



EFFECTS OF DIETARY SUPPLEMENTATION WITH PHYTOBIOTIC ENCAPSULATED PLANT EXTRACTS ON BROILERS' PERFORMANCE PARAMETERS, WELFARE TRAITS AND MEAT CHARACTERISTICS

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Abstract

Background: Medicinal plants exhibit significant effects on broiler physiology and production. This trial aimed to evaluate the effects of dietary supplementation with an aqueous or cyclodextrin extract derived from a mixture of *Origanum vulgare* L., *Camelina sativa* L. Crantz, *Crithmum maritimum* L., and *Allium sativum* L. at the level of 0.1% per kg of dry matter (DM) on broiler growth performance, welfare, meat chemical composition, and oxidative stability.

Results: Cyclodextrin treated group presented significantly higher final body weight, while feed intake and FCR were unaffected by the treatments. Both aqueous and cyclodextrin treated groups had significantly lower pododermatitis score compared to the control group. Additionally, both treatment groups presented increased thigh meat protein content and lower fat content, likewise meat color was also positively affected by both treatments. Supplementation of broilers diet with the cyclodextrin extract also resulted in Σ n-3 fatty acids and Σ n-6 fatty acids increase. The PUFA/SFA and H/H ratios were also increased in the thigh meat of both supplemented groups compared to the control group.

Conclusion: The application of aqueous or cyclodextrin extracts yielded positive results on meat composition, and supported growth performance, welfare, and the oxidative status of the chicken meat.

Key words: broilers, phytobiotics, cyclodextrin extract, performance, meat protein and fat oxidation

The excessive use of antibiotic substances, during the last decades, in the intensive broiler management system has led to financial losses and increased consumer concern regarding the quality of broiler meat and pathogen resistance to common medicine (Nhung et al., 2017). Since strict legislative regulations concerning antibiotic residues in broilers' meat have been decreed, the need for alternative practices to increase production, health status, and product quality has become crucial, not only in the scientific community but also in the commercial world (Chishti et al., 2013). The use of phytobiotic extracts as an unconventional feed supplement to achieve these goals remains an ongoing global experimental process with encouraging results (Grashorn, 2010).

The use of plant extracts in food technology is multifunctional, as they exhibit antioxidant and antibacterial effects and have beneficial effects on the texture of meat and meat products (Giannenas et al., 2018). Edible

wild herbs growing naturally on uncultivated lands, or as weeds on farms, have traditionally played an important role in supplementing staple foods, and they might do so again in the future.

An innovative approach of phytobiotics being incorporated in poultry diets occurs through encapsulated forms. It is evident that these forms exhibit superior characteristics compared to non-encapsulated ones (Giannenas et al., 2018), such as protection against oxidation and slow-release rate. Among delivery systems, used for the encapsulation of bioactive compounds from medicinal plants through formation of molecular inclusion complexes, are cyclodextrins. Cyclodextrins, a group of cyclic oligosaccharides that have the structure of a truncated cone, can act as host molecules that form inclusion complexes with polyphenols. The encapsulation of bioactive compounds in the cavity of cyclodextrins can effectively protect them from oxidation phenomena and

prolong their antioxidant activity. These multifunctional ingredients offer various advantages, increased stability rate to essential oils that are abundant in volatile compounds (Dhifi et al., 2016). Apart from encapsulation, cyclodextrin solutions have been efficiently applied as extraction media for the extraction of polyphenols from various plant matrices. In fact, aqueous solutions of cyclodextrins could be considered as alternative green solvents, with the ability to extract a variety of bioactive compounds, both hydrophobic and hydrophilic, thus increasing the extraction yield (Bennett et al., 2011). The hydrophobic cavities of cyclodextrins become hosts for hydrophobic polyphenols, forming inclusion complexes, whereas the solvent (water) extracts hydrophilic compounds. However, it should be noted that the affinity between cyclodextrin molecules and bioactive compounds of the plant materials plays a crucial role on the final extraction yield and the selection of the appropriate cyclodextrin solution based on the compounds of interest should be carefully investigated.

Origanum vulgare L. (*Lamiaceae*) is a perennial herb, native to temperate Western and Southwestern Eurasia and the Mediterranean region (Bakkali et al., 2008). It is a medicinal plant with numerous applications and great worldwide economic importance (Baydar et al., 2004). *O. vulgare* L. extracts exhibit antimicrobial, antioxidant, and antifungal properties (Mastelić et al., 2008; Stefanakis et al., 2014, 2013). These properties are attributed to the phenolic fraction of its essential oil and especially to carvacrol and thymol (Wogiatzi et al., 2009; Burt et al., 2004; Vollmann et al., 2007; Obour et al., 2018). *Camelina sativa*, an herbaceous plant belonging to the *Brassicaceae* family, has raised significant interest (Rodríguez et al., 2021). *Camelina sativa* (L.) Crantz, has potential to diversify cropland and benefit the environment by providing forage resources for native and non-native pollinators (Atia et al., 2011). The seeds of camelina are rich in oil (Meot-Duros et al., 2010), which contains the polyunsaturated linoleic and linolenic fatty acids, accounting for up to 55% of the total oil fatty acids, as well as in very long-chain fatty acids like gondoic and erucic acid (Pilgeram et al., 2007). *Crithmum maritimum* L. (*Umbelliferae*), commonly known as sea fennel or rock samphire, is an aromatic, edible, and medicinal halophyte common in marine coastal ecosystems along the European and North African Atlantic, Mediterranean and Black Sea (Tchorzewska et al., 2017; Ruberto et al., 2000). Sea fennel has been considered as a functional food, largely used for its nutritional and health promoting value. This is due to its high content of vitamins, carotenoids, polyphenols, and other bioactive constituents, which could be used for aromatic, medicinal, and other applications (Najda et al., 2016). *Allium sativum* L. (garlic) has been grown on a massive scale on all continents for thousands of years. The presence of many biologically active substances in garlic, especially in its underground bulb, makes the plant suitable for use in alternative medicine. The properties of garlic are as-

sociated with its composition. It contains approximately 33 sulfur compounds, 17 amino acids, enzymes, mineral salts (e.g., germanium, selenium, phosphates, calcium, and iron salts), vitamins (e.g., ascorbic acid, riboflavin, niacin, thiamine, folic acid), and valuable essential oils (Ariga and Seki, 2006; Singleton et al., 1999; Brauer-Vigoderis et al., 2014). Essential oils mode of action is attributed to their biological active compounds, like carvacrol that can effectively modulate digestion through enzyme activation or intestinal microflora modification (Saad et al., 2013). Supplementary, essential oils have the ability to increase tissue oxidative stability resulting in superior final product quality (Giannenas et al., 2018). Various aromatic and medicinal plants have been reported in the literature to provide beneficial outcomes in poultry producibility, yet scientific community is exploring the possibility to enhance essential oils potency and overall bioactivity, through their combination, as a result of synergy among different compounds (Tak and Isman, 2015).

The main goal of the present study was to investigate the possible effects of a phytobiotic extract, prepared by the combination of the aforementioned medicinal plants on broilers' performance parameters and welfare indexes. The effects of the mixture on meat chemical composition, color, oxidative stability, as well as fatty acid profile were also investigated. A secondary target was to compare the effectiveness of the cyclodextrin plant mixture compared to the respective aqueous one.

