birds in the OG group grew more than 7 g/d than those receiving OX, with a 30 g higher final BW. Also, birds in the C group grew more than those receiving OX, with approximately 6 g/d more, and reaching a 23 g higher final BW than the OX group. Consequently, compared to the other groups OX had the lowest CW (Table 3), but a not statistically different DY.

This result is consistent with Jamroz et al. (2009) and Schiavone et al. (2008) who noted that the supplementation showed antinutritive effects but did not affect the carcass quality.

The literature reports contrasting information on the effect of polyphenols on broiler performance and gut health. Several studies have reported the efficiency of polyphenols in exercising antimicrobial activities and in ameliorating bird performance when used as additives in poultry feeding (Schiavone et al., 2008). In contrast, other studies have reported that polyphenols reduce protein digestibility in monogastric species, decreasing the productive performance in accordance with a lower availability of nitrogen for the animal nutritional requirements (Griffiths and Moseley, 1980; Ortiz et al., 1993; Ortiz et al., 1994; Smulikowska et al., 2001; Garcia et al., 2004). In fact, Mansoori and Acamovic (2006) noted that polyphenols (belonging to the tannin species) may stimulate the hypersecretion of endogenous enzymes leading to losses in sulfur amino acids in poultry.

The differences between the performance of the experimental groups can be partially explained by the use of the OX additive, which affected LW (Table 3), and, consequently, the FI (Table 2). As reported in Table 2, after the fifth week, the birds fed with the OX diet consumed a significantly lower amount of feed than the C and OG groups, had a lower average daily consumption, and consequently had the lowest LW (both wk 5 and 6, Table 3). In fact, birds in the OX group had an overall higher FCR compared to the C and OG groups.

This thus suggests that phenolic supplementation negatively affected the feed intake of OX birds. This is not surprising as phenol compounds have been considered as antinutritional factors (Chamorro et al., 2013; Scicutella et al., 2021). Considering the capability of polyphenols to bind proteins and the fact that LW and BWG decreased only at the end of the trial without affecting the birds' behavior or ethology, the differences observed are probably related to the level of polyphenol inclusion (Oxilem 100% in OX Oxilem 80% in OG) or to the mixed additive effect (Oxilem plus Sanitres) as reviewed by Scicutella et al. (2021).

Surprisingly, the OG diets showed an opposite effect compared to the OX diet. As the OX and OG were supplied with about same quantity of phenols, it is possible that the gum rosin contained in Oxigem limited the negative effect of the phenolic compounds. There is much

Table 3. Animal growth and slaughter performance.

	Body weight (kg)					Average body weight gain (g/d)				
Wk	C	OX	OG	SE	S	C	OX	OG	SE	S
1	0.18	0.18	0.18	0.01	ns	19.80	18.95	19.76	0.70	ns
2	0.44	0.43	0.45	0.01	ns	36.89	37.45	37.55	0.91	ns
3	0.88	0.85	0.89	0.02	ns	62.18	59.43	63.86	1.79	ns
4	1.45	1.36	1.36	0.05	ns	82.03	73.00	66.59	4.03	ns
5	1.94[a]	1.78[b]	1.95[a]	0.04	*	70.52[b]	59.88[c]	85.07[a]	5.16	*
6	2.34[a]	2.11[b]	2.41[a]	0.05	**	80.98[b]	66.30[c]	90.47[a]	4.05	**
Total	-	-	-	-		57.62[a]	51.81[b]	59.05[a]	1.16	**
CW	1.76[a]	1.57[b]	1.76[a]	0.04	*	-	-	-	-	
DY (%)	74.82	73.73	74.24	0.66	ns	-	-	-	-	

C, control diet; OX, Oxilem; OG, OxiGem; SE, standard error; S, significance; ns, not significant.
CW, carcass weight; DY, dressing yield.
*$0.01 < P < 0.05$.
**$0.01 < P < 0.001$
different superscript letters indicate significant differences ($P < 0.05$) between treatments.

evidence that essential oils and/or the lipid fraction of plants can affect the growth of poultry by interacting with the microbiota (Amerah et al., 2011; Kettunen et al., 2015; Haapakorva et al., 2018; Vienola et al., 2018; Aguirre et al., 2019). The effectiveness of pure resin acids in improving the performance and health of poultry has recently been recognized (Apajalahti et al., 2020). The mechanism explaining such an effect has not yet been clarified, but it could be associated with a repressed growth of pathogen microorganisms (Vienola et al., 2018).

Meat Quality

Physicochemical Properties Table 4 reports the proximate composition, total cholesterol content, and the pH of the chicken breast. Supplementation proved to be a significant variation factor for the crude protein content, expressed as a percentage of meat dry matter. Birds fed the OG diet showed the highest concentration of total protein (over 1 percentage point higher than the other 2 groups). Such findings confirmed the patterns reported for BWG (Table 2), as well as for FI and FCR (Table 3). This thus suggests an additive effect in OG compared to OX in determining a distinct protein accumulation in the breast, which might be attributed, at least in part, to the gum rosin from pine tree compounds, which was only contained in the OG diet. On the other hand, the lower CP in the OG breast might have been a secondary effect after the lower fat deposition, even though it was not significant, which may also have been caused by the OG additive compounds. An increased protein content with a simultaneous decrease in crude fat content was found in Ross 308 breast fed with 7.5% of fermented dried olive pomace, which is naturally rich in simple phenolics, polyphenols, oleuropeosides, and flavonoids (Ibrahim et al., 2021). The authors suggested that the role of the bioactive compound might have been to increase the protein synthesis in meat. However, other multiple concomitant causes might have caused such an effect, and further investigations are needed to clarify this.

Overall, this effect was interesting, as the growth and development of broiler muscle (approximately 40% of the total body mass) are important economic traits since they are strongly linked to the productivity of the poultry industry.

Table 2 also shows the color values of the meat. This important physical parameter is defined by lightness (L^*), which represents the reflectance of the color, and the combination of the redness and yellowness, measured using the red index (a^*) and the yellow index (b^*), respectively. These indexes give the hue angle (h), which is an angular measure identifying the type of pure color, and chrome (C*) which represents the intensity of pure color.

Supplementation was a significant variation factor with respect to the yellow index and, therefore, h°. Meat from the OX group showed a higher yellow index than that from the C group, while OG yellowness fell between C and OX. This effect might be linked to the reducing activity of phenolic compounds on metmyoglobin (**MetMb**) to oxymyoglobin ($\mathbf{MbO_2}$), as previously found in vitro conditions (Miura et al., 2014). Indeed, many compounds contained in Oxilem (Maggiolino et al., 2019) and consequently, in higher concentrations in OX diets, have a catechol substructure. This substructure can reduce MetMb to MbO_2 in the presence of cysteine, which is naturally contained in chicken meat (Miura et al., 2014). Accordingly, OX had the lowest hue angle, followed by OG and C, thus indicating a greater redness, though not significant.

Lipid Profile Table 5 shows the fatty acid composition of the chicken breast. The diet significantly affected the FA profile, especially with respect to the linoleic acid (C18:2 n-6, LA) and γ-linolenic (C18:3 n-6, γ-LNA) content, which increased in OX compared to C and OG. In line with this, supplementation of active compounds (i.e., curcumin) has been reported to increase unsaturated FAs, probably because of enhanced desaturase enzyme efficiency (Galli et al., 2020). In fact, C17-1 9c expressed as g/100 g of fatty acids was also significantly higher in the OX compared to C groups.

Regarding the composition of fatty acids expressed as a percentage (g/100 g of fatty acids, Table 5), the diet quality also significantly affected the amount of C23:0 and EPA (C20:5 n-3).

Chickens have enzymes (i.e., elongases and desaturases) that convert essential FAs, such as LA (for the omega 6 series) and a-linolenic acid (**ALA**, C18:3 n-3 for the omega 3 series), into long-chain omega 6 and omega 3 derivatives. Of these, particular importance is given to FAs with 20 carbon atoms, namely arachidonic (**ARA**, C20:4 n-6) and eicosapentaenoic (**EPA**, C20:5 n-3), due to the bioactive role that they play. ARA and EPA serve as precursors of eicosanoids.

The eicosanoids deriving from the ARA are generally proinflammatory, whereas those deriving from the EPA show high anti-inflammatory properties. Among the

Table 4. Proximate composition, cholesterol, energetic value, and color of chicken breast.

Parameter	C	OX	OG	SE	S
Moisture (g/100 g)	74.96	74.27	73.69	0.34	ns
Ash (g/100 g)	1.19	1.16	1.25	0.03	ns
Total lipids (g/100 g)	1.00	1.12	0.95	0.17	ns
Crude protein (g/100 g)	22.08^{b}	22.24^{b}	23.36^{a}	0.26	**
Energy (kcal/100 g)	98.39	100.17	102.93	1.62	ns
Cholesterol (g/100 g di TL)	3.62	3.16	3.39	0.23	ns
Cholesterol (mg/100 g)	48.34	47.72	47.67	3.82	ns
pH	5.91	5.92	5.88	0.05	ns
Color					
Lightness (L^*)	57.11	57.43	55.23	0.7	ns
Yellow index (a^*)	2.33^{b}	3.70^{a}	3.14^{ab}	0.29	***
Red index (b^*)	13.25	14.24	14.00	0.35	ns
Hue angle (h°)	80.50^{a}	75.33^{b}	77.51^{ab}	0.95	**
Chrome (C*)	13.57	14.73	14.37	0.40	ns

C, control diet; OX, Oxilem; OG, OxiGem; SE, standard error; S, significance; ns, not significant.
*$0.01 < P < 0.05$.
**$0.01 < P < 0.001$.
***$P < 0.001$; different superscript letters indicate significant differences ($P < 0.05$) between treatments.

Table 5. Fatty acid composition of chicken breast.

	g/100 g of fatty acids					mg/100 g of meat				
Fatty acids	C	OX	OG	SE	S	C	OX	OG	SE	S
C14	0.29	0.32	0.30	0.01	ns	3.18	3.93	3.03	0.34	ns
C16	16.69	16.19	16.84	0.28	ns	178.04	196.98	168.15	16.03	ns
C16-1 7*c*	0.31	0.32	0.34	0.01	ns	4.02	4.06	3.46	0.50	ns
C16-1 9*c*	1.69	1.64	1.58	0.07	ns	19.38	20.71	16.78	2.54	ns
C17	0.10	0.11	0.11	0.00	ns	1.11	1.28	1.11	0.11	ns
C17-1 9*c*	0.14^{b}	0.17^{a}	0.16^{ab}	0.01	*	1.61	2.17	1.64	0.20	ns
C18	7.52	7.00	7.56	0.26	ns	78.53	84.05	73.12	5.98	ns
C18-1 12*t*	0.05	0.05	0.05	0.01	ns	0.51	0.57	0.60	0.08	ns
C18-1 9c	29.96	29.94	29.86	0.49	ns	328.42	370.08	304.50	33.76	ns
C18-1 11*c*	1.47	1.35	1.45	0.05	ns	15.99	16.51	14.23	1.52	ns
C18-2 n-6	33.00^{b}	34.97^{a}	33.37^{ab}	0.49	*	361.76^{b}	432.96^{a}	339.68^{b}	38.02	*
C18-3 n-6	0.26^{a}	0.27^{a}	0.23^{b}	0.01	*	2.87^{b}	3.45^{a}	2.42^{b}	0.37	*
C20-1 11*c*	0.32^{a}	0.29^{b}	0.32^{a}	0.01	**	3.39	3.56	3.21	0.33	ns
C18-3 n-3	0.32	0.35	0.33	0.02	ns	3.69	4.42	3.54	0.49	ns
C20-2 n-6	0.87	0.72	0.83	0.08	ns	8.91	8.52	7.55	0.85	ns
C20-3 n-6	0.66	0.57	0.61	0.04	ns	7.24	6.92	6.07	0.82	ns
C20-4 n-6	3.48	3.17	3.29	0.25	ns	40.02	39.43	31.03	5.52	ns
C23	0.09^{ab}	0.07^{b}	0.12^{a}	0.01	**	0.84^{ab}	0.79^{b}	1.12^{a}	0.07	*
C20-5 n-3	0.04^{a}	0.03^{b}	0.02^{b}	0.01	***	0.35	0.33	0.17	0.06	ns
C22-4 n-6	1.46	1.33	1.40	0.12	ns	16.98	16.21	13.20	2.31	ns
C22-5 n-6	0.40	0.36	0.39	0.04	ns	4.16	4.29	3.81	0.58	ns
C22-5 n-3	0.14	0.14	0.12	0.02	ns	1.45	1.70	1.13	0.22	ns
C22-6 n-3	0.06	0.06	0.07	0.01	ns	0.70	0.73	0.72	0.10	ns
Total	100	100	100	100	ns	1089.01	1228.66	1006.64	106.21	ns
Fatty acid classes										
SFA	25.30	23.77	25.20	0.49	ns	264.97	290.34	249.28	22.63	ns
UFA	74.69	76.23	74.79	0.49	ns	823.71	938.39	757.39	83.83	ns
MUFA	33.07	33.85	33.91	0.72	ns	374.85	420.22	346.28	38.72	ns
PUFA	40.65	42.62	40.93	0.79	ns	447.92	518.26	411.22	46.24	ns
PUFA n-6	39.89	41.92	40.29	0.76	ns	439.19	509.82	404.83	45.33	ns
PUFA n-3	0.76	0.65	0.61	0.09	ns	8.36	7.90	5.88	1.04	ns
n-6/n-3	52.48	64.49	66.04	4.94	ns	52.48	64.49	68.84	4.94	ns

C, control diet; MUFA, monounsaturated fatty acids; ns, not significant; OX, Oxilem; OG, OxiGem; PUFA, polyunsaturated fatty acids; S, significance; SE, standard error; SFA, saturated fatty acids; UFA, unsaturated fatty acids.

*$0.01 < P < 0.05$.

**$0.01 < P < 0.001$.

***$P < 0.001$;different superscript letters indicate significant differences ($P < 0.05$) between treatments.

omega-6 series, LNA plays a positive role in human health as a precursor of dihomo-γ-linolenic acid (**DGLA**, C20:3 n-6). Through the action of cyclooxygenase enzyme, DGLA produces prostaglandin E1, which is known for its anti-inflammatory, vasodilator, antipain, bronchial dilator and anti-impotence effects in males (Wang et al., 2012).

As shown in Table 5, birds fed a diet supplemented with OX were higher in LA and γ-LNA compared to the C group, while the OG group showed an intermediate value. Since γ-LNA acid is produced from linoleic acid by the action of **Δ**-6 desaturase enzyme, it is not surprising that γ-LNA was significantly higher in the OX group. It is conceivable that the diet affected the further elongation and desaturation steps that would lead to the formation of DGLA (elongation) and ARA through the **Δ**-5 desaturase enzyme. Indeed, DGLA and ARA showed no differences between groups (Table 5). The higher concentration of LA found in breast meat from birds fed OX despite their lower FI, could be related to a possible different use in the muscle of LA, which is the main FA in the lipid supplement of the diets. This could explain the worse FCR in the OX group compared to the other 2 groups, suggesting a lower feed efficiency in converting nutrients/energy in the muscle.

The higher content of LA in the meat lipid fraction from birds fed OX and the similar percentage of ARA in all meats, regardless of the diet, suggest that the LA elongation to ARA was not enhanced by the higher percentage of the ARA precursor according to the downregulation of genes related to the next elongation steps. The downregulation of genes involved in ARA endogenous syntheses could be due to the direct effect of gum rosin or to the negative one caused by the LA accumulation.

In mammals, the inhibition of LA on the elongation of FA (from C4 to C16) in cells has been demonstrated (Ungerfeld et al., 2019). Moreover, the differences in concentration between C and OX could also be partially explained by the lesser extent of oxidation of LA to hexanal (see Table 7).

The OG supplementation caused no significant changes in the FA profile compared to the control diet except for EPA, which showed a comparable concentration level with OX but lower than in the C meat. This finding could be attributed to the upregulation effect by gum rosin on certain genes involved in the elongation of this FA to DHA, which is primarily incorporated in the phospholipids of the nervous system or brain.

Table 6. Color parameters in burgers made with meat from 3 diets at 0 and 7 d of storage.

	C				OX				OG			
Color parameter	0	7	SE	S	0	7	SE	S	0	7	SE	S
Lightness (L)	57.11	45.70	0.67	***	57.43	47.16	1.13	***	55.23	45.96	1.58	***
Red index (a^*)	2.33	1.21	0.45	*	3.70	3.07	0.55	ns	3.14	3.15	0.81	ns
Yellow index (b^*)	13.25	11.28	0.71	***	14.24	13.63	0.80	ns	14.00	13.84	1.57	ns
Hue angle (h°)	80.50	83.16	1.59	*	75.33	77.67	1.44	*	77.51	78.36	1.62	ns
Chrome (C*)	13.57	11.45	0.74	***	14.73	14.08	0.87	ns	14.37	14.25	1.72	ns

C, control diet; OX, Oxilem; OG, OxiGem; SE, standard error; S, significance; ns, not significant.
*$0.01 \le P < 0.05$.
**$0.01 < P \le 0.001$.
***$P < 0.001$.

Dietary treatments had no effect on the fatty acid classes (i.e., PUFA, MUFA, SFA, etc.).

Lipid Oxidation The effects on lipid oxidation protection, influenced by the addition of phenolic compounds, were analyzed in burgers stored for 7 d at 4°C. Table 6 shows the color parameters of each group, whereas Table 7 lists the FA and cholesterol oxidation data in burgers during the 7-day storage period.

The data reported in Table 6 are particularly interesting, as all the color parameters were significantly affected by the storage time of the C burgers, but not the OX and OG burgers. Lightness was the sole parameter to markedly decrease in all burgers, regardless of the additive supplementation. On the other hand, it is well known that lightness is not a particularly informative proxy of meat discoloration, whereas red and yellow indexes can be easily employed to assess the color loss of meat. A decrease in the red index and an increase in the hue angle indicate a deterioration in the color of the meat (Luciano et al., 2013b). As previously mentioned, burgers made with meat from birds fed only with the standard diet underwent a highly significant decrease in the red index and, consequently, an increase in the hue angle. Interestingly, burgers made with the OX meat showed a good color stability during storage. The OX burgers, instead, showed good resistance in terms of the red index but suffered a significant increase in the hue angle.

These results are important since color is a key physical characteristic that strongly influences consumers during purchasing. Protecting color remains a primary objective in conservation techniques and technologies (Font-i-Furnols and Guerrero, 2014).

An interesting parameter considered was $\Delta E*$ as an indicator of the total color differences of the meat. According to Sanz et al. (2009), color differences are only visible to the human eye when $\Delta E* > 3$. All burgers showed an eye-visible difference of color when the variation from 0 to 7 d of cold-storage period was calculated (Figure 1A). Figure 1B reports the difference in color between OG or OX burgers compared to C burgers. A significantly lower color difference was obtained with burgers from birds fed OG compared to C burgers, suggesting an effect of OxiGem on limiting the alteration in meat color.

The deterioration in color has been associated with the oxidation of the lipid component (Xiong et al., 2020). The meat color is a result of the interaction between the myoglobin type, oxidation status of the iron in the eme-group, and the oxidation status of FAs. Plant extracts containing phenolic compounds have antioxidant activities that may reduce the formation of free radicals and, consequently, the oxidation of PUFAs (Galli et al., 2020). Assessing lipid oxidation is complicated and needs more than one proxy to be correct and complete. The oxidation of FAs is usually estimated through the thiobarbituric acid reactive substances (**TBARS**) test, which only accounts for malonaldehyde (**MDA**). MDA is one of the major products of the oxidation of FAs with 3 or more double bonds, as it comes from breaking the cyclic peroxides produced during the oxidation of this kind of FA. Therefore, TBARS is a good test for estimating the oxidation of LA and/or longer-chain polyunsaturated FAs with 3 or more double bonds (i.e., ARA, EPA, DHA, etc.). The most represented FAs of intramuscular fat of the chicken breast analyzed in our experiment were LA (about 32−33%) and oleic acid (about 30%) (Table 5). They have 2 and 1 double bonds, respectively. In contrast, LNA represents less than 0.5% of the total FAs (Table 5).

It was thus not surprising that secondary oxidation products of FAs were not significantly affected by the diets. Conversely, one of the largest products of LA

Table 7. Oxidation of fatty acids and cholesterol in chicken burgers after 7 d of storage (mg/kg).

Oxidation parameter	C	OX	OG	SE	S
TBARS	0.39	0.47	0.39	0.05	ns
Hexanal	0.93^a	0.26^b	0.27^b	0.27	*
Nonanal	0.48^a	0.29^b	0.14^c	0.09	**
Total volatile aldehydes	2.08^a	1.04^b	1.13^b	0.42	**
Cholesterol	521.26	457.80	455.43	22.80	ns
7 α-hydroxicholesterol	1.43	0.39	0.77	0.37	ns
7 β-hydroxicholesterol	2.27	1.21	1.20	0.50	ns
β-epoxycholesterol	1.52	0.70	0.82	0.26	ns
α-epoxicholesterol	0.42	0.25	0.26	0.07	ns
Triol	7.94^a	1.83^b	2.6^b	1.62	*
7-ketocholesterol	1.84	0.94	1.13	0.33	ns
Total COPs	15.43^a	5.31^b	6.78^b	1.80	**
COPS/Cholesterol (%)	2.91^a	1.17^b	1.66^{ab}	0.38	*

C, control diet; OX, Oxilem; OG, OxiGem; SE, standard error S, significant; ns, not significant.
*$0.01 \le P < 0.05$.
**$0.01 < P \le 0.001$.
different superscript letters indicate significant differences ($P < 0.05$) between treatments.

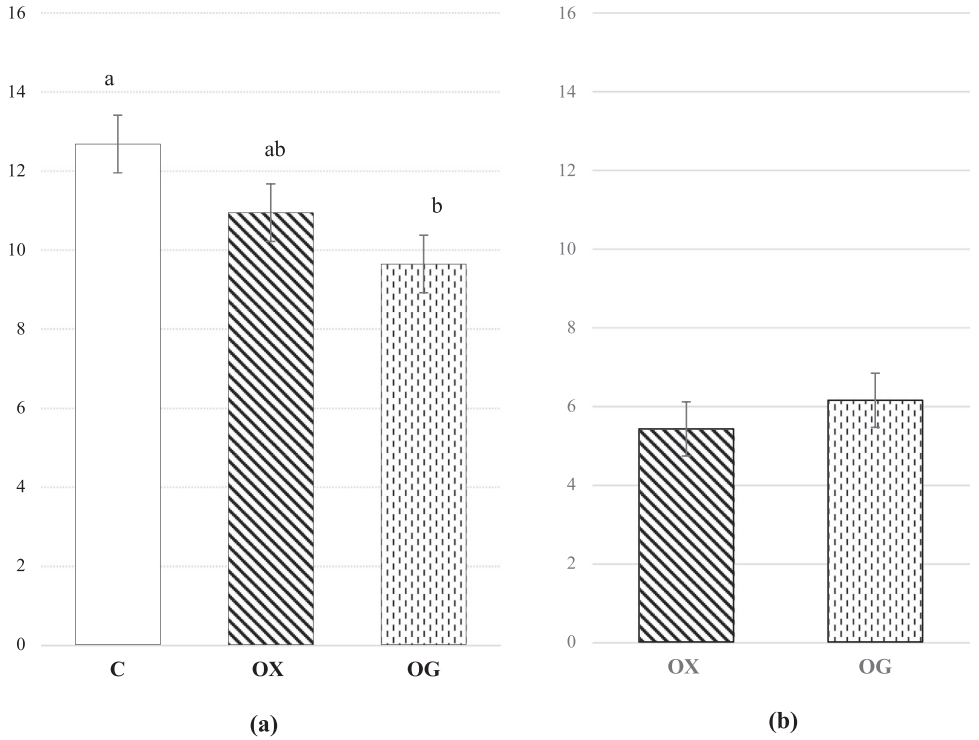

Figure 1. Total color differences calculated as $\Delta E* = [(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2]^{1/2}$ (Sanz et al., 2009). (a) ΔE∗ between 0 and 7 d of storage in 3 groups; (b) ΔE∗ of group OX or OG as difference with respect to C. Different superscript letters indicate significant differences ($P < 0.05$).

oxidation is hexanal (Cossignani et al., 2014) and one of the major oxidation products of oleic acid is nonanal (Tavassoli−Kafrani et al., 2016). Both are volatile aldehydes, which in our study were significantly affected by the supplementation (Table 7).

In fact, the hexanal of OX and OG burgers was 3 times lower than in the C burger. The supplements fed to OX and OG groups were protective mainly against the oxidation of LA, but also against the oxidation of oleic acid. The excellent protection against FA oxidation of OX and OG was also supported by the amount of total volatile aldehydes, which was almost half than in the C burgers.

Oxidation products affect both the length of the storage period (a key parameter for the food industry) as well as the nutritional quality of the product itself.

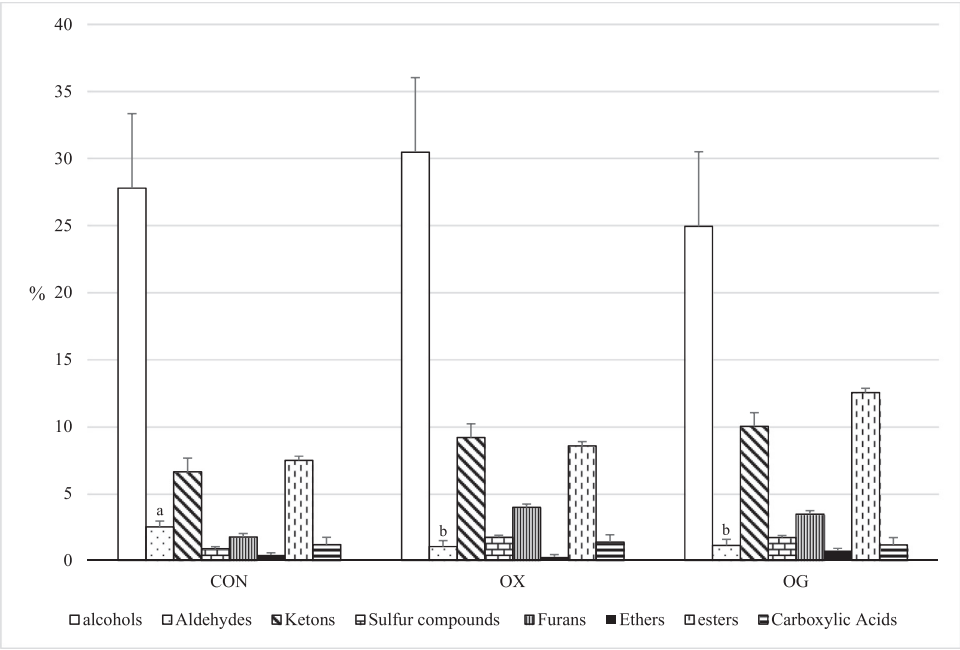

Figure 2. VOCs profile of burger stored 7 d. Values are tested within VOCs and between diets. Different superscript letters indicate significant differences ($P < 0.05$).

Aldehydes are the substances most implicated in the deterioration of the aroma of food (Morales et al., 1997). They are also highly reactive compounds, which represent "secondary toxic messengers" able to react with proteins, thus favoring the production of substances that negatively affect their stability and structure (Pizzimenti et al., 2013).

Figure 2 shows the profile of the volatile organic substances (**VOCs**), which defines the burgers' aroma stored at 4°C for 7 d. In our study, the most represented categories were alcohols, followed by esters and ketones. Aldehydes were the only category affected by diet; thus, confirming that such substances are excellent estimators of FA oxidation. Their determination is therefore important to ensure a correct and exhaustive assessment of meat lipid oxidation.

Our study also aimed to verify the effect of the diet on the oxidative stability of cholesterol. Cholesterol is an unsaturated lipid which undergoes oxidization through a mechanism similar to the oxidation of FAs. However, as the products of cholesterol oxidation are odorless (COPs are not volatile) and colorless, they cannot influence the technological quality of the meat and are not perceived by consumers. On the other hand, COPs dramatically affect the nutritional quality of meat, as they show higher pathological and cytotoxic effects than unoxidized cholesterol, as they are involved in the development of several human diseases. COPs are implicated in all steps of the atherosclerosis process, and the oxidized LDL-c plays a more negative role than nonoxidized LDL-c (Maldonado-Pereira et al., 2018). Therefore, minimization of COP formation is desirable.

In this study, 6 different products of cholesterol oxidation (**COPs**) were detected: 7 α-hydroxycholesterol, 7 β-hydroxycholesterol, β-epoxycholesterol, α-epoxycholesterol, triol, and 7-ketocholesterol. Of these, triol and the total content of COPs were significantly lower in OX and OG compared to C meat. These data thus confirm the effectiveness of our proposed supplementation in preventing lipid oxidation.

CONCLUSIONS

The inclusion of OX and OG in the poultry diets at a level of 2 g/kg of DM did not affect the birds' performance until the last week of the trial. Introducing both additives in the feeding strategy reduced the lipid oxidation of the meat during storage, extending its shelf-life. Broilers fed with the diet supplemented with OX and OG showed lower levels of total volatile aldehydes, triol and COPs in their meat. The color preservation in the meat from birds fed OX and OG suggested better freshness maintenance. Supplementing with Oxilem (biopolymer of hydrolyzed lignin extracted from *Pinus taeda* spp. or loblolly pine) or Oxigem (gum rosin from pine) could thus enhance the nutritional quality of broiler meat, while maintaining animal welfare and in vivo performance. Further investigations are needed to determine the minimum effective concentration of these additives for cost-effective commercial use.

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DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in the present study.

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