

Article

Dietary Supplementation with Pomegranate and Onion Aqueous and Cyclodextrin Encapsulated Extracts Affects Broiler Performance Parameters, Welfare and Meat Characteristics

Stelios Vasilopoulos¹, Stella Dokou¹, Georgios A. Papadopoulos² , Soumela Savvidou³, Stamatia Christaki⁴ , Anastasia Kyriakoudi⁴ , Vassilios Dotas⁵ , Vasilios Tsiouris⁶ , Eleftherios Bonos⁷ , Ioannis Skoufos⁷, Ioannis Mourtzinis⁴  and Ilias Giannenas^{1,*} 

- ¹ Laboratory of Nutrition, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece; svasilopoulos@gmail.com (S.V.); dokoustella@vet.auth.gr (S.D.)
 - ² Laboratory of Animal Husbandry, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece; geopaps@vet.auth.gr
 - ³ Research Institute of Animal Science, Hellenic Agricultural Organisation-DEMETER, Paralimni, 58100 Giannitsa, Greece; savidousumela@gmail.com
 - ⁴ Laboratory of Food Chemistry and Biochemistry, Department of Food Science and Technology, School of Agriculture, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece; stamatiach02@gmail.com (S.C.); ankyria@chem.auth.gr (A.K.); mourtzinis@agro.auth.gr (I.M.)
 - ⁵ Laboratory of Nutrition Physiology & Applied Farm Animal Nutrition, Department of Animal Production, School of Agriculture, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece; vdotas@agro.auth.gr
 - ⁶ Unit of Avian Medicine, Clinic of Farm Animals, School of Veterinary Medicine, Aristotle University of Thessaloniki, 54627 Thessaloniki, Greece; biltsiou@vet.auth.gr
 - ⁷ Laboratory of Animal Science, Nutrition and Biotechnology, Department of Agriculture, School of Agriculture, University of Ioannina, Kostakioi Artas, 47100 Artas, Greece; eleftherios.bonos@gmail.com (E.B.); jskoufos@uoi.gr (I.S.)
- * Correspondence: igiannenas@vet.auth.gr; Tel.: +30-2310999937



Citation: Vasilopoulos, S.; Dokou, S.; Papadopoulos, G.A.; Savvidou, S.; Christaki, S.; Kyriakoudi, A.; Dotas, V.; Tsiouris, V.; Bonos, E.; Skoufos, I.; et al. Dietary Supplementation with Pomegranate and Onion Aqueous and Cyclodextrin Encapsulated Extracts Affects Broiler Performance Parameters, Welfare and Meat Characteristics. *Poultry* **2022**, *1*, 74–93. <https://doi.org/10.3390/poultry1020008>

Academic Editor: Alessandro Dal Bosco

Received: 9 March 2022

Accepted: 15 April 2022

Published: 5 May 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: The purpose of this trial was to evaluate the effects of *Punica granatum* L. and *Allium cepa* L. peels aqueous and cyclodextrin extracts on broiler chicks' performance and welfare status, as well as on the meat chemical composition and oxidative stability. A total of 120 one-day-old male Ross-308 chicks were randomly allocated to three treatments with four replicate pens (10 chicks per pen). Broiler chicks in the control group were fed typical commercial rations in mash form, based on maize and soybean meal. The rations of the other two treatments were further supplemented with the mixture of *Punica granatum* and *Allium cepa* aqueous and cyclodextrin extracts at the level of 0.1% of the feed, respectively. At the end of the trial (day 35), tissue samples were collected for analysis. Body weight (BW), feed intake (FI), average daily gain (ADG) and the feed conversion ratio (FCR) during the period of 1–10 days, 11–24 days, 25–35 days and 1–35 days were evaluated. Litter score, dry matter in litter, pododermatitis and feather score were also assessed at the end of the trial. Data were analyzed with ANOVA using SPSS v25 software. The results showed that BW, FI and FCR values did not differ among the groups. Scoring of pododermatitis, diarrhea, feather, fecal moisture, wooden breast and white stripping did not differ ($p \geq 0.05$) among the groups. *Punica granatum* and *Allium cepa* aqueous and cyclodextrin extracts favorably affected ($p < 0.05$) meat composition, color parameters, TBARS and protein carbonyls. Diet supplementation also increased ($p < 0.05$) $\Sigma n-3$ fatty acids as well as $\Sigma n-6$ fatty acids in the thigh meat. The cis-4,7,10,13,16,19-Docosahexaenoic acid fatty acids in the breast meat of broilers fed with diets supplemented with the aqueous pomegranate and onion peel extracts were found to be higher ($p < 0.05$), while these fatty acids in the thigh meat were found increased ($p < 0.05$) in the cyclodextrin group. Aqueous and cyclodextrin pomegranate and onion peel extracts may provide a promising additive to the broilers diet with functional properties, in the absence of stressful conditions.

Keywords: *Punica granatum* and *Allium cepa*; encapsulated extracts; meat protein and fat oxidation; fatty acid profile

1. Introduction

Poultry meat possesses the primary position in the global meat market, owing to the higher consumption rates per year compared to other types of meat, such as pork or beef [1]. Nowadays, in the poultry meat market, emphasis is congregated on increasing meat's quality characteristics [2] and production sustainability [1]. Novel findings outline the role of poultry meat as a functional food. For this purpose, the nutrient profile of broiler meat can be altered through the supplementation of broilers diets. More specifically, broiler diet supplementation with natural feed additives, such as different phytobiotics, are proven to exert positive effects on animals' health performance and stress response. In addition, these phytobiotics are natural, non-toxic and chemical-residue-free [3–5].

Phytobiotics include a huge variety of plant-derived products such as extracts, essential oils, herbs and oleoresins [6]. The dietary supplementation of commercial animal diets with phytobiotics has been related to increased animal productivity, elevated welfare indices and improved final product quality [7]. The incorporation of aromatic plants and their derivatives that are considered the main source of phytobiotics, to a broilers diet is an interesting tool for providing supplements with biologically active compounds [8]. *Punica granatum* and *Allium cepa* are both widely known plants that present extensive antioxidant and antimicrobial properties and are considered promising feed additives for application in poultry diets [9,10].

The employment of phytobiotics to retard the broiler meat oxidation process and subsequently to increase its nutritional value, has been verified by several researchers [8,11–13]. In vitro trials have elucidated the extensive antioxidant effect of pomegranate, which is attributed to its rich content of ellagitannins [14]. Ahmed et al. [15] reported that dietary supplementation with pomegranate, up to 2%, could improve the breast and thigh muscle composition (in terms of protein and fat content) and fatty acid profile, as well as decrease the TBARS values of broiler meat. Additionally, the positive impact of onion on broiler chickens' meat oxidative stability and color was highlighted by Aditya et al. [16] and was attributed to the rich flavonoid content of onion. Overall, only a few studies in the literature evaluate the pomegranate and onion contribution as feed additives in poultry nutrition.

Different plant extracts may have variable efficiency based on the absorption site, metabolism and biodegradation. An efficient application of cyclodextrin solutions is their use as extraction tools for the extraction and encapsulation of polyphenols. Aqueous solutions of cyclodextrins are considered as alternative green solvents as the formation of complexes between the hydrophobic cavity of cyclodextrin and polyphenols could enhance the extraction yield [17], in contrast to aqueous solutions that mostly hold hydrophilic compounds. In a study by Mourtzinis et al. [18] 2-hydroxypropyl- β -cyclodextrin was used as a co-solvent during the extraction of polyphenols from olive leaves resulting in the formation of a water-soluble inclusion complex between polyphenol and cyclodextrin. Cyclodextrins have also been used as a holistic approach for the exploitation of edible and non-edible parts of the pomegranate. The presence of cyclodextrin during extraction enhances the yield of total phenolics and the radical scavenging activity of pomegranate extracts [19]. Similarly, the employment of aqueous cyclodextrin solution as a media for the extraction of polyphenols from onion solid waste increases their solubility while maintaining the typical phenolic profile [18].

The main goal of the present study was the evaluation of a combination of *Punica granatum* and *Allium cepa* peel extracts on broiler performance, welfare status and meat quality. Secondly, a comparison of aqueous and cyclodextrin *Punica granatum* and *Allium cepa* peel extracts, regarding their effectiveness, was performed.

2. Materials and Methods

2.1. Ethics and Procedures

Husbandry, euthanasia, experimental procedures, and biosecurity precautions were conducted in accordance with the 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.

2.2. Animals, Diet Composition 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 three equal groups with four replicates of 10 birds each. All treatment replicates were housed in separate floor pens, each equipped with an infrared lamp for heating, in a specially designed experimental room at the Research 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®, ROSS Nutrition Specifications; Aviagen: Huntsville, AL, USA). The health of the chicks was monitored twice daily by a veterinary surgeon. Birds were vaccinated against Newcastle disease (ND) and infectious bronchitis (IB) by spray vaccination, as well as against infectious bursal disease (IBD) by subcutaneous vaccination, on the 1st day in the 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 the breeding company recommendations [20]. Based on this basal diet, additional diets were prepared by incorporating either pomegranate and onion peel aqueous extract (POM-ON-AQ) at 0.1% per kg of dry matter (DM) or cyclodextrin extract (POM-ON-CD) at the same concentration.

Table 1. Basal diets of broilers.