Material and methods

Ethics and procedures

Husbandry, euthanasia, experimental procedures, and biosecurity precautions were conducted in accordance with Greek legislation governing experimental animals and were approved by the local Public Veterinary Service (Reg.489181(3254)/07.02.2018) in research experimental facilities. All institutional and national guidelines for the care and use of laboratory animals were followed. This research was supported from EU and national Greek Funds, under the research program Bilateral R&T Cooperation Greece-China, 2014–2020 (*T7ΔKI-00313-GreenPro*).

Materials

2,2-Diphenyl-1-picrylhydrazyl (DPPH• >97.0%) and β-cyclodextrin (β-CD, 98.0%) were supplied from TCI (Tokyo, Japan). Folin-Ciocalteu reagent, sodium carbonate (Na₂CO₃, 99.8%) chloroform, methanol and n-hexane were purchased from Chem-Lab NV (Zedelgem, Belgium). Standard compounds, Trolox™ (97%) and gallic acid (98+%) were obtained from Sigma-Aldrich (Darmstadt, Germany) and Alfa Aesar (Heysham, United Kingdom), respectively, whereas a standard mixture containing 37 fatty acids was from AccuStandard (New Haven, USA).

Animals, diet, and experimental design

A total of 120 as hatched one-day-old Ross-308 chicks, kindly donated by PINDOS APSI hatchery, were randomly allocated into 3 equal groups with 4 replicates of 10 birds each. All subgroups were housed in separate floor pens, each equipped with an infrared lamp, in a specially designed experimental room at the Institute of Animal Science, Hellenic Agricultural Organisation-DEMETER, Paralimni (latitude 40.45°, longitude 22.27°), Giannitsa, Greece, during the period of September and October 2020, where the temperature, the relative humidity, and the lighting program were controlled, following the recommendations of the breeding company (Aviagen®). Temperature gradually decreased from 36°C on day 1 to 24°C on day 21 and then kept constant. The lighting regime provided 24 h of continuous light per day until the 2nd day of the experiment and 23 h thereafter. The health of the animals was monitored twice daily by a veterinary surgeon. Birds were vaccinated against Newcastle disease and infectious bronchitis by spray vaccination, as well as against infectious bursal disease by subcutaneous vaccination on the first day of hatchery. Table 1 provides the detailed composition of the control diet that is based on maize and soybean meal in mash form, formulated according to breeder company recommendations (Aviagen®, ROSS Nutrition Specifications; Aviagen: Huntsville, AL, USA). Based on this basal diet, additional diets were prepared by incorporating the phytobiotic mixture composed either of aqueous extracts at 0.1% per kg of dry matter (DM) or cyclodextrin extracts at the same level. All diets were equal in terms of energy and protein values. The three groups were named as control, OGCC-AQ (diet supplemented with aqueous extract), and OGCC-CD (diet supplemented with cyclodextrin extract).

Preparation of aqueous and cyclodextrin quadruple extracts

A. sativum peels were ground in a mill (Janke and Kunkel, IKA Labortechnik, Germany). The particle size of the ground garlic peels was determined at 0.1 mm. Extractions of *O. vulgare* L., *C. maritimum* L., *C. sativa* L. plant materials, and *A. sativum* L. peels were performed using double distilled water and saturated aqueous solution of β -cyclodextrin or β -CD (1.85 g/100 mL) as solvents. The solid/liquid ratio (S/L) was determined at 1/10 w/v for each plant material based on preliminary experiments. The cyclodextrin solution was prepared by dissolving the β -CD in double distilled water through magnetic stirring (700 rpm) at 40°C for 1 h. After complete dissolution, the plant material was added and the stirring continued at 700 rpm and 25°C for 3 h. The obtained mixtures were filtered, and the extracts were stored at -18°C until use. The extract of each plant material was prepared separately, and the individual extracts were then mixed before storage. The volume of the final extract was 1000 mL (Table 2).

Table 1. Broiler chicken basal diets

Ingredients (%)	Starter	Grower	Finisher
	Days 1–14	Days 15–28	Days 29–35
Maize	55.50	60.00	61.00
Soybean meal	35.77	30.70	28.62
Soybean oil	3.50	3.50	4.50
Palm fat	-	1.00	1.50
Calcium phosphate	1.46	1.33	1.28
Limestone (calcium carbonate)	1.86	1.68	1.53
Salt	0.28	0.23	0.23
Sodium carbonate	0.21	0.21	0.19
Lysine	0.41	0.40	0.35
Methionine	0.39	0.35	0.31
Threonine	0.22	0.21	0.15
Valine	0.15	0.14	0.09
Vitamin and mineral premix*	0.25	0.25	0.25
Total (kg)	100.00	100.00	100.00
Calculated analysis (As fed basis)	3000	3070	3150
Metab. energy (Kcal/kg)			
Moisture (%)	10.15	10.55	11.14
Protein (%)	22.00	21.00	20.00
Crude fiber (%)	2.85	2.65	2.55
Crude fat (%)	4.85	6.11	6.65
Ash (%)	6.12	5.65	5.58
Lysine (%)	1.41	1.28	1.15
Methionine+Cystine (%)	1.08	0.99	0.92
Methionine (%)	0.73	0.67	0.62
Threonine (%)	0.98	0.89	0.79
Tryptophan (%)	0.28	0.25	0.24
Valine (%)	1.10	1.02	0.92
Total NSP (%)	9.50	7.50	6.50
Calcium (%)	0.99	0.93	0.85
Total phosphorus (%)	0.71	0.65	0.62
Sodium (%)	0.24	0.23	0.22
Chloride (%)	0.24	0.23	0.22

*Supplying per kg feed: 12,000 IU vitamin A, 5,000 IU vitamin D₃, 30 mg vitamin E, 3 mg vitamin K, 3 mg thiamin, 7 mg riboflavin, 6 mg pyridoxine, 0.035 mg vitamin B₁₂, 40 mg niacin, 13 mg pantothenic acid, 1.5 mg folic acid, 0.13 mg biotin, 340 mg choline chloride, 55 mg Zn, 155 mg Mn, 20 mg Fe, 12 mg Cu, 0.2 mg Co, 1 mg I, 0.2 mg Se, and phytase 0.01 g.

Determination of total phenolic content and DPPH radical scavenging activity of quadruple extracts

The TPC of the extracts was determined by Folin-Ciocalteu method (Singleton et al., 1999). The results were expressed as mg gallic acid equivalents (GAE)/g plant material. A method described by Vasilopoulos et al. (2022), was applied to determine antiradical activity. Trolox™ was used to create a standard curve, and the results were presented as μ mol Trolox equivalents (TRE)/g plant material (mM TRE).

Table 2. Composition of quadruple extracts

Plant material (dried and powdered form)	Solvent	Final volume
50 g <i>Origanum vulgare</i> L. 50 g <i>Crithmum maritimum</i> L. 50 g <i>Camelina sativa</i> L. Crantz 10 g <i>Allium sativum</i> L.	1000 mL β -CD aqueous solution (1.85mg/mL)	1000 mL (300 mL oregano extract, 300 mL samphire extract, 300 mL camelina extract and 100 mL garlic peels extract)
50 g <i>Origanum vulgare</i> L. 50 g <i>Crithmum maritimum</i> L. 50 g <i>Camelina sativa</i> L. Crantz 10 g <i>Allium sativum</i> L.	1000 mL double distilled water	1000 mL (300 mL oregano extract, 300 mL samphire extract, 300 mL camelina extract and 100 mL garlic peels extract)

Performance parameters

All chicks were individually weighed when placed into the pens and subsequently at weekly intervals. Feeding was withdrawn four hours prior to weighing, and feed consumption within each subgroup was determined. The feed conversion ratio was calculated weekly, and mortality was recorded daily in each subgroup.

Welfare status

Footpad dermatitis was evaluated on days 10, 24 and 35. Footpads from 2 birds per pen, randomly selected, were cleaned with brush and both pads were evaluated at the same time. The presence of hock burns and their severity was evaluated, using scoring categories from 0 to 2 (where 0 represents no evidence of pododermatitis and 2 represents severe pododermatitis). Feathering condition was evaluated on 2 birds per pen on days 10, 24 and 35 through a 3-point scoring system ranging from 1 to 3 (where 1 refers to clean feathers and 3 to very dirty feathers). Diarrhea scores were evaluated on day 10, on 2 birds per pen, using categories from 1 to 3 (where 1 indicates absence of diarrhea and 3 severe diarrhea). On days 10, 24 and 35, fecal scores were evaluated on surface per pen, using categories from 1 to 4 (where 1 represents firm and well-formed feces and 4 represents watery liquid feces) (Vasilopoulos et al., 2022). On days 10, 24 and 35, litter scores were evaluated on 2 samples, coming from pooled samples of 3 locations per pen, using categories from 1 to 5 (where 1 illustrates dry and crumbly litter and 5 illustrates capping or completely wet litter). Fresh litter (wheat straw) was added weekly to the pens after day 21, and at the same quantity in each pen. This happened because the birds could spoil some water through the bell drinkers, increasing the moisture of the litter (Brauer-Vigoderis et al., 2014). On days 10, 24 and 35, litter and fecal DM and litter NH_3 were analyzed as follows. From each pen, 5 litter samples of 100 g each were collected (four samples from corners and one from the centre). The 5 litter samples were pooled twice and homogenized prior to DM analysis (2 values of litter DM per pen, each sample taken as a mix of all five locations). For litter DM analysis, the samples were weighed using precision scales, dried at 120°C for 6 h and then weighed again to determine the weight difference (Patsoukis et al., 2004). The DM was also analyzed on fresh fecal 10 g sample collected from each pen. For litter and fecal

sampling, at least 5 subsamples for litter and at least 3 subsamples for feces were collected to reach a final sample of litter or feces, respectively. For litter pH analysis: 10 g of each sample were placed in a beaker and then 100 ml of distilled water was added; after mixing the samples with a glass rod and allowing them to stand for 30 min, the pH value was determined using a pH meter (Vasilopoulos et al., 2022). Kjeldahl nitrogen (%) was determined by the micro-Kjeldahl method (Guebel et al., 1991). Litter NH_3 was determined on fresh litter samples collected from each pen. Spleen, bursa and thymus of the two selected birds per replicate were weighed after slaughter on day 35.

Carcass characteristics and breast and thigh meat composition and oxidative status

Two birds per replicate were used for the analysis of the meat chemical composition, which were individually marked (leg bands) and then transported and processed in a commercial slaughterhouse, according to local practices. Carcasses were handled similarly to Vasilopoulos et al. (2022). Wooden breast and white striping scores were evaluated on the same birds, using categories from 0 to 2 (for wooden breast: 0 represents good and 2 severe; and for white striping: 0 represents normal without any distinctive white lines and 2 represents severe presence of white lines in parallel to muscle fibers that were > 1 mm thick). The breast meat and the thigh meat of the same birds were carefully separated from skin and bones, and then they were ground using an industrial large meat grinder. Fresh samples of 200 g of the minced meat were analyzed for moisture, crude protein and fat content, by near infra-red spectroscopy using a DA 7250 (Perten, Sweden) in transmittance mode, by the reference method 2007.04 for meat and meat products (Anderson et al., 2007). Thiobarbituric acid-reactive substances (TBARS) in broiler meat samples were determined according to Ahn et al. (2006) with minor modifications.

Determination of meat fatty acids

Aliquots of the meat samples of the same birds per subgroup were freeze-dried using a HyperCOOL HC8080 freeze-dryer (Gyrozen Co., Ltd, Korea) (−95°C, 0.1 mbar) for 48 h. Freeze-dried samples were ground with a household blender and stored in the freezer till further analysis. Total lipids were extracted using the Folch

method (Folch et al., 1957) and after transesterification, they were analyzed by a gas chromatography system (Trace GC 2000 Series, Thermo Quest CE Instruments) with a flame ionization detector (FID) equipped with an autosampler (TriPlus AS ThermoQuest CE Instruments) on a BPX70 GC column (30 m length, 0.32 mm i.d., 0.25 μ m film thickness, SGE Analytical Science) as previously described in detail by Vasilopoulos et al. (2022). Apart from individual fatty acids, parameters useful for evaluating the nutritional value of the fatty acid profile were also determined. In particular, the sum of saturated fatty acids (Σ SFA), monounsaturated fatty acids (Σ MUFA), polyunsaturated fatty acids (Σ PUFA), n3 fatty acids (Σ n3) and n6 fatty acids (Σ n6) and the ratios of PUFA to SFA (PUFA/SFA), n6 to n3 (n3/n6) and hypocholesterolemic to hypercholesterolemic (H/H) fatty acid ratio. The H/H ratio was determined as follows: $H/H = \Sigma C18:1 \text{ cis-9}, C18 \text{ n-6}, C20:4 \text{ n-6}, C18:3 \text{ n-3}, C20:3 \text{ n-6}, C20:5 \text{ n-3}, C22:6} / \Sigma C14:0, C16:0$.

Color meat evaluation

The CIE L* (lightness), a* (redness), b* (yellowness) color parameters of breasts and thighs were determined by a colorimeter (Konica Minolta CR-400/410, Kyoto, Japan). Every measurement was performed in accordance with the procedure outlined by Vasilopoulos et al. (2022).

Statistical analysis

The basic study design was RCB (random complete block design) and the replication (pen) was considered as the experimental unit. Prior to the onset of the experiment, the minimum required total sample size was calculated using the "Power analysis for one-way ANOVA" methodology (Charan and Kantharia, 2013) with G*Power 3.1.9.2 software and power ≥ 0.80 . Statistical comparisons of the mean values were performed by one-way ANOVA, followed by the Tukey's test ($P < 0.05$ confidence level) using the IBM SPSS Statistics for Windows software, Version 27.0 (Armonk, NY: IBM Corp).

Results

Total phenolic content and antiradical activity of extracts

The total phenolic content of the extracts was 152.5 ± 1.46 mg GAE/g plant material for the aqueous quadruple extract and 206.6 ± 2.77 mg GAE/g plant material for the β -CD quadruple extract. Based on the results, a small difference was observed between the extracts regarding their total phenolic content, with the β -CD one presenting the highest value. The total phenolic content of control feed was 0.51 ± 0.15 mg GAE/g dry matter. The results regarding antiradical activity present a different and interesting pattern. More specifically, the value for the aqueous extract was 690.38 ± 0.68 μ mol TRE/g plant material, whereas the value for the β -CD extract

was equal to 620.87 ± 0.07 μ mol TRE/g plant material. Although the β -CD extract presented slightly higher total phenolic content compared to the aqueous one, the antiradical activity was lower. Based on these results, it seems that the yield of components presenting scavenging activity against DPPH is higher when water is used as extraction solvent compared to aqueous β -CD solution. The antiradical activity of control feed was 41.5 ± 2.24 μ mol TRE/g feed.

Performance parameters

Broiler chickens' performance parameters are represented in Table 3. At the end of the experimental period, on day 35, the cyclodextrin treated group had significantly increased live body weight compared to the other groups ($P < 0.05$), while no significant alterations were noticed on days 1, 10, and 24 ($P > 0.05$). Similarly, feed intake remained analogous among the different groups at the following time periods: 1–10, 25–35 and 1–35 days ($P > 0.05$), while OGCC-CD group had increased feed intake during the interval of 11–24 days compared to the control group ($P < 0.05$). Feed conversion ratio was unaffected by both aqueous and cyclodextrin phytobiotic treatments during the overall experimental period ($P > 0.05$). Although feed intake did not fluctuate significantly between treatments, the cyclodextrin group tended to show increased values ($P = 0.063$) for a 1–35 day period.

Welfare traits

All welfare traits were recorded on days 10, 24, and 35 of the trial. Broilers dietary supplementation with the quadruple phytobiotic mixture did not have any adverse effect on litter DM and score, as displayed in Table 4. Litter moisture was significantly lower for the groups supplemented with either the aqueous or the cyclodextrin phytobiotic mixture in contrast to the control ($P < 0.05$).

Pododermatitis scores were similar for all groups on day 10. OGCC-AQ group marked a significant lower score on day 24 among the three treatments ($P < 0.05$), while on day 35, both phytobiotic treated groups presented lower pododermatitis score values compared to the control group ($P < 0.05$) (Table 4). The diarrhea score was unaffected by the dietary supplementation.

Table 4 displays also the results referring to feather score, fecal score and fecal litter moisture. Insignificant differences among the three treatment groups were noted indicating that the inclusion of the phytobiotics did not interfere with these welfare traits. Similar results were obtained according to the proportion of litter NH_3 where dietary supplementation had no interaction with NH_3 scores (Table 5). There were insignificant differences in terms of immune organs among all groups (Table 6).

As shown in Table 7 carcass yield was significantly higher for OGCC-CD group in contrast to control and OGCC-AQ groups ($P < 0.05$). Wooden breast and white striping scores had insignificant differences among the treatment groups.

Table 3. Effect of dietary supplementation on broiler chicken performance

Live body weight (g)	Control ¹	OGCC-AQ ¹	OGCC-CD ¹	SEM ²	P-value
Day 1	45.87	46.08	44.87	0.361	0.346
Day 10	362.92	367.07	364.40	3.860	0.907
Day 24	1407.9	1403.17	1380.62	13.49	0.681
Day 35	2287.75 b	2264.50 b	2295.20 a	0.905	<0.001
Feed intake per chicken (g)	Control	OGCC-AQ	OGCC-CD	SEM	P-value
Days 1–10	381.52	393.80	384.35	40.69	0.478
Days 11–24	1877.15 b	1900.27 ab	1938.97 a	99.28	0.016
Days 25–35	2046.47	2083.55	2086.25	98.69	0.192
Days 1–35	4305.15	4377.62	4409.55	369.67	0.063
Feed conversion ratio (g feed/g weight gain)	Control	OGCC-AQ	OGCC-CD	SEM	P-value
Days 1–10	1.205	1.248	1.203	0.011	0.853
Days 11–24	1.800	1.839	1.910	0.027	0.281
Days 25–35	2.379	2.446	2.348	0.084	0.907
Days 1–35	1.922	1.974	1.980	0.021	0.538

¹Control: Basal diet; OGCC-AQ: Diet supplemented with aqueous extract at 0.1% per kg of DM; OGCC-CD: Diet supplemented with cyclodextrin extract at 0.1% per kg of DM. ²SEM: Standard error of mean. a, b – values in the same line with the same letters do not differ significantly.

Table 4. Effect of dietary supplementation on litter dry matter, litter moisture and litter score, pododermatitis, diarrhea, feather and fecal score and fecal litter moisture

Litter dry matter	Control ¹	OGCC-AQ ¹	OGCC-CD ¹	SEM ²	P-value
Day 10	64.675	64.781	66.006	0.631	0.653
Day 24	73.837	74.856	73.837	0.442	0.519
Day 35	74.287	75.475	74.850	0.898	0.875
Litter moisture	Control	OGCC-AQ	OGCC-CD	SEM	P-value
Day 10	33.668	35.534	34.331	0.448	0.542
Day 24	27.418 a	23.818 b	24.612 b	0.529	0.008
Day 35	26.443	23.425	25.543	0.808	0.615
Litter score	Control	OGCC-AQ	OGCC-CD	SEM	P-value
Day 10	1.380	1.440	1.560	0.099	0.753
Day 24	1.310	1.130	1.250	0.052	0.339
Day 35	1.000	1.130	1.130	0.049	0.508
PD ³ score	Control	OGCC-AQ	OGCC-CD	SEM	P-value
Day 10	0.130	0.690	0.440	0.098	0.057
Day 24	0.310 a	0.190 b	0.290 a	0.024	0.043
Day 35	1.060 a	0.500 b	0.540 b	0.123	0.049
Diarrhea score	Control	OGCC-AQ	OGCC-CD	SEM	P-value
Day 10	1.690	1.500	2.000	0.120	0.238
Feather score	Control	OGCC-AQ	OGCC-CD	SEM	P-value
Day 10	2.690	2.690	2.560	0.070	0.723
Day 24	2.750	2.810	2.940	0.049	0.296
Day 35	2.750	2.690	2.750	0.060	0.895
Fecal score	Control	OGCC-AQ	OGCC-CD	SEM	P-value
Day 10	1.130	1.440	1.190	0.091	0.342
Day 24	1.310	1.380	1.190	0.067	0.526
Day 35	1.440	1.190	1.190	0.074	0.289
Fecal litter moisture	Control	OGCC-AQ	OGCC-CD	SEM	P-value
Day 10	23.100	23.112	23.587	0.153	0.351
Day 24	23.726	22.325	22.912	0.438	0.422
Day 35	21.162	20.650	21.012	0.316	0.808

¹Control: Basal diet; OGCC-AQ: Diet supplemented with aqueous extract at 0.1% per kg of DM; OGCC-CD: Diet supplemented with cyclodextrin extract at 0.1% per kg of DM. ²SEM: Standard error of mean. ³PD: pododermatitis a, b – values in the same line with the same letters do not differ significantly.

Table 5. Effect of dietary supplementation on NH₃

NH ₃	Control ¹	OGCC-AQ ¹	OGCC-CD ¹	SEM ²	P-value
Day 10	1.196	1.191	1.187	0.009	0.942
Day 24	1.136	1.166	1.152	0.006	0.143
Day 35	1.216	1.201	1.211	0.011	0.869

¹Control: Basal diet; OGCC-AQ: Diet supplemented with aqueous extract at 0.1% per kg of DM; OGCC-CD: Diet supplemented with cyclodextrin extract at 0.1% per kg of DM. ²SEM: Standard error of mean.

Table 6. Effect of dietary supplementation on broiler chicken spleen, bursa and thymus weight expressed as a percentage of the total body weight at day 35

Spleen weight (%)	Control ¹	OGCC-AQ ¹	OGCC-CD ¹	SEM ²	P-value
Day 35	0.10	0.10	0.09	0.095	0.421
Bursa weight (%)	Control	OGCC-AQ	OGCC-CD	SEM	P-value
Day 35	0.09	0.10	0.09	0.029	0.895
Thymus weight (%)	Control	OGCC-AQ	OGCC-CD	SEM	P-value
Day 35	0.06	0.06	0.06	0.066	0.668

¹Control: Basal diet; OGCC-AQ: Diet supplemented with aqueous extract at 0.1% per kg of DM; OGCC-CD: Diet supplemented with cyclodextrin extract at 0.1% per kg of DM. ²SEM: Standard error of mean.

Table 7. Effect of dietary supplementation on broiler chicken carcass yield, wooden breast score and white striping score

Carcass yield (%)	Control ¹	OGCC-AQ ¹	OGCC-CD ¹	SEM ²	P-value
Day 35	83.35 b	84.21 b	89.18 a	24.84	0.021
Wooden breast score	Control	OGCC-AQ	OGCC-CD	SEM	P-value
Day 35	0.375	0.437	0.250	0.101	0.763
White striping score	Control	OGCC-AQ	OGCC-CD	SEM	P-value
Day 35	0.562	0.500	0.875	0.082	0.137

¹Control: Basal diet; OGCC-AQ: Diet supplemented with aqueous extract at 0.1% per kg of DM; OGCC-CD: Diet supplemented with cyclodextrin extract at 0.1% per kg of DM. ²SEM: Standard error of mean. a, b – values in the same line with the same letters do not differ significantly.

Table 8. Effect of dietary supplementation on broiler chicken breast and thigh meat composition, MDA values and protein carbonyls

Breast meat	Control ¹	OGCC-AQ ¹	OGCC-CD ¹	SEM ²	P-value
Moisture %	73.525 b	77.162 a	73.987 b	0.396	<0.001
Protein %	23.975	24.075	24.337	0.173	0.696
Fat %	1.678	1.177	1.288	0.116	0.185
Thigh meat	Control	OGCC-AQ	OGCC-CD	SEM	P-value
Moisture %	74.765	74.370	74.825	0.126	0.291
Protein %	20.298 b	21.552 a	21.503 a	0.165	<0.001
Fat %	4.193 a	2.870 b	2.773 b	0.165	<0.001
Breast meat TBARS	Control	OGCC-AQ	OGCC-CD	SEM	P-value
Day 1	0.065 a	0.045 c	0.050 b	0.001	<0.001
Day 3	0.067	0.033	0.049	0.012	0.546
Thigh meat TBARS	Control	OGCC-AQ	OGCC-CD	SEM	P-value
Day 1	0.156	0.059	0.075	0.052	0.169
Day 3	0.025 b	0.042 a	0.024 b	0.002	<0.001
Breast meat protein carbonyls	Control	OGCC-AQ	OGCC-CD	SEM	P-value
	0.419 a	0.166 b	0.187 c	0.037	<0.001
Thigh meat protein carbonyls	Control	OGCC-AQ	OGCC-CD	SEM	P-value
	0.272 b	0.217 b	0.732 a	0.055	<0.001

¹Control: Basal diet; OGCC-AQ: Diet supplemented with aqueous extract at 0.1% per kg of DM; OGCC-CD: Diet supplemented with cyclodextrin extract at 0.1% per kg of DM. ²SEM: Standard error of mean. a, b – values in the same line with the same letters do not differ significantly.

Table 9. Breast and thigh meat color parameters

Color parameters	Control ¹	OGCC-AQ ¹	OGCC-CD ¹	P-value
Breast meat				
L* ²	41.46±0.71 a	57.15±1.09 c	55.66±0.50 b	<0.05
a*	3.55±0.35 b	3.34±0.59 b	1.58±0.21 a	<0.05
b*	5.81±0.05 a	7.40±1.47 b	6.72±0.10 b	<0.05
Thigh meat				
L*	40.76±0.19 a	50.76±1.79 b	52.48±0.64 c	<0.05
a*	3.83±0.06 a	5.10±0.48 b	5.93±0.23 c	<0.05
b*	5.81 ± 0.04 b	3.31±0.74 a	6.50±0.27 c	<0.05

¹Control: Basal diet; OGCC-AQ: Diet supplemented with aqueous extract at 0.1% per kg of DM; OGCC-CD: Diet supplemented with cyclodextrin extract at 0.1% per kg of DM. ²L* stands for lightness, a* for redness and b* for yellowness. Values in the same line with the same letters do not differ significantly. Each value is the mean of triplicate determinations ± S.D. Different lowercase letters within the same row indicate significant differences according to Duncan's test (P<0.05).

Breast and thigh meat composition, lipid, and protein oxidation

Chemical analysis of chicken breast and thigh meat are shown in Table 8. Regarding breast meat, the dietary supplementation significantly affected moisture content, where OGCC-AQ group displayed the highest value compared to all groups (P<0.001). The other examined parameters (protein and fat percentages) were not significantly affected. According to the results, broilers' thigh meat protein and fat percentages were significantly affected by the phytobiotic treatment. Both phytobiotic supplemented groups (OGCC-AQ and OGCC-CD) noted higher protein content compared to the control (P<0.001), while simultaneously their fat content was significantly lower compared to the control (P<0.001). The moisture percentage was not significantly affected.

Based on Table 8, TBARS concentrations of breast meat were significantly affected by the interaction of phytobiotic supplementation and meat oxidative status on day 1 of refrigeration. OGCC-AQ group had the lowest TBARS values, and at the same time, OGCC-CD group had lower values compared to the control (P<0.001). In contrast to day 1, on day 3, no interaction was noted, with TBARS values being similar among the treatments. Results referring to TBARS concentrations of thigh meat were divergent compared to breast meat. On day 1 of refrigeration, no differences were noted among the groups, while after two days (day 3) OGCC-AQ group marked the lowest TBARS concentration among all three treatments (P<0.001). Control and OGCC-CD had similar values.

According to Table 8, OGCC-CD had considerably higher protein carbonyls in both breast and thigh meat (P<0.001), whereas OGCC-AQ had the lowest protein carbonyls value for the breast meat (P<0.001). Thigh meat samples from the OGCC-AQ and control treatments produced comparable protein carbonyl levels (P>0.05).

Color parameters

Breast and thigh meat color parameters were affected by the dietary supplementation with the aqueous and

cyclodextrin quadruple extracts. As presented in Table 9, breast meat lightness (L*) was significantly lower for the control group compared to the other two, while OGCC-AQ presented the highest value among the three samples (P<0.05). Regarding redness (a*), OGCC-CD group displayed the lowest value (P<0.05), whereas the values of the other two groups, control and OGCC-AQ, had no statistically significant differences. In terms of yellowness (b*), control group presented the lowest value (P<0.05) compared to OGCC-AQ and OGCC-CD which displayed similar values (P>0.05). According to the results of the thigh meat color parameters, OGCC-CD group exhibited the highest values regarding all three parameters L*, a* and b*. On the other hand, control group displayed the lowest overall values, except for the b* parameter, where OGCC-AQ presented the lowest one.

Breast and thigh meat color parameters were affected by the dietary supplementation with the aqueous and cyclodextrin quadruple extracts. As presented in Table 9, breast meat lightness (L*) was significantly lower for the control group compared to the other two, while OGCC-AQ presented the highest value among the three samples (P<0.05). Regarding redness (a*), OGCC-CD group displayed the lowest value (P<0.05), whereas the values of the other two groups, control and OGCC-AQ, had no statistically significant differences. In terms of yellowness (b*), control group presented the lowest value (P<0.05) compared to OGCC-AQ and OGCC-CD which displayed similar values (P>0.05). According to the results of the thigh meat color parameters, OGCC-CD group exhibited the highest values regarding all three parameters L*, a* and b*. On the other hand, control group displayed the lowest overall values, except for the b* parameter, where OGCC-AQ presented the lowest one.

Meat fatty acids composition

The effects of dietary treatments on the fatty acid composition of broilers' breast and thigh meat are shown in Tables 10 a and 10 b. Regarding breast meat, dietary

supplementation with an aqueous extract of a combination of the four above mentioned medicinal plants or its encapsulated form in cyclodextrin, resulted in a significant reduction in stearic acid ($P<0.05$). In terms of Σ SFA (saturated fatty acids), both the OGCC-AQ and OGCC-CD groups presented similar values which were significantly ($P<0.05$) lower compared to the control one. Regarding individual MUFAs (monounsaturated fatty acids), the oleic acid content was found to be similar in all of the examined groups whereas the Σ MUFA content was found to be increased in both supplemented groups. The Σ PUFA (polyunsaturated fatty acids) content of the OGCC-CD group was similar to the control one, but it displayed increased values compared to the OGCC-AQ group ($P<0.05$). Moreover, the OGCC-CD group exhibit-

ed also a significantly ($P<0.05$) increased content in Σ n-3 fatty acids compared to the OGCC-AQ group; however, it was similar to that of the control group. The content of the cis-4,7,10,13,16,19-docosahexaenoic acid (DHA) fatty acid in the breast meat of the OGCC-CD supplemented broilers was also found to be higher ($P<0.05$) compared to the other groups, while the OGCC-AQ group showed the lowest value ($P<0.05$). The OGCC-AQ group showed also the lowest value regarding the Σ n-6 fatty acids content ($P<0.05$). The PUFA/SFA ratio was found similar in all of the examined groups, whereas the H/H (hypocholesterolemic/hypercholesterolemic) ratio was found to be increased ($P\leq 0.05$) in the OGCC-AQ group due to an increase of the oleic acid content, as previously mentioned.

Table 10 a. Fatty acid composition (g/100 g of total fatty acids) of broilers' breast meat

Fatty acid	Control ^{a,b}	OGCC-AQ ^{a,b}	OGCC-CD ^{a,b}	SEM ^c
Myristic (C14:0)	0.45 a	0.46 a	0.47 a	0.003
Palmitic (C16:0)	22.28 b	21.97 a	23.28 b	0.395
Palmitoleic (C16:1 cis)	0.38 a	2.26 b	2.19 b	0.615
Heptadecanoic (C17:0)	0.17 a	0.20 ab	0.13 a	0.020
Stearic (C18:0)	11.84 c	10.58 b	8.31 a	1.033
Oleic (C18:1 cis ω 9)	26.36 a	27.96 b	26.79 a	0.478
Linoleic (C18:2 cis ω 6)	25.93 a	26.55 b	26.79 b	0.256
Arachidic (C20:0)	0.11 a	0.15 a	0.13 a	0.012
g-Linolenic (C18:3 cis ω 6)	1.44 a	1.54 a	1.49 a	0.029
Linolenic (C18:3 cis ω 3)	0.35 a	0.35 a	0.37 a	0.007
Heneicosanoic (C21:0)	1.02 a	0.93 a	1.41 b	0.147
cis-11,14-Eicosadienoic (C20:2 cis ω 6)	0.17 a	0.11 a	0.19 a	0.024
Behenic (C22:0)	0.92 ab	0.73 a	1.12 b	0.113
cis-8,11,14-Eicosatrienoate (C20:3 cis ω 6)	6.26 b	4.07	6.14 a	0.711
Erucic (C22:1 cis ω 9)	0.19 b	0.16 a	0.23 b	0.020
Arachidonic (C20:4 cis ω 6)	0.26 b	0.15 a	0.31 b	0.047
Nervonic (C24:1 cis ω 9)	1.01 b	0.73 a	1.02 b	0.095
cis-4,7,10,13,16,19-Docosahexaenoic (C22:6 cis ω 3)	0.64 b	0.49 c	0.78 a	0.084
Σ SFA ^d	37.00 b	35.31 a	35.58 a	0.524
Σ MUFA ^e	27.94 a	31.26 c	28.47 b	1.030
Σ PUFA ^f	35.05 a	33.43 a	35.95 a	0.737
Σ n-3 ^g	0.99 ab	0.84 a	1.03 b	0.058
Σ n-6 ^h	8.13 b	6.04 a	8.13 b	0.697
PUFA/SFA	0.95 a	0.95 a	1.01 a	0.020
n-6/n-3	8.19 b	7.18 a	8.16 b	0.332
H/H ⁱ	2.40 a	2.53 a	2.28 a	0.072

^aEach value is the mean of triplicate determinations $\pm$ s.d. ^bDifferent lowercase letters within the same raw indicate significant differences according to Duncan's test ($P<0.05$). ^cStandard error of mean, ^dSaturated fatty acids, ^eMonounsaturated fatty acids, ^fPolyunsaturated fatty acids, ^gomega-3 fatty acids, ^homega-6 fatty acids, ⁱHypocholesterolemic/Hypercholesterolemic fatty acid ratio = Σ C18:1 cis-9, C18 n-6, C20:4 n-6, C18:3 n-3, C20:3 n-6, C20:5 n-3, C22:6/ Σ C14:0, C16:0.

Table 10 b. Fatty acid composition (g/100 g of total fatty acids) of broilers' thigh meat

Fatty acid	Control ^{a,b}	OGCC-AQ ^{a,b}	OGCC-CD ^{a,b}	SEM ^c
Myristic (C14:0)	0.542 a	0.52 a	0.41 a	0.040
Myristoleic acid (C14:1)	0.13 a	0.08 a	0.10 a	0.015
Palmitic (C16:0)	22.00 b	24.48 c	20.48 a	1.166
Palmitoleic (C16:1 cis)	4.31 c	2.82 b	1.99 a	0.679
Heptadecanoic (C17:0)	0.16 a	0.23 b	0.25 b	0.027
Stearic (C18:0)	11.76 a	11.04 a	8.95 a	0.843
Oleic (C18:1 cis ω 9)	30.37 b	27.49 a	26.31 a	1.206
Linoleic (C18:2 cis ω 6)	25.85 a	27.74 a	32.54 b	1.991
Arachidic (C20:0)	0.19 a	0.16 a	0.17 a	0.009
g-Linolenic (C18:3 cis ω 6)	2.12 b	1.68 a	1.82 a	0.130
Linolenic (C18:3 cis ω 3)	0.37 a	0.34 a	0.27 a	0.030
Heneicosanoic (C21:0)	0.36 a	0.60 b	0.58 b	0.077
Behenic (C22:0)	0.26 a	0.54 b	0.50 b	0.087
cis-8,11,14-Eicosatrienoate (C20:3 cis ω 6)	1.09 a	3.43 b	3.56 b	0.803
Nervonic (C24:1 cis ω 9)	0.21 a	0.59 b	0.81 c	0.175
cis-4,7,10,13,16,19-Docosahexaenoic (C22:6 cis ω 3)	0.11 a	0.34 b	0.44 c	0.098
Σ SFA ^d	35.28 c	34.87 b	31.75 a	1.115
Σ MUFA ^e	35.021 b	31.22 a	29.40 a	1.656
Σ PUFA ^f	29.70 a	33.91 b	38.85 c	2.644
Σ n3 ^g	0.48 a	0.68 b	0.74 b	0.079
Σ n6 ^h	3.36 a	5.49 b	5.57 b	0.724
PUFA/SFA	0.84 a	0.97 b	1.22 b	0.112
n6/n3	7.05 b	8.11 b	7.97 a	0.332
H/H ⁱ	2.60 a	2.61 a	2.93 b	0.108

^aEach value is the mean of triplicate determinations $\pm$ s.d. ^bDifferent lowercase letters within the same raw indicate significant differences according to Duncan's test ($P < 0.05$). ^cStandard error of mean, ^dSaturated fatty acids, ^eMonounsaturated fatty acids, ^fPolyunsaturated fatty acids, ^gomega-3 fatty acids, ^homega-6 fatty acids, ⁱHypocholesterolemic/Hypercholesterolemic fatty acid ratio = Σ C18:1 cis-9, C18 n-6, C20:4 n-6, C18:3 n-3, C20:3 n-6, C20:5 n-3, C22:6/ Σ C14:0, C16:0.

In the thigh meat of broilers, the palmitic acid content, the stearic acid content, as well as the Σ SFA content were significantly reduced in the OGCC-CD group compared to the OGCC-AQ and the control ones ($P < 0.05$). Oleic acid was found to be decreased in both groups with a concomitant increase in linoleic acid and cis-8,11,14-eicosatrienoate (C20:3 cis ω 6) and cis-4,7,10,13,16,19-docosahexaenoic (C22:6 cis ω 3) content ($P < 0.05$). A significant increase in the Σ PUFA content was also observed in the thigh meat of broilers fed with the supplemented diets compared to the control one ($P < 0.05$). Between the supplemented groups, the OGCC-CD group showed significantly higher values ($P < 0.05$). Supplementation of the broilers diet with the cyclodextrin extract also resulted in an increase in the Σ n-3 fatty acids, as well as the Σ n-6 fatty acids ($P < 0.05$). The PUFA/SFA and H/H ratios were also increased in the thigh meat of both supplemented groups compared to the group that was fed with the unsupplemented diet ($P < 0.05$).

Discussion

Natural products are being currently identified as reliable resources to overcome broilers' husbandry stress and final product's oxidative stress. According to the findings of these studies, the incorporation of phytobiotic extracts combination in encapsulated form had a significant effect on broilers' final body weight. Likewise, Peng et al., (2016) found that the use of *Origanum* essential oil had a positive influence over the intestinal health, and thereby improved the growth performance and carcass traits of broilers. Nevertheless, several studies examining dried oregano or *Origanum* essential oil supplementation, presented similar results where there was no direct effect on animal performance (Barreto et al., 2008; Bampidis et al., 2005; Cerisuelo et al., 2014). Similarly, the effects of garlic powder application exhibited great variance when integrated in the dietary habits of chickens. Broilers fed with different levels of garlic powder (1.5%, 3%, and 4.5%) did not show improved body weight gain or feed

conversion ratio (FCR) (Konjufca et al., 1997). Supplementation with garlic powder (1%, 3% or 5%) had no effect on growth performance (e.g., weight gain, feed intake, and FCR) of chickens (Choi et al., 2010). In addition, garlic did not influence average feed intake, weight gain and FCR of chickens, but reduced abdominal fat contents in broilers (Onibi et al., 2009). However, Horton et al. (1991), added dried garlic (0.01%, 0.1% and 1%) to broiler diets and found that daily average gain increased at the two intermediary levels of garlic (0.01% and 0.1%) during the first 21 days. Direct comparison with existing studies is difficult since no studies have reported dietary supplementation to broilers' diet with mixture of herbs. The results of the present study could be attributed to synergistic effects of bioactive compounds present in the mixture. In addition, the variability of bioactive compounds depending on the type of plant material as well as the extraction process and condition could highly affect the outcome. Last but not least, the present trial facilities do not fully reflect commercial facilities where birds' population is significantly higher thus hushing stress is addressed to a higher extend.

Meat plays a crucial role in human evolution and is an important component of a healthy and balanced diet (Horton et al., 1991). Thus, consumers' awareness regarding meat quality is constantly increasing and they are searching for quality products with health benefits. Plant food products from selected herbs contain biologically active substances which exert health benefits. These crops are sources usually rich in fatty acids, sterols, phenolic compounds, and dietary fibers, which mainly have the ability to increase satiety and obesity control and prevent various diseases (Pereira and Vicente, 2013; Hernández-Pérez et al., 2021; Farinon et al., 2020). Medicinal plants were found to significantly alter fat and protein content in favor of human nutritional requirements. For example, breast muscle protein content showed an increase of 1.31–1.56% contrariwise to fat that decreased by 2.23–2.97% (Egorov and Egorova, 2021). Additionally, fat represents one of the major nutrient components of meat products. Poultry meat is characterized by low fat content of high quality and thus it meets modern consumer demands (Kralik et al., 2018). Nevertheless, lipids are susceptible to oxidation, which is described as a main deterioration process through a free radical chain reaction, leading to diminished quality (Min and Ahn, 2005). The use of phytobiotics as means to overcome this harmful process has been exploited over the last decades creating an optimistic point of view (Fellenberg and Speisky, 2006). Our results indicate that the quadruple plant mixture encapsulated in cyclodextrin was found to inhibit lipid oxidation. Previous experiments exploring *O. vulgare* supplementation of broilers' diet demonstrated oregano's ability to decrease oxidative phenomena (Weerawatanakorn et al., 2017). Similar investigations regarding the antioxidant properties of camelina meal in broiler diets could lead to development of n-3- and antioxidant-rich poultry feeds while producing wholesome

foods for human consumption (Aziza et al., 2010). Furthermore, various authors have studied *C. maritimum* essential oil, revealing antioxidant and antibiotic activities. The aromatic plants used in our experiment are rich in phenolic compounds that have been acknowledged to exhibit antioxidant properties due to their mechanism of action as hydrogen donors and radical scavengers (Marongiu et al., 2007). In the present study, the employment of cyclodextrin as means of encapsulation was investigated for the protection of extracts' bioactive compounds and the mechanism of action improvement. Generally, the encapsulated materials are protected from environmental factors while passing through animals' digestive tract. An *in vitro* experiment conducted by Smith et al. (2012), revealed that microencapsulation had the ability to postpone butyric acid absorption, allowing it to reach the intestine attached and provide higher availability of the desirable organic acid.

Phytobiotics produced by green technologies may be further exploited as performance enhancers; in that case, the aim of enhanced growth in the absence of antibiotics can be reached through targeting different directions, as phytobiotics act by various mechanisms of action (Christaki et al., 2020). One approach to accomplish this is the innovative technology of extracts encapsulation. Among the advantages that encapsulated ingredients present are increased bioavailability and solubility of core compounds, prevention of their degradation while being processed or during storage and coverage of undesirable properties like unpleasant odor or flavor (Gómez-Estaca et al., 2018). Additionally, it was proven that microencapsulation, by spray drying, had a beneficial effect on oregano essential oil by enhancing the speed and amount of its bioactive compounds release (Partheniadis et al., 2017). Supplementary encapsulation techniques could be a convenient method to supplement feed with extracts or essential oils characterized by intense odor that could lead to a decrease in DM intake.

As meat color is often associated with freshness, it is regarded as an important factor in meat quality and consumer acceptance of meat products (Cong et al., 2017). To this respect, meat color is one of the main parameters that should be determined and can be used as an indirect marker for evaluating meat quality parameters (Pathare et al., 2013). Among other factors, poultry diet and individual ingredients present in the mixture affect to a great degree the respective color of the meat (Pokoo-Aikins et al., 2022). In addition, the employment of different plant materials and ingredients applied as antioxidants, antimicrobials, and flavorings in poultry meat plays an important role in the determination of the final meat color (Wideman et al., 2016). These changes can be attributed not only to the ability of some compounds to act as natural colorants but also to their antioxidant capacity, which can affect oxidation processes and subsequently the color of the final product during storage (Mancini and Hunt, 2005). However, despite the importance of diet ingredients in the color and quality of meat, color parameters are

not usually determined in the case of dietary supplementation of broilers diet. It would be of great importance to determine whether these components have the ability to reach the meat or skin tissues.

Generally, the interpretation of color results in meat products based on the effect of different dietary ingredients is ambiguous. In the case of the present study, the dietary supplementation of broilers diet with aqueous and β -CD mixture of oregano, camelina, samphire and garlic extracts (quadruple extract) had an impact on the meat color based on the results of the color parameters L^* , a^* and b^* . Regarding lightness (L^*), in the cases of both OGCC-AQ and OGCC-CD the presented values were higher regardless of the body part (breast or thigh), compared to control. This indicates that dietary supplementation resulted in more pale colorations in the final product (Vasilopoulos et al., 2022). In most cases, higher L^* values are linked to higher oxidation, however in some studies it was stated that possibly the original extract color and the individual ingredients were responsible for this result (Ahn et al., 2007; Gao et al., 2021). The results for a^* value followed a different pattern depending on the body part. More specifically, OGCC-CD breast samples had the lowest a^* value whereas the respective thigh samples had the highest a^* value. Low meat redness is generally linked to lower hemoglobin concentration (Pokoo-Aikins et al., 2022; Wideman et al., 2016; Mancini and Hunt, 2005; Ahn et al., 2004, 2007; Gao et al., 2021; Khan et al., 2021). Since oxymyoglobin is responsible for meat redness, the oxidation of myoglobin to metmyoglobin, leads to a reduction in meat redness (Khan et al., 2021). Also, thigh meat parts are naturally redder than breast meat parts due to higher myoglobin content in the red fibers of the thigh (Wideman et al., 2016). The parameter of yellowness (b^*) is the most important regarding poultry meat since the deep yellow skin coloration is preferred by many consumers (Castañeda et al., 2005). Dietary supplementation with the quadruple extract OGCC-CD increased the yellowness in breast and thigh samples compared to control, but OGCC-AQ extract had a different effect on the yellowness based on the body part. This result could be attributed to the extracted compounds in the OGCC-AQ extract that possibly did not intervene with the thigh tissue in order to affect the yellowness of the meat.

However, it should be noted that to the best of our knowledge no previous studies have reported the dietary supplementation of broiler diet with a quadruple extract, aqueous or with cyclodextrin, of oregano, camelina, samphire and garlic. To this respect, no direct comparisons can be made regarding the color parameters of the final meat product. In the present study, it can be stated that the compounds presented in the extract affected the color parameters, in a different way based on the extraction solvent (water or β -cyclodextrin) and body part (breast or thigh), indicating that there are some dietary interventions that may be valuable to influence the color of poultry meat.

The effects of dietary supplementation with an aqueous or encapsulated extract of oregano, samphire, camelina and garlic peels, on the fatty acid composition of the breast and thigh meat of broilers are presented in Tables 10 a and 10 b, respectively. Dietary saturated fatty acids, and particularly stearic, myristic and palmitic acids, have been associated with coronary heart diseases due to their hypercholesterolemic properties (Ahmed et al., 2015). In the present study, stearic acid as well as total saturated fatty acids content were decreased with a concomitant increase in the polyunsaturated fatty acids content of the breast and thigh meat of broilers upon incorporation of both the aqueous and cyclodextrin extracts in the diet compared to the control group. Similar observations were also made by our research group after broilers' dietary supplementation with aqueous or cyclodextrin extracts of pomegranate and onion peels (Vasilopoulos et al., 2022). Analogous changes in fatty acid composition upon thymol and carvacrol broilers' feed supplementation have also been reported (Hashemi-pour et al., 2013). In another study Forte et al. (2018) claimed that diet supplementation with oregano was not found to have any impact on the total fatty acid composition of breast meat, even though it affected meat composition in terms of total phenolic content, antioxidant capacity, and thiobarbituric acid-reactive substances, improving meat resistance to oxidation. The PUFA/SFA ratio is generally used to evaluate the nutritional value of fat. Fats with a low PUFA/SFA ratio (≤ 0.4) are considered unfavorable because they may induce an increase in cholesterolemia. In the present study, the thigh meat of broilers fed with a diet supplemented with oregano, samphire, camelina and garlic peel extracts exhibited higher PUFA/SFA ratios compared to the control group. Another approach for the nutritional evaluation of fat, taking into consideration the MUFA content as well, is the estimation of the hypocholesterolemic/hypercholesterolemic fatty acid (H/H) ratio. The values obtained for this ratio in the present study were found to be higher in the breast and thigh meat of broilers fed with the examined extracts, indicating a positive effect on broilers' meat.

Conclusions

The incorporation of the cyclodextrin extract derived from a mixture composed of *Origanum vulgare* L., *Camelina sativa* L. Crantz, *Crithmum maritimum* L., and *Allium sativum* L. to broilers diet, beneficially affected final body weight, while both aqueous or cyclodextrin extracts had a positive impact on meat composition by increasing protein and lowering fat content. Increased $\Sigma n-3$ fatty acids, as well as $\Sigma n-6$ fatty acids, were detected in the thigh meat of broilers that consumed the enriched diets. Both fatty acid composition and color parameters were altered by the incorporation of the extracts, with the cyclodextrin one being more efficient compared to the aqueous. A further application of the herbal mixture as antioxidant agent has been identified in this study. Nev-

ertheless, additional investigation is required to highlight the possible application of phyto-genics encapsulation as means to enhance their potency.

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