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
L-Lysine	0.41	0.40	0.35
DL-Methionine	0.39	0.35	0.31
L-Threonine	0.22	0.21	0.15
L-Valine	0.15	0.14	0.09
Vitamin, mineral and enzyme premix ¹	0.25	0.25	0.25
Total (kg)	100.00	100.00	100.00
Calculated Analysis (As fed basis)			
M. Energy ² , Kcal/kg	3000	3070	3150
Moisture, %	10.15	10.55	11.14
Crude protein, %	22.00	21.00	20.00
Crude fiber, %	2.85	2.65	2.55
Crude fat, %	4.84	6.11	6.65
Ash, %	6.12	5.65	5.58

Table 1. *Cont.*

Ingredients (%)	Starter	Grower	Finisher
	Days 1–14	Days 15–28	Days 29–35
Total Lysine, %	1.41	1.28	1.15
Total 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 NSPs ³ , %	9.5	7.5	6.5
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

¹ Supplying per kg feed: 12,000 IU vitamin A, 5000 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, ² M. Energy: Metabolizable Energy, ³ NSPs: Non-Starch-Polysaccharides.

2.3. Preparation of Aqueous and Cyclodextrin Extracts from Pomegranate and Onion Peels

Pomegranate peels were dried for 48 h at 40 °C. Both onion and pomegranate peels were subsequently ground in a mill (Janke and Kunkel, IKA Labortechnik, Germany). The particle size of the ground plant material was determined at 0.1 mm. Extractions were performed using either double distilled water or a saturated aqueous solution of β -cyclodextrin (98.0%, TCI, Tokyo, Japan) as solvents. The solid/liquid ratio (S/L) was determined at 1/10 *w/v* for each plant material. The solution of β -CD was prepared by dissolving 1.85 mg/mL in double distilled water through magnetic stirring at 40 °C for 1h. After complete dissolution, the plant material was added into the solution and the stirring continued for 3 h at 25 °C. The obtained extracts were filtered and stored at –18 °C until use. The extract of each plant material was prepared separately, and the extracts were mixed before storage. The volume of the final extract was 1000 mL (Table 2).

Table 2. Solid–liquid ratio for extracts' preparation.

Plant Material (Dried and Ground Form)	Solvent	Final Extract
50 g pomegranate peels 50 g onion peels	1000 mL aqueous β -CD solution (1.85 mg/mL)	1000 mL (500 mL pomegranate-peel extract and 500 mL onion-peel extract)
50 g pomegranate peels 50 g onion peels	1000 mL double distilled water	1000 mL (500 mL pomegranate-peel extract and 500 mL onion-peel extract)

2.4. Determination of Total Phenolic Content (TPC)

The total phenolic content of the extracts was determined by the Folin–Ciocalteu method [21]. An aliquot of 0.01 mL of the sample was added to 0.79 mL of double distilled water and then 0.05 mL of the Folin–Ciocalteu reagent (CHEM-LAB NV, Zedelgem, Belgium) was added. The mixture was vortexed for 10 sec and, after 1 min, 0.15 mL of 20% *w/v* aqueous Na₂CO₃ solution (99.8%, CHEM-LAB NV, Zedelgem, Belgium) was added. The final mixture was vortexed again and kept in the dark for 120 min. For each sample, three replications were performed. At the end of the incubation time, the absorbance of the samples was measured at 750 nm using a UV-Vis spectrophotometer (UV-1800, UV/Visible Scanning Spectrophotometer, SHIMADZU, Kyoto, Japan). A standard curve

was constructed using gallic acid (Gallic acid 98 +%, Alfa Aesar, Heysham, United Kingdom) and the total phenolic content was expressed as mg gallic acid equivalents (GAE) per gram of plant material.

2.5. Determination of Antiradical Activity

For the determination of antiradical activity, an aliquot of 0.025 mL of the sample was added to 0.975 mL 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution (100 μ M in MeOH) and the absorbance was measured after incubation in the dark for 30 min at 515 nm using a UV-Vis spectrophotometer (UV-1800, UV/Visible Scanning Spectrophotometer, SHIMADZU, Kyoto, Japan). DPPH \cdot solution with no sample addition was used as the control. A standard curve was constructed using TroloxTM (97%, Sigma-Aldrich, Darmstadt, Germany) and the results were expressed as μ mol Trolox equivalent (mM TRE) per g of plant material.

2.6. Performance Parameters

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

2.7. Welfare Status

Footpad dermatitis was evaluated at days 10, 24 and 35. Footpads from two birds per pen, randomly selected, were cleaned with a 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) [22]. Feathering condition was evaluated on two birds per pen at days 10, 24 and 35, through a three-point scoring system ranging from 1 to 3 (where 1 refers to clean feathers and 3 to very dirty feathers) [22]. Diarrhea scores were evaluated at day 10, on two birds per pen, using categories from 1 to 3 (where 1 indicates the absence of diarrhea and 3 indicates severe diarrhea) [22]. At days 10, 24 and 35, fecal scores were evaluated on the surface of each pen, using categories from 1 to 4 (where 1 represents firm and well-formed feces and 4 represents watery liquid feces) [22]. At days 10, 24 and 35, litter scores were evaluated on two samples, coming from pooled samples of three locations per pen, using categories from 1 to 5 (where 1 illustrates dry and crumbly litter and 5 illustrates capping or a completely wet litter) [18]. Fresh litter (wheat straw) was added to the pens after day 21, weekly and of 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. At days 10, 24 and 35, litter and fecal DM and litter NH₃ were analyzed as follows: From each pen, five litter samples of 100 g each were collected (four samples from the corners and one from the center). The five litter samples were pooled twice and homogenized prior to dry matter analysis (two values of litter dry matter per pen, each sample taken as a mix of all five locations). For litter dry matter analysis, the samples were weighed using precision scales, they were dried at 120 °C for 6 h and then weighed again to determine the weight difference [23]. The dry matter was also analyzed on a fresh fecal 10 g sample collected from each pen. For litter and fecal sampling, at least five subsamples for litter and at least three subsamples for feces were collected to reach a final sample of 10 g of litter or 50 g of feces. For the litter pH analysis: 10 g of each sample were placed in a beaker and then 100 mL of distilled water was added; after shaking the samples with a glass rod and allowing them to stand for 30 min, the pH value was obtained using a pH meter [24]. Kjeldahl nitrogen (%) was determined by the Micro-Kjeldahl method [23]. Litter NH₃ was determined on fresh litter samples collected from each pen. The spleen, bursa and thymus of the two selected birds per replicate were weighed after slaughter on day 35.

2.8. 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. They were individually marked (leg bands) and then transported and processed in a commercial slaughterhouse, according to local practices. Carcasses were scalded at 61–65 °C for 60 s, defeathered in a rotary drum picker for 25 s and whole carcasses (head, feet, without intestines) were air chilled at 4 °C. After chilling, carcasses were weighed 24 h postmortem. From each carcass, the breast and the leg (with back attached) were initially cut. Wooden breast & white striping scores were evaluated on the same birds, using categories 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, exhibiting white lines in parallel to the 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 the transmittance mode, by the reference method 2007.04 for meat and meat products [25]. Thiobarbituric acid reactive substances (TBARS) in broiler meat samples were determined according to Ahn et al. [26] with minor modifications. Briefly, after keeping the carcasses refrigerated (4 °C) for one or four days accordingly, breast and thigh meat subsamples were collected (5 g) and were homogenized in 15 mL of distilled water with Ultra-Turrax T25 (Janke & Kunkel, IKA Labor Technik) for 15 s. Then, 5 mL aliquots of the homogenates were transferred into a test tube and 50 µL of butylated hydroxyanisole (7.2%) and 5 mL of TBA-trichloroacetic acid solution (20 mM TBA in 15% trichloroacetic acid) were added. The sample mixtures were vortex-mixed and incubated in boiling water for 15 min. Following cooling, the samples were centrifuged at 1000× g for 15 min and the absorbance of each supernatant was measured at 532 nm with a spectrophotometer (UV 1700 PharmaSpec, Shimadzu, Japan). Lipid oxidation was determined as the thiobarbituric acid reactive substances (TBARS) value, expressed as nanograms of malondialdehyde per gram of meat.

2.9. Protein Carbonyls

For the determination of protein carbonyls, the method of Patsoukis et al. was applied [27] to meat samples of the same birds. In particular, 50 µL of 20% TCA was added to 50 µL of sample homogenate (diluted 1:2 v/v), the mixture was incubated in an ice bath and then centrifuged. The supernatant was discarded, and 2,4-dinitrophenylhydrazine (DNPH) was added in the pellet. The samples were incubated at room temperature, at darkness and then centrifuged. The supernatant was discarded and 1 mL of 10% TCA was added, vortexed and centrifuged. Then, the supernatant was discarded, and 1 mL of ethanol-ethyl acetate (1:1 v/v) was added, vortexed and centrifuged. Afterwards, the supernatant was discarded and 1 mL of 5 mol/L urea (pH 2.3) was added, vortexed and incubated at 37 °C for 15 min. The samples were centrifuged at 15,000 g for 3 min at 4 °C. In this assay, carbonyl formation is detected by the reaction of protein carbonyls with 2,4-dinitrophenylhydrazine (DNPH) and its subsequent conversion to 2,4-dinitrophenylhydrazone (DNP-hydrazone) that is measured at 375 nm. Calculation of protein carbonyl concentration was based on the molar extinction coefficient of DNPH ($22 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$).

2.10. 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 [28]. In particular, 2 g of each freeze-dried and ground sample was mixed with 40 mL of a solution of chloroform:methanol (2:1, v/v) (ChemLab, Zedelgem, Belgium) and homogenized with the aid of Ultra-Turrax (IKA, Stanfen, Germany) at 11,000 rpm for 3 min. The sample temperature was kept at ~15 °C in an ice bath. The extraction was repeated twice. After

filtering, water was added for the phase separation. The upper phase was removed and the lower chloroform one was collected, dehydrated with anhydrous Na_2SO_4 and rotary-evaporated to dryness. Afterwards, transesterification was carried out to the samples for subsequent gas chromatographic analysis. In particular, 0.1 g of the extracted lipids were weighed in a test tube with a screw cap and 2 mL of n-hexane (ChemLab, Zedelgem, Belgium) were added, followed by 0.2 mL of a 2 M methanolic solution of potassium hydroxide for the fatty acid methyl esters (FAMES) preparation. The mixture was vortexed for 1 min and was left to settle until the upper phase that contains the FAMES became transparent. The phase that contained the methyl esters was collected, filtered (0.45 μm PTFE hydrophobic filters) and 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 Thermo Quest CE Instruments). FAMES were analyzed on a BPX70 GC column (30 m length, 0.32 mm i.d., 0.25 μm film thickness, SGE Analytical Science). Helium was the carrier gas at a flow rate of 2.0 mL/min. The injector port and detector temperature were maintained at 250 °C. The split ratio was 1:20. The column oven was initially set at 46 °C for 2 min, then increased to 130 °C at a rate of 50 °C/min for 10 min, then increased to 175 °C at 2 °C/min and maintained at that temperature for 2 min, then increased to 200 °C at 3 °C/min and maintained at that temperature for 3.5 min, before increasing to a plateau of 240 °C at a rate of 10 °C/min for 5 min. The total run time was 60 min. The identification of FAMES was carried out by comparing the retention times (RT) with those of a standard mixture (AccuStandard, New Haven, USA) containing 37 fatty acids analyzed under the same chromatographic conditions. Chromatograms were acquired and processed with the aid of Chrom Quest 5.0 software (ver. 3.2.1, Thermo Separation Products).

Parameters useful for evaluating the nutritional value of the fatty acid profile were also determined. In particular, the sum of the saturated fatty acids (ΣSFA), monounsaturated fatty acids (ΣMUFA), polyunsaturated fatty acids (ΣPUFA), n3 fatty acid (Σn3) and n6 fatty acids (Σn6), 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: $\text{H/H} = \Sigma\text{C18:1 cis-9, C18 n-6, C20:4 n-6, C18:3 n-3, C20:3 n-6, C20:5 n-3, C22:6} / \Sigma\text{C14:0, C16:0}$.

2.11. Color Meat Evaluation

The CIE L* (lightness), a* (redness) and b* (yellowness) color parameters of the breasts and thighs were determined by a colorimeter (Konica Minolta CR-400/410, Kyoto, Japan). All measurements were performed ten times at different points in each sample and the conditions remained the same during the measurements.

2.12. Statistical Analysis

The basic study design was RCBD (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 [29,30] with G*Power 3.1.9.2 software (Faul et al., Universitat Kiel, Germany) with power ≥ 0.80 . Experimental data were subjected to analysis of variance (ANOVA) using the statistical package of SPSS software v.27.0.1. (SPSS Inc./IBM Corp., Armonk, New York, NY, USA), using ANOVA (Tukey's or Duncan's post-hoc test). The statistical significance (p) was set at 0.05%.

3. Results

3.1. Total Phenolic Content and Antiradical Activity of Extracts

The total phenolic content of the extracts was calculated at 203.10 ± 4.36 mg GAE/g plant material for the aqueous extract and 207.80 ± 7.72 mg GAE/g plant material for the β -cyclodextrin extract. Based on the results, a slight difference was observed between the extracts regarding the total phenolic content, with the β -cyclodextrin one presenting the

highest value. As is mentioned above, β -cyclodextrin is employed as an extraction media for extracting polyphenols from a variety of plant materials and it presents many benefits compared to organic solvents. The total phenolic content of the control feed was found to be 0.51 mgGAE/g dry matter.

The results regarding antiradical activity present a different and interesting pattern. More specifically, the value for the aqueous extract was calculated at $945.42 \pm 16.33 \mu\text{mol TRE/g}$ plant material, whereas the value for the β -cyclodextrin extract was equal to $899.65 \pm 7.86 \mu\text{mol TRE/g}$ plant material. Although the β -cyclodextrin extract presented a 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 \cdot is higher when water is used as the extraction media, compared to the β -cyclodextrin. The value of DPPH in the control feed was found to be $41.5 \mu\text{mol TRE/g}$ feed.

3.2. Performance Parameters

No mortality was observed in either of the three groups throughout the starter, grower and finisher phases. The results regarding the performance parameters are presented in Table 3. There was no difference in terms of live body weight among the three groups ($p \geq 0.05$). During the total trial period, no differences in feed intake and feed conversion ratio were noted among the three groups ($p \geq 0.05$).

Table 3. Effect of dietary supplementation with aqueous and cyclodextrin encapsulated herbal extracts on broilers performance.

Live Body Weight (g)	Control ¹	POM-ON-AQ ¹	POM-ON-CD ¹	SEM ²	<i>p</i> Value
Day 1	46.38	46.14	46.39	4.4	0.973
Day 10	365.32	364.37	347.12	49.64	0.261
Day 24	1404.5	1420.37	1379.72	125.91	0.455
Day 35	2287.75	2268.75	2267.25	246.49	0.943
Feed Intake per Chicken (g)	Control	POM-ON-AQ	POM-ON-CD	SEM	<i>p</i> Value
Days 1–10	381.10	389.05	382.10	31.18	0.572
Days 11–24	1746.97	1797.57	1853.72	194.81	0.065
Days 25–35	2116.85	2168.32	2199.12	426.65	0.766
Days 1–35	4244.92	4354.95	4354.95	499.89	0.325
Body Weight Gain (g)	Control	POM-ON-AQ	POM-ON-CD	SEM	<i>p</i> Value
Days 1–10	318.94	303.67	305.75	7.06	0.677
Days 11–24	1039.17	1079.12	1038.42	13.26	0.395
Days 25–35	883.25	835.20	885.55	24.32	0.678
Days 1–35	2241.37	2217.99	2229.73	25.15	0.942
Feed Conversion Ratio (g Feed/g Weight Gain)	Control	POM-ON-AQ	POM-ON-CD	SEM	<i>p</i> Value
Days 1–10	1.195	1.225	1.276	0.024	0.432
Days 11–24	1.683	1.709	1.798	0.034	0.405
Days 25–35	2.407	2.562	2.496	0.060	0.627
Days 1–35	1.895	1.959	2.000	0.027	0.303

¹ Control: Basal diet; POM-ON-AQ: Diet supplemented with aqueous extract at 0.1% per kg of DM; POM-ON-CD: Diet supplemented with cyclodextrin extract at 0.1% per kg of DM. ² SEM: Standard Error of Mean.

3.3. Welfare Status

The litter score was similar among all groups in terms of the litter score during the total trial period ($p \geq 0.05$) (Table 4). Similarly, the litter dry matter did not differ among the groups, although litter moisture was significantly higher for the POM-ON-AQ group compared to the other two groups at day 24 of the trial period ($p < 0.05$). However, no differences were observed on any other sampling day. Table 5 displays the pododermatitis and diarrhea score results, where all three groups had minor differences referring to the entire trial period ($p \geq 0.05$). The feather score displayed insignificant differences among the three groups during the overall trial period ($p \geq 0.05$) (Table 6). No differences were observed during the trial concerning the fecal score among the three treatment groups ($p \geq 0.05$). Additionally, no differences were detected in terms of fecal litter moisture concerning the three groups ($p \geq 0.05$) (Table 7). There was no difference among the three groups regarding the weight of the immune organs ($p \geq 0.05$) (Table 8). At day 24 of the experimental period, a significant difference was noted regarding the litter NH_3 between the POM-ON-AQ and the control group, where POM-ON-AQ presented significantly higher litter NH_3 compared to the control ($p < 0.05$) (Table 9). According to the wooden breast and white stripping scores no differences were observed among the trial groups during the experimental period ($p \geq 0.05$). Finally, there was no difference among the three groups regarding the carcass yield ($p \geq 0.05$) (Table 10).

Table 4. Effect of dietary supplementation with aqueous and cyclodextrin encapsulated herbal extracts on litter dry matter, litter moisture and litter score.

Litter Dry Matter (%)	Control ¹	POM-ON-AQ ¹	POM-ON-CD ¹	SEM ²	<i>p</i> Value
Day 10	64.687	64.662	66.162	0.630	0.559
Day 24	74.912	75.075	74.025	0.489	0.661
Day 35	75.012	75.725	74.412	0.835	0.827
Litter Moisture	Control	POM-ON-AQ	POM-ON-CD	SEM	<i>p</i> Value
Day 10	34.750	35.725	34.462	0.412	0.444
Day 24	23.762 ^b	27.487 ^a	24.700 ^b	0.537	0.007
Day 35	25.600	26.437	24.375	0.785	0.580
Litter Score	Control	POM-ON-AQ	POM-ON-CD	SEM	<i>p</i> Value
Day 10	1.312	1.500	1.500	0.091	0.649
Day 24	1.250	1.187	1.250	0.060	0.895
Day 35	1.062	1.187	1.125	0.054	0.662

¹ Control: Basal diet; POM-ON-AQ: Diet supplemented with aqueous extract at 0.1% per kg of DM; POM-ON-CD: Diet supplemented with cyclodextrin extract at 0.1% per kg of DM. ^{a,b} values in the same line with the same superscript do not differ significantly. ² SEM: Standard Error of Mean.

Table 5. Effect of dietary supplementation on broiler pododermatitis and diarrhea scores.

PD ¹ Score	Control ²	POM-ON-AQ ²	POM-ON-CD ²	SEM ³	<i>p</i> Value
Day 10	0.187	0.687	0.437	0.096	0.104
Day 24	0.312	0.187	0.312	0.073	0.743
Day 35	0.937	0.500	0.937	0.123	0.259
Diarrhea Score ⁴	Control	POM-ON-AQ	POM-ON-CD	SEM	<i>p</i> Value
Day 10	1.750	1.500	1.937	0.120	0.345

¹ PD: pododermatitis. ² Control: Basal diet; POM-ON-AQ: Diet supplemented with aqueous extract at 0.1% per kg of DM; POM-ON-CD: Diet supplemented with cyclodextrin extract at 0.1% per kg of DM. ³ SEM: Standard Error of Mean. ⁴ Diarrhea score: 3-point scoring system ranging from 1 to 3 (1 indicates absence of diarrhea and 3 severe diarrhea).

Table 6. Effect of dietary supplementation on broiler feather score.

Feather Score ¹	Control ²	POM-ON-AQ ²	POM-ON-CD ²	SEM ³	<i>p</i> Value
Day 10	2.687	2.687	2.625	0.083	0.944
Day 24	2.750	2.875	3.000	0.045	0.071
Day 35	2.750	2.687	2.750	0.060	0.895

¹ Feather score: 3-point scoring system ranging from 1 to 3 (where 1 refers to clean feathers and 3 to very dirty feathers). ² Control: Basal diet; POM-ON-AQ: Diet supplemented with aqueous extract at 0.1% per kg of DM; POM-ON-CD: Diet supplemented with cyclodextrin extract at 0.1% per kg of DM. ³ SEM: Standard Error of Mean.

Table 7. Effect of dietary supplementation on litter fecal score and litter fecal moisture.

Fecal Score ¹	Control ²	POM-ON-AQ ²	POM-ON-CD ²	SEM ³	<i>p</i> Value
Day 10	1.750	1.500	1.937	0.089	0.442
Day 24	1.125	1.375	1.125	0.089	0.526
Day 35	1.437	1.187	1.125	0.073	0.191

Fecal Litter Moisture (%)	Control	POM-ON-AQ	POM-ON-CD	SEM	<i>p</i> Value
Day 10	22.993	23.175	23.375	0.156	0.629
Day 24	23.612	22.306	22.818	0.405	0.435
Day 35	21.131	20.700	20.956	0.321	0.870

¹ Fecal score: 3-point scoring system ranging from 1 to 4 (1 represents firm and well-formed feces and 4 represents watery liquid feces). ² Control: Basal diet; POM-ON-AQ: Diet supplemented with aqueous extract at 0.1% per kg of DM; POM-ON-CD: Diet supplemented with cyclodextrin extract at 0.1% per kg of DM. ³ SEM: Standard Error of Mean.

Table 8. Effect of dietary supplementation on broiler spleen, bursa and thymus weight on day 35.

Spleen Weight (g)	Control ¹	POM-ON-AQ ¹	POM-ON-CD ¹	SEM ²	<i>p</i> Value
	2.331	2.355	2.066	0.090	0.368

Bursa Weight (g)	Control	POM-ON-AQ	POM-ON-CD	SEM	<i>p</i> Value
	2.273	2.307	2.298	0.029	0.898

Thymus Weight (g)	Control	POM-ON-AQ	POM-ON-CD	SEM	<i>p</i> Value
	1.418	1.440	1.377	0.036	0.788

¹ Control: Basal diet; POM-ON-AQ: Diet supplemented with aqueous extract at 0.1% per kg of DM; POM-ON-CD: Diet supplemented with cyclodextrin extract at 0.1% per kg of DM. ² SEM: Standard Error of Mean.

Table 9. Effect of dietary supplementation on NH₃.

Litter NH ₃	Control ¹	POM-ON-AQ ¹	POM-ON-CD ¹	SEM ²	<i>p</i> Value
Day 10	1.186	1.186	1.190	0.009	0.985
Day 24	1.130 ^b	1.168 ^a	1.150 ^{ab}	0.006	0.042
Day 35	1.213	1.201	1.211	0.010	0.887

¹ Control: Basal diet; POM-ON-AQ: Diet supplemented with aqueous extract at 0.1% per kg of DM; POM-ON-CD: Diet supplemented with cyclodextrin extract at 0.1% per kg of DM. ^{a,b} values in the same line with the same superscript do not differ significantly. ² SEM: Standard Error of Mean.

3.4. Protein Carbonyls

Breast and thigh meat protein carbonyls were affected by the dietary supplementation with pomegranate and onion peel extracts. The POM-ON-CD and the control group displayed significantly higher values regarding breast meat protein carbonyls in contrast to the POM-ON-AQ group ($p < 0.05$), while the POM-ON-CD group presented higher values compared to the control group ($p < 0.05$). Regarding thigh meat protein carbonyls, the POM-ON-CD group had higher values compared to the other two treatment groups ($p < 0.05$) (Table 11).

Table 10. Effect of dietary supplementation on broiler carcass yield, wooden breast score and white stripping score.

Carcass Yield (g)	Control ²	POM-ON-AQ ²	POM-ON-CD ²	SEM ³	<i>p</i> Value
Day 35	1907.31	1906.05	2014.96	21.77	0.057
Wooden Breast Score ¹	Control	POM-ON-AQ	POM-ON-CD	SEM	<i>p</i> Value
Day 35	0.375	0.437	0.250	0.101	0.763
White Stripping Score ¹	Control	POM-ON-AQ	POM-ON-CD	SEM	<i>p</i> Value
Day 35	0.562	0.500	0.875	0.082	0.137

¹ Wooden breast and white striping scores, using categories from 0 to 2 (where 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, exhibiting white lines in parallel to muscle fibers, that were > 1 mm thick). ² Control: Basal diet; POM-ON-AQ: Diet supplemented with aqueous extract at 0.1% per kg of DM; POM-ON-CD: Diet supplemented with cyclodextrin extract at 0.1% per kg of DM. ³ SEM: Standard Error of Mean.

Table 11. Effect of dietary supplementation on broiler breast and thigh meat composition, TBARS ¹ values and protein carbonyls.

Breast Meat	Control ²	POM-ON-AQ ²	POM-ON-CD ²	SEM ²	<i>p</i> Value
Moisture %	73.412 ^b	77.087 ^a	74.050 ^b	0.397	<0.001
Protein %	24.000	24.100	24.325	0.167	0.738
Fat %	1.696	1.180	1.288	0.119	0.182
Thigh Meat	Control	POM-ON-AQ	POM-ON-CD	SEM	<i>p</i> Value
Moisture %	74.766	74.446	74.833	0.133	0.469
Protein %	20.322 ^b	21.561 ^a	21.530 ^a	0.161	<0.001
Fat %	4.206 ^a	2.858 ^b	2.750 ^b	0.133	<0.001
Breast Meat TBARS (ng/g of Sample)	Control	POM-ON-AQ	POM-ON-CD	SEM	<i>p</i> Value
Day 1	0.065 ^a	0.045 ^c	0.048 ^b	0.001	<0.001
Day 3	0.067	0.033	0.049	0.012	0.542
Thigh Meat TBARS (ng/g of Sample)	Control	POM-ON-AQ	POM-ON-CD	SEM	<i>p</i> Value
Day 1	0.087 ^a	0.059 ^c	0.075 ^b	0.003	<0.001
Day 3	0.025 ^b	0.043 ^a	0.024 ^b	0.002	<0.001
Breast Meat Protein Carbonyls (nmol/mg of Sample)	Control	POM-ON-AQ	POM-ON-CD	SEM	<i>p</i> Value
	0.421 ^b	0.168 ^c	0.582 ^a	0.037	<0.001
Thigh Meat Protein Carbonyls (nmol/mg of Sample)	Control	POM-ON-AQ	POM-ON-CD	SEM	<i>p</i> Value
	0.273 ^b	0.217 ^b	0.735 ^a	0.054	<0.001

¹ TBARS: Thiobarbituric acid reactive substances. ² Control: Basal diet; POM-ON-AQ: Diet supplemented with aqueous extract at 0.1% per kg of DM; POM-ON-CD: Diet supplemented with cyclodextrin extract at 0.1% per kg of DM. ^{a,b,c} values in the same line with the same superscript do not differ significantly. ² SEM: Standard Error of Mean.

3.5. Breast and Thigh Meat Composition

The breast meat moisture percentage was significantly increased for the POM-ON-AQ group compared to the POM-ON-CD group and the control group ($p < 0.05$). No differences were noted in terms of breast meat protein and fat content among all groups. The thigh meat moisture percentage did not show any significant difference ($p \geq 0.05$). The POM-ON-AQ group and the POM-ON-CD group displayed a substantially higher protein content

compared to the control group ($p < 0.05$). On the contrary, the control group exhibited an elevated fat content compared to the other two treatment groups ($p < 0.05$) (Table 11).

3.6. Determination of TBARS

Determination of TBARS in the breast meat kept under refrigeration revealed significantly higher values for the control group compared to the other two groups ($p < 0.05$), whereas the POM-ON-CD group presented higher TBARS values compared to the POM-ON-AQ group ($p < 0.05$). TBARS values of the breast meat at day 3 of refrigerated storage showed no significant difference among the treatments ($p \geq 0.05$). However, the thigh meat TBARS values were affected by the treatments. At day 1 of refrigeration, the control group had the highest values compared to the other two groups ($p < 0.05$), whereas the POM-ON-CD group also had significantly higher values compared to the POM-ON-AQ group ($p < 0.05$). In contrast to day 1, at day 3 of refrigeration, the POM-ON-AQ group showed the highest TBARS values compared to the other two treatments ($p < 0.05$) (Table 11).

3.7. Meat Fatty Acid Composition

The effects of dietary treatments on the fatty acid composition of broiler breast and thigh meat are shown in Tables 12 and 13, respectively. Dietary supplementation with an aqueous or with a cyclodextrin pomegranate and onion peel extract resulted in a significant reduction in stearic acid ($p < 0.05$). In terms of Σ SFA (saturated fatty acids) in broiler breast meat, the POM-ON-CD group presented significantly lower values ($p < 0.05$). Regarding individual MUFAs (monounsaturated fatty acids), the oleic acid content was found to be significantly increased in the POM-ON-CD group, whereas the Σ MUFA were found to be increased in both supplemented groups. The cis-4,7,10,13,16,19-docosahexaenoic acid (DHA) fatty acids in the breast meat of the POM-ON-AQ supplemented broilers was found to be higher ($p < 0.05$) compared to the other groups, while the POM-ON-CD group showed the lowest value ($p < 0.05$). Σ PUFA (polyunsaturated fatty acids) content displayed increased values for the control group compared to the other two ($p < 0.05$). Moreover, the POM-ON-AQ group had significantly higher Σ n-3 fatty acids concentrations compared to the POM-ON-CD group ($p < 0.05$). A decrease was observed in the Σ n-6 fatty acids content in the case of both supplemented diets compared to the control one, while the POM-ON-AQ group had a higher content compared to the POM-ON-CD group ($p < 0.05$). The PUFA/SFA and H/H (hypocholesterolemic/hypercholesterolemic) ratios were found to be increased ($p \leq 0.05$) in both supplemented groups compared to the control one due to the decrease in the Σ SFA content as previously mentioned.

Table 12. Fatty acid composition (g/100 g of total fatty acids) of broiler breast meat.

Fatty Acid	Control ^{1,2,3}	POM-ON-AQ ^{1,2,3}	POM-ON-CD ^{1,2,3}
Myristic (C14:0)	0.45 ± 0.05 ^b	0.53 ± 0.01 ^a	0.51 ± 0.00 ^a
Palmitic (C16:0)	22.28 ± 0.15 ^b	23.34 ± 0.16 ^a	22.22 ± 0.20 ^b
Palmitoleic (C16:1 cis)	0.38 ± 0.02 ^c	2.01 ± 0.02 ^b	3.45 ± 0.03 ^a
Heptadecanoic (C17:0)	0.17 ± 0.03 ^b	0.20 ± 0.03 ^a	0.20 ± 0.01 ^a
Stearic (C18:0)	11.84 ± 0.01 ^a	10.95 ± 0.02 ^b	6.90 ± 0.90 ^c
Oleic (C18:1 cis ω9)	26.36 ± 0.02 ^b	25.16 ± 0.13 ^c	30.08 ± 0.27 ^a
Linoleic (C18:2 cis ω6)	25.93 ± 0.03 ^b	25.48 ± 0.33 ^b	29.54 ± 0.31 ^a
Arachidic (C20:0)	0.11 ± 0.01 ^b	0.14 ± 0.02 ^b	0.22 ± 0.02 ^a
γ-Linolenic (C18:3 cis ω6)	1.44 ± 0.03 ^b	1.42 ± 0.02 ^b	2.30 ± 0.03 ^a
Linolenic (C18:3 trans ω3)	0.35 ± 0.02 ^a	0.32 ± 0.01 ^a	0.31 ± 0.02 ^a
Heneicosanoic (C21:0)	1.02 ± 0.01 ^a	1.17 ± 0.01 ^a	0.55 ± 0.03 ^b

Table 12. Cont.

Fatty Acid	Control ^{1,2,3}	POM-ON-AQ ^{1,2,3}	POM-ON-CD ^{1,2,3}
cis-11,14-Eicosadienoic (C20:2 cis ω6)	0.17 ± 0.02 ^a	0.15 ± 0.00 ^a	0.08 ± 0.02 ^b
Behenic (C22:0)	0.92 ± 0.05 ^a	1.03 ± 0.01 ^a	0.46 ± 0.00 ^b
cis-8,11,14-Eicosatrienoate (C20:3 cis ω6)	6.26 ± 0.09 ^a	5.54 ± 0.26 ^b	2.31 ± 0.03 ^c
Erucic (C22:1 cis ω9)	0.19 ± 0.03 ^a	0.16 ± 0.04 ^a	0.08 ± 0.03 ^b
Arachidonic (C20:4 cis ω6)	0.26 ± 0.01 ^a	0.25 ± 0.00 ^a	0.10 ± 0.01 ^b
Lignoceric (C24:0)	0.21 ± 0.01 ^b	0.32 ± 0.00 ^a	0.11 ± 0.01 ^c
Nervonic (C24:1 cis ω9)	1.01 ± 0.02 ^a	1.08 ± 0.05 ^a	0.38 ± 0.09 ^b
cis-4,7,10,13,16,19-Docosahexaenoic (C22:6 cis ω3)	0.64 ± 0.10 ^b	0.76 ± 0.00 ^a	0.21 ± 0.05 ^c
∑SFA ⁴	37.00 ± 0.14 ^a	37.80 ± 0.12 ^a	31.15 ± 0.68 ^b
∑MUFA ⁵	27.94 ± 0.15 ^c	28.50 ± 0.25 ^b	33.99 ± 0.31 ^a
∑PUFA ⁶	35.05 ± 0.06 ^a	34.02 ± 0.24 ^b	34.86 ± 0.37 ^b
∑n-3 ⁷	0.99 ± 0.02 ^{a,b}	1.08 ± 0.15 ^a	0.52 ± 0.02 ^b
∑n-6 ⁸	8.13 ± 0.03 ^a	7.37 ± 0.25 ^b	4.79 ± 0.05 ^c
PUFA/SFA	0.95 ± 0.00 ^a	0.90 ± 0.01 ^a	1.12 ± 0.04 ^a
n-6/n-3	8.19 ± 0.17 ^a	6.87 ± 0.65 ^b	9.19 ± 0.06 ^a
H/H ⁹	2.40 ± 0.05 ^a	2.22 ± 0.03 ^a	2.74 ± 0.04 ^a

¹ Control: Basal diet; POM-ON-AQ: Diet supplemented with aqueous extract at 0.1% per kg of DM; POM-ON-CD: Diet supplemented with cyclodextrin extract at 0.1% per kg of DM. ² Each value is the mean of triplicate determinations ± s.d. ³ Different lowercase letters within the same row indicate significant differences according to Duncan's test ($p < 0.05$). ⁴ Saturated fatty acids, ⁵ Monounsaturated fatty acids, ⁶ Polyunsaturated fatty acids, ⁷ omega-3 fatty acids, ⁸ omega-6 fatty acids, ⁹ Hypocholesterolemic/hypercholesterolemic fatty acid ratio = $\frac{\sum C18:1 \text{ cis-9, C18 n-6, C20:4 n-6, C18:3 n-3, C20:3 n-6, C20:5 n-3, C22:6}}{\sum C14:0, C16:0}$.

Table 13. Fatty acid composition (g/100 g of total fatty acids) of broiler thigh meat.

Fatty Acid	Control ^{1,2,3}	POM-ON-AQ ^{1,2,3}	POM-ON-CD ^{1,2,3}
Myristic (C14:0)	0.54 ± 0.02 ^a	0.52 ± 0.03 ^a	0.48 ± 0.05 ^a
Myristoleic acid (C14:1)	0.13 ± 0.05 ^a	0.09 ± 0.01 ^a	0.07 ± 0.01 ^a
Palmitic (C16:0)	22.00 ± 0.38 ^b	23.75 ± 1.87 ^a	20.91 ± 0.25 ^c
Palmitoleic (C16:1 cis)	4.31 ± 0.06 ^a	3.80 ± 0.11 ^b	1.90 ± 1.41 ^c
Heptadecanoic (C17:0)	0.16 ± 0.03 ^a	0.18 ± 0.01 ^a	0.20 ± 0.03 ^a
Stearic (C18:0)	11.76 ± 0.91 ^a	8.61 ± 0.31 ^c	9.14 ± 0.12 ^b
Oleic (C18:1 cis ω9)	30.37 ± 0.42 ^b	30.51 ± 1.92 ^b	31.65 ± 0.72 ^a
Linoleic (C18:2 cis ω6)	25.85 ± 0.51 ^b	26.90 ± 0.85 ^b	28.57 ± 0.38 ^a
Arachidic (C20:0)	0.19 ± 0.01 ^a	0.19 ± 0.03 ^a	0.17 ± 0.02 ^a
g-Linolenic (C18:3 cis ω6)	2.12 ± 0.04 ^a	2.08 ± 0.08 ^a	1.85 ± 0.03 ^b
Linolenic (C18:3 trans ω3)	0.37 ± 0.04 ^a	0.32 ± 0.03 ^b	0.33 ± 0.05 ^b
Heneicosanoic (C21:0)	0.36 ± 0.03 ^c	0.47 ± 0.05 ^b	0.58 ± 0.07 ^a
Behenic (C22:0)	0.26 ± 0.07 ^c	0.34 ± 0.03 ^b	0.46 ± 0.05 ^a
cis-8,11,14-Eicosatrienoate (C20:3 cis ω6)	1.09 ± 0.05 ^b	1.53 ± 0.09 ^b	2.58 ± 0.07 ^a
Nervonic (C24:1 cis ω9)	0.21 ± 0.05 ^c	0.32 ± 0.03 ^b	0.47 ± 0.09 ^a
cis-4,7,10,13,16,19-Docosahexaenoic (C22:6 cis ω3)	0.11 ± 0.05 ^b	0.16 ± 0.03 ^b	0.32 ± 0.07 ^a
∑SFA ⁴	35.28 ± 0.64 ^a	34.06 ± 2.03 ^b	31.94 ± 0.39 ^c
∑MUFA ⁵	35.02 ± 0.39 ^a	34.74 ± 1.51 ^b	34.09 ± 0.83 ^b
∑PUFA ⁶	29.70 ± 0.55 ^b	31.21 ± 0.64 ^b	33.97 ± 0.44 ^a
∑n3 ⁷	0.48 ± 0.04 ^b	0.48 ± 0.02 ^b	0.65 ± 0.07 ^a
∑n6 ⁸	3.36 ± 0.07 ^b	3.83 ± 0.21 ^b	4.75 ± 0.06 ^a
PUFA/SFA	0.84 ± 0.05 ^b	0.92 ± 0.06 ^b	1.06 ± 0.09 ^a
n6/n3	7.05 ± 0.59 ^b	7.99 ± 0.22 ^a	7.33 ± 0.07 ^b
H/H ⁹	2.60 ± 0.05 ^b	2.47 ± 0.27 ^b	2.92 ± 0.05 ^a

¹ Control: Basal diet; POM-ON-AQ: Diet supplemented with aqueous extract at 0.1% per kg of DM; POM-ON-CD: Diet supplemented with cyclodextrin extract at 0.1% per kg of DM. ² Each value is the mean of triplicate determinations ± s.d. ³ Different lowercase letters within the same row indicate significant differences according to Duncan's test ($p < 0.05$). ⁴ Saturated fatty acids, ⁵ Monounsaturated fatty acids, ⁶ Polyunsaturated fatty acids, ⁷ omega-3 fatty acids, ⁸ omega-6 fatty acids, ⁹ Hypocholesterolemic/hypercholesterolemic fatty acid ratio = $\frac{\sum C18:1 \text{ cis-9, C18 n-6, C20:4 n-6, C18:3 n-3, C20:3 n-6, C20:5 n-3, C22:6}}{\sum C14:0, C16:0}$.

In the thigh meat of broilers fed with diets supplemented with both extracts, the palmitoleic acid content, the stearic acid content, as well as the Σ SFA content were significantly reduced compared to those fed with the non-supplemented diet. Furthermore, palmitoleic acid ($p < 0.05$) and Σ SFA displayed lower values for the POM-ON-CD group, while stearic acid displayed lower values for the POM-ON-AQ group ($p < 0.05$). Oleic acid, linoleic acid, 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 were significantly higher in the POM-ON-CD supplemented group in contrast to the other two groups ($p < 0.05$). Palmitic acid was found to be significantly higher in the POM-ON-AQ group ($p < 0.05$) and reduced in the POM-ON-CD group compared to the control group. However, the Σ MUFA content was found to be slightly reduced ($p < 0.05$) in the supplemented groups. Linolenic acid was also found to be low in the supplemented groups compared to the control ($p < 0.05$). A significant increase in the Σ PUFA content was also observed in the thigh meat of broilers fed with the cyclodextrin supplemented diet compared to the control and the aqueous one ($p < 0.05$). Between the supplemented groups, the POM-ON-CD group showed significant higher values ($p < 0.05$). Supplementation of the broilers diet with the cyclodextrin extracts (POM-ON-CD) 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 the POM-ON-CD group in response to dietary supplementation with the cyclodextrin extract ($p < 0.05$).

3.8. Color Parameters

Breast and thigh meat color parameters were affected by the dietary supplementation with pomegranate and onion peel extracts (Table 14). Breast meat lightness (L^*) was significantly lower for the control group compared to the treated ones, while the POM-ON-CD group had the highest value ($p < 0.05$). The POM-ON-AQ group displayed the highest redness (a^*) value among the three groups and, at the same time, the POM-ON-CD group had the lowest ($p < 0.05$). In terms of yellowness (b^*), the POM-ON-CD group showed significantly increased values, while the POM-ON-AQ group had the second highest value ($p < 0.05$).

Table 14. Meat color parameters.

Color Parameters	Control ²	POM-ON-AQ ²	POM-ON-CD ²
Breast Meat			
L^*	41.46 \pm 0.7 ^{c1}	42.45 \pm 0.20 ^b	44.77 \pm 0.81 ^a
a^*	3.55 \pm 0.35 ^b	4.27 \pm 0.11 ^a	3.00 \pm 0.15 ^c
b^*	5.81 \pm 0.05 ^c	6.44 \pm 0.12 ^b	7.94 \pm 0.31 ^a
Thigh Meat			
L^*	40.76 \pm 0.19 ^c	42.54 \pm 0.30 ^a	41.34 \pm 0.20 ^b
a^*	3.83 \pm 0.06 ^c	6.77 \pm 0.11 ^a	4.78 \pm 0.12 ^b
b^*	5.81 \pm 0.04 ^c	9.70 \pm 0.15 ^a	7.62 \pm 0.36 ^b

¹ Each value is the mean of triplicate determinations \pm s.d. Different superscript letters within the same row indicate significant differences according to Duncan's test ($p < 0.05$). ² Control: Basal diet; POM-ON-AQ: Diet supplemented with aqueous extract at 0.1% per kg of DM; POM-ON-CD: Diet supplemented with cyclodextrin extract at 0.1% per kg of DM. L^* stands for lightness, a^* for redness and b^* for yellowness.

According to the thigh meat color parameters, the POM-ON-AQ group exhibited significantly increased L^* , a^* and b^* values, while the control group had the lowest ($p < 0.05$).

4. Discussion

In the present study, pomegranate and onion peel extracts were employed as phytochemicals and their effect as dietary supplements in a broilers diet was evaluated. Apart from performance parameters, plausible effects on litter quality and welfare parameters of broilers were investigated. It was hypothesized that supplementation with plant extracts could impact gastrointestinal function and positively influence litter characteristics, for

the evaluation of which, indicators commonly applied at the experimental and industrial level were used. Furthermore, the nutrient profile of broiler meat was assessed, focusing primarily on the lipid profile and lipid oxidation. Based on the results of the study, the tested extracts' combination can be used as dietary supplements to improve the quality of broiler meat. However, as poultry meat quality can be affected by other dietary factors as well, further research is needed regarding the effect of the tested phytobiotics. For example, phytobiotics should be incorporated into diets with other ingredients, in order to evaluate the possible interactions with different components, as well as diets with different fat contents for the evaluation of antioxidant activity.

No growth promoting effect was observed on the supplemented groups compared to the control group. This result could be attributed to the fact that the broilers were raised under experimental conditions that cannot totally resemble field conditions. On the contrary, Akuru et al. [31] described a significant improvement in the average final body weight and daily gain of birds consuming 2 or 4 g of pomegranate peel powder meal per kilogram of feed, compared with the tocopherol-treated control group. This improvement could be attributed to the rich properties of pomegranate in proanthocyanidin [31]. Proanthocyanidin's mechanism of action is linked to the improvement of pancreatic function and digestive enzyme secretion in the small intestine. Moreover, proanthocyanidin acts as an antioxidant and has the ability to scavenge free radicals, thus leading to intestinal cell protection. Baset et al. [32] found no significant differences in feed intake and feed conversion ratio among groups supplemented or not with pomegranate peels during all the experimental periods. Similar were the results of Bostami et al. [33], who revealed that the daily feed intake and feed to gain ratio remained unaltered among the treatments throughout the different experimental periods.

Onion is known to be a rich source of bioactive compounds, including phenols, polyphenols, terpenoids and essential oils with antioxidant properties that stimulate digestion and promote growth [34,35]. Diets enriched with onion extracts have been shown to increase feed intake in broilers, thus leading to body weight gain, as confirmed by various studies [16,36]. The increase in feed intake could be attributed to the improvement of its taste, due to the sweet taste and flavor of bispropenyl disulfide. This compound is produced during heating from the alteration of the sulfur-containing components of onion [37].

Welfare status is an indicator of the overall flock health status and is positively correlated with the final products of advanced quality. Apart from housing and environmental factors, a broilers diet displays a significant regulating role in terms of welfare status. In the past, antibiotics were a cost-efficient resource that could amplify broiler health status [38]. Aromatic and medicinal plants and herbs have been identified as an additional and more acceptable way to increase broiler welfare. For example, the use of *Curcuma longa* powder as a feed additive in broiler nutrition was proved to have beneficial outcomes in terms of the diarrhea score when birds were infected with *Eimeria tenella* [39]. In our trial, the diarrhea score showed insignificant differences among the control and treated groups, an outcome that can be attributed to the lack of external diarrhea predisposing factors, such as coccidiosis.

Wet litter is known to prompt foot pad lesions or pododermatitis, a common condition affecting contemporary chicken breeding systems [40]. Pododermatitis results in decreased animal welfare status that could lead to reduced performance parameters [41]. Increased litter moisture is a multifactorial consequence leading to accelerated moisture [42]. In the present study, all treated groups displayed minor differences in terms of litter, fecal and pododermatitis score. However, in different commercial breeding environments, characterized by massive broiler populations and a burdened atmosphere, pomegranate and onion peel extracts would likely display their protective effect against pododermatitis.

Color plays a substantial role regarding the quality of meat products and may influence the consumers' preferences. Meat color is affected by many parameters such as chemical, biochemical, microbial and physical changes that occur during the stages of

animals' feeding, growth, maturation and storage of the carcass. The measurement of color parameters has been used as an indirect marker for the evaluation of meat quality, including oxidation, taste and pigment content [43]. This method is preferred because it is simple, fast and gives useful information regarding other physicochemical properties [43]. The effect of phytobiotics on the color of meat products has not been thoroughly investigated.

Different plant materials have been applied to broiler meat, primarily as flavorings, in the form of herbs and spices. Additionally, since several natural extracts have been known to retard lipid oxidation [44], they are employed as natural antioxidants in meat. The dietary supplementation of broiler feed with different plant extracts or bioactive compounds is responsible for color changes in the carcass [45]. These changes are attributed not only to the antioxidant capacity of these compounds but also to the ability of some to act as natural pigments, which can eventually affect the color of the meat as well as the final product. It would be interesting to unravel if such components or their metabolites reach the meat or skin tissues.

The dietary supplementation of the broiler diet with pomegranate and onion extracts affected the color parameters L^* , a^* and b^* of the final meat products in our trial (breasts and thighs). More specifically, both in the breast and thigh samples, incorporation of POM-ON-AQ and POM-ON-CD extracts significantly increased ($p < 0.05$) the L^* values (lightness) of the respective samples compared to the control group. An increase in lightness (L^*) is linked to oxidation reactions and transformation of myoglobin to metmyoglobin, leading to pale colorations in broiler meat [46]. In this regard, the presence of antioxidant bioactive compounds could delay metmyoglobin formation. In contrast to our results, the supplementation of antioxidants in broiler diets in the form of natural extracts should decrease the L^* value [22,31,47]. Even so, the L^* values in the samples of the present study remained inside the normal range for standard broiler meat according to [48]. In addition, increased L^* values in this case should not be associated with oxidation reactions since the TBARS values were lower in samples from the POM-ON-AQ group and the POM-ON-CD group compared to the control group. Regarding a^* values (redness), a different pattern compared to the L^* parameter was observed. In breast samples, the POM-ON-CD group presented the lowest a^* value while the highest was observed in the POM-ON-AQ group, whereas in thigh samples, the POM-ON-AQ group and the POM-ON-CD group presented significantly higher a^* values compared to the control group. Redness in meat products is positively linked to their quality as it is usually associated with freshness. Different interpretations have been presented regarding the values of the a^* parameter in meat. On one hand, the presence of antioxidant compounds may inhibit the formation of oxymyoglobin to metmyoglobin in the surface of the meat, thus the red color is preserved through storage [45]. However, other researchers have found that higher a^* values represent a higher oxidative capacity of muscles [49,50]. In this case, both the original color of pomegranate and onion extracts, as well as compounds that can act as natural pigments present in these extracts (e.g., anthocyanins), may be responsible for the different effects in color parameters of broiler meat and the increase in a^* values [51]. Lastly, the b^* values increased in the POM-ON-AQ group and the POM-ON-CD group meat samples, compared to the control group. This result is in agreement with Castañeda et al. [52], who suggested that natural pigments from plant extracts generally increase the b^* value, which enhances the yellowness in broiler meat.

Dietary saturated fatty acids (SFA), and particularly stearic, myristic and palmitic acids, have a pronounced importance due to their hypercholesterolemic properties that are associated with coronary heart diseases [15]. In the present study, dietary supplementation with either an aqueous or cyclodextrin extract significantly reduced the stearic acid and Σ SFA content of broiler breast and thigh meat. This could be explained by the increased content of oleic acid and total monounsaturated fatty acids (Σ MUFA) content in broiler meat, considering that plant antioxidant compounds enhanced the rapid conversion of stearic acid into oleic acid. Similar observations were made by Ahmed et al. [15] after the supplementation of the broiler diet with pomegranate by-products, as well as by

Ramiah et al., who studied the effects of dietary supplementation with herbal extracts containing garlic on broiler meat fatty acid composition [53]. The n-3 and n-6 fatty acids play also a phenomenal role in human nutrition as they are precursors of prostaglandins, leucotriens and thromboxanes, regulating the cardiovascular system. An increase in the n-3 fatty acids can cause a decrease in the n-6 fatty acids in meat as these two families of fatty acids compete for the same enzymes in their elongation and desaturation metabolism. The polysaturated fatty acids (PUFA)/SFA ratio is usually 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 breast and thigh meat of broilers fed a diet enriched with pomegranate and onion peel extracts had 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 were found to be higher in the breast and thigh meat of broilers fed with POM-ON-CD, indicating a positive effect on broiler meat. Possibly, the metabolomic and lipidomic analysis of oily and fatty compounds could provide more detailed information on the composition of the meat and the deposition of phenolic compounds on breast and thigh broiler meat.

In the present study, saturated cyclodextrin solutions were employed as extraction solvents for the recovery of bioactive compounds from pomegranate and onion peels. The results showed that β -cyclodextrin was not as effective as water regarding the extraction of antioxidant compounds, although. This could be attributed to the affinity of the β -cyclodextrin molecule and the compounds present in the plant materials [54]. However, the POM-ON-CD extract was able to further enhance some of the meat quality characteristics compared to the POM-ON-AQ extract, such as TBARS values and fatty acid composition. Although aqueous extracts from pomegranate and onion peels as dietary supplements have been mentioned in other studies before [16,31,55], supplementation of the broiler diet with aqueous cyclodextrin extracts, as well as the combination of extracts from pomegranate and onion peels, has not been previously reported in the literature.

5. Conclusions

The present study showed that the enrichment of broilers' diet with a mixture of *Punica granatum* and *Allium cepa* aqueous or cyclodextrin extracts beneficially modified the meat composition by increasing protein and lowering fat. Both aqueous and cyclodextrin herbal extracts reduced lipid oxidation, while protein carbonyls were beneficially affected by the aqueous herbal mixture. The examined performance parameters and welfare status were not affected. Increased Σ n-3 fatty acids, as well as Σ n-6 fatty acids, were detected in the thigh meat of broilers consuming the enriched diets. Both fatty acid composition and color parameters were altered by the incorporation of the extracts, the cyclodextrin extract being more efficient compared to the aqueous one. It is worth further investigation as to whether the polyphenolic extracts of pomegranate and onion, either in aqueous or cyclodextrin encapsulated forms, can enhance the performance of broiler chickens in large scale trials or health indices under various bacterial, viral or protozoal infectious agents.

Author Contributions: I.G., E.B. and I.S. conceived and designed the experiment. S.V., S.D., G.A.P., S.S., V.D., V.T. and I.G. collected raw materials. S.V., S.D., S.C., A.K. and I.M. performed the analytical experiments. S.V., G.A.P., V.T., E.B., S.C., A.K. and I.M. analyzed the data and interpreted the results. S.D., S.C., A.K., V.D., V.T., I.M. and I.G. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: 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).

Institutional Review Board Statement: The animal study protocol was in accordance with Greek legislation governing experimental animals and was 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.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Mottet, A.; Tempio, G. Global poultry production: Current state and future outlook and challenges. *World's Poult. Sci. J.* **2017**, *73*, 245–256. [[CrossRef](#)]
- Mir, N.A.; Rafiq, A.; Kumar, F.; Singh, V.; Shukla, V. Determinants of broiler chicken meat quality and factors affecting them: A review. *J. Food Sci. Technol.* **2017**, *54*, 2997–3009. [[CrossRef](#)] [[PubMed](#)]
- Grashorn, M.A. Use of phytobiotics in broiler nutrition—an alternative to infeed antibiotics. *J. Anim. Feed Sci.* **2010**, *19*, 338–347. [[CrossRef](#)]
- Gavriil, A.; Zilelidou, E.; Papadopoulou, A.-E.; Siderakou, D.; Kasiotis, K.M.; Haroutounian, S.A.; Gardeli, C.; Giannenas, I.; Skandamis, P.N. Evaluation of antimicrobial activities of plant aqueous extracts against *Salmonella Typhimurium* and their application to improve safety of pork meat. *Sci. Rep.* **2021**, *11*, 21971. [[CrossRef](#)] [[PubMed](#)]
- Kalaitsidis, K.; Sidiropoulou, E.; Tsiftoglou, O.; Mourtzinou, I.; Moschakis, T.; Basdagianni, Z.; Vasilopoulos, S.; Chatzigavriel, S.; Lazari, D.; Giannenas, I. Effects of *Cornus* and Its Mixture with *Oregano* and *Thyme* Essential Oils on Dairy Sheep Performance and Milk, Yoghurt and Cheese Quality under Heat Stress. *Animals* **2021**, *11*, 1063. [[CrossRef](#)] [[PubMed](#)]
- Grigoriadou, K.; Krigas, N.; Lazari, D.; Maloupa, E. Sustainable use of mediterranean medicinal-aromatic plants. In *Feed Additives: Aromatic Plants and Herbs in Animal Nutrition and Health*; Florou-Paneri, P., Christaki, E., Giannenas, I., Eds.; Elsevier: London, UK, 2020; pp. 57–74.
- Windisch, W.; Schedle, K.; Plitzner, C.; Kroismayr, A. Use of phytogetic products as feed additives for swine and poultry. *J. Anim. Sci.* **2010**, *86*, E140–E148. [[CrossRef](#)] [[PubMed](#)]
- Jin, L.-Z.; Dersjant-Li, Y.; Giannenas, I. Application of aromatic plants and their extracts in diets of broiler chickens. In *Feed Additives: Aromatic Plants and Herbs in Animal Nutrition and Health*; Florou-Paneri, P., Christaki, E., Giannenas, I., Eds.; Elsevier: London, UK, 2020; pp. 159–185.
- Viuda-Martos, M.; Fernández-López, J.; Pérez-Álvarez, J.A. Pomegranate and its many functional components as related to human health: A review. *Compr. Rev. Food Sci. Food Saf.* **2010**, *9*, 635–654. [[CrossRef](#)]
- Santas, J.; Almajano, M.P.; Carbó, R. Antimicrobial and antioxidant activity of crude onion (*Allium cepa*, L.) extracts. *Int. J. Sci. Technol.* **2010**, *45*, 403–409. [[CrossRef](#)]
- Christaki, E.; Giannenas, I.; Bonos, E.; Florou-Paneri, P. Innovative uses of aromatic plants as natural supplements in nutrition. In *Feed Additives: Aromatic Plants and Herbs in Animal Nutrition and Health*; Florou-Paneri, P., Christaki, E., Giannenas, I., Eds.; Elsevier: London, UK, 2020; pp. 19–31.
- Tashla, T.; Puvača, N.; Pelić, D.L.; Prodanović, R.; Bošković, J.; Ivanišević, D.; Jahić, M.; Mahmoud, O.; Giannenas, I.; Lević, J. Dietary medicinal plants enhance the chemical composition and quality of broiler chicken meat. *JHVMS* **2019**, *70*, 1823–1832. [[CrossRef](#)]
- Giannenas, I.; Tsinas, A.; Bartzanas, T.; Skoufos, I.; Tzora, A.; Karamoutsios, A. *Oregano* and *sage* essential oils improve antioxidant status of raw and cooked breast and thigh chicken meat: In vivo investigation of antioxidant constituents of *oregano* and *sage* on chicken breast and thigh meat. *Agro Food Ind. Hi-Tech.* **2016**, *27*, 24–27.
- Johanningsmeier, S.D.; Harris, G.K. Pomegranate as a Functional Food and Nutraceutical Source. *Annu. Rev. Food Sci. Technol.* **2011**, *2*, 181–201. [[CrossRef](#)] [[PubMed](#)]
- Ahmed, S.T.; Islam, M.M.; Bostami, A.R.; Mun, H.-S.; Kim, Y.-J.; Yang, C.-J. Meat composition, fatty acid profile and oxidative stability of meat from broilers supplemented with pomegranate (*Punica granatum* L.) by-products. *Food Chem.* **2015**, *188*, 481–488. [[CrossRef](#)] [[PubMed](#)]
- Aditya, S.; Ahammed, M.; Jang, S.H.; Ohh, S.J. Effects of dietary onion (*Allium cepa*) extract supplementation on performance, apparent total tract retention of nutrients, blood profile and meat quality of broiler chicks. *Asian-Australas. J. Anim. Sci.* **2017**, *30*, 229–235. [[CrossRef](#)] [[PubMed](#)]
- Chemat, F.; Vian, M.A.; Cravotto, G. Green Extraction of Natural Products: Concept and Principles. *Int. J. Mol. Sci.* **2012**, *13*, 8615–8627. [[CrossRef](#)] [[PubMed](#)]
- Mourtzinou, I.; Salta, F.; Yannakopoulou, K.; Chiou, A.; Karathanos, V.T. Encapsulation of Olive Leaf Extract in β -Cyclodextrin. *J. Agric. Food Chem.* **2007**, *55*, 8088–8094. [[CrossRef](#)] [[PubMed](#)]
- Diamanti, A.C.; Igoumenidis, P.E.; Mourtzinou, I.; Yannakopoulou, K.; Karathanos, V.T. Green extraction of polyphenols from whole pomegranate fruit using cyclodextrins. *Food Chem.* **2017**, *214*, 61–66. [[CrossRef](#)] [[PubMed](#)]
- Aviagen. *ROSS Nutrition Specifications*; Aviagen: Huntsville, AL, USA, 2019.
- Singleton, V.L.; Orthofer, R.; Lamuela-Raventios, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol.* **1999**, *299*, 152–178.

22. Welfare Quality, R. *Welfare Quality R Assessment Protocol for Poultry (Broilers, Laying Hens)*; Welfare Quality R Consortium: Lelystad, The Netherlands, 2009.
23. AHPA. *Standard Methods for the Examination of Water and Wastewater*, 17th ed.; American Public Health Association: Washington, DC, USA, 1989.
24. Brauer-Vigoderis, R.; Ferreira-Tinôco, I.D.F.; Pandorfi, H.; Bastos-Cordeiro, M.; de Souza-Júnior, J.P.; de Carvalho-Guimarães, M.C. Effect of heating systems in litter quality in broiler facilities in winter conditions. *Dyna* **2014**, *81*, 36–40. [[CrossRef](#)]
25. Anderson, S.; Aldana, S.; Beggs, M.; Birkey, J.; Conquest, A.; Conway, R.; Hemminger, T.; Herrick, J.; Hurley, C.; Ionita, C.; et al. Determination of Fat, Moisture, and Protein in Meat and Meat Products by Using the FOSS FoodScan Near-Infrared Spectrophotometer with FOSS Artificial Neural Network Calibration Model and Associated Database: Collaborative Study. *J. AOAC Int.* **2007**, *90*, 1073–1083. [[CrossRef](#)]
26. Ahn, D.U.; Olson, D.G.; Jo, C.; Love, J.; Jin, S.K. Volatiles Production and Lipid Oxidation in Irradiated Cooked Sausage as Related to Packaging and Storage. *J. Food Sci.* **1999**, *64*, 226–229. [[CrossRef](#)]
27. Patsoukis, N.; Zervoudakis, G.; Panagopoulos, N.T.; Georgiou, C.D.; Angelatou, F.; Matsokis, N.A. Thiol redox state (TRS) and oxidative stress in the mouse hippocampus after pentylenetetrazol-induced epileptic seizure. *Neurosci. Lett.* **2004**, *357*, 83–86. [[CrossRef](#)] [[PubMed](#)]
28. Folch, J.; Lees, M.; Stanley, G.H.S. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **1957**, *226*, 497–509. [[CrossRef](#)]
29. Charan, J.; Kantharia, N.D. How to calculate sample size in animal studies? *J. Pharmacol. Pharmacother.* **2013**, *4*, 303–306. [[CrossRef](#)]
30. IDRE. G*POWER. Available online: <https://stats.idre.ucla.edu/other/gpower/> (accessed on 2 February 2021).
31. Akuru, E.A.; Mpendulo, C.T.; Oyeagu, C.E.; Nantapo, C.W.T. Pomegranate (*Punica granatum* L.) peel powder meal supplementation in broilers: Effect on growth performance, digestibility, carcass and organ weights, serum and some meat antioxidant enzyme biomarkers. *Ital. J. Anim. Sci.* **2021**, *20*, 119–131. [[CrossRef](#)]
32. Baset, S.A.; Ashour, E.A.; El-Hack, M.E.A.; El-Mekkawy, M.M. Effect of different levels of pomegranate peel powder and probiotic supplementation on growth, carcass traits, blood serum metabolites, antioxidant status and meat quality of broilers. *Anim. Biotechnol.* **2020**, 1–11. Available online: <https://www.webofscience.com/wos/woscc/full-record/WOS:000574208300001> (accessed on 9 January 2022). [[CrossRef](#)] [[PubMed](#)]
33. Bostami, A.B.M.R.; Ahmed, S.T.; Islam, M.M.; Mun, H.S.; Ko, S.S.; Kim, S.; Yang, C.J. Growth performance, fecal noxious gas emission and economic efficacy in broilers fed fermented pomegranate byproducts as residue of fruit industry. *Int. J. Adv. Res.* **2015**, *3*, 102–114.
34. Cross, D.E.; McDevitt, R.M.; Hillman, K.; Acamovic, T. The effect of herbs and their associated essential oils on performance, dietary digestibility and gut microflora in chickens from 7 to 28 days of age. *Br. Poult. Sci.* **2007**, *48*, 496–506. [[CrossRef](#)]
35. Lee, K.-W.; Everts, H.; Kappert, H.J.; Beynen, A.C. Addition of β -ionone to the diet fails to affect growth performance in female broiler chickens. *Anim. Feed Sci. Technol.* **2003**, *106*, 219–223. [[CrossRef](#)]
36. An, B.K.; Kim, J.Y.; Oh, S.T.; Kang, C.W.; Cho, S.; Kim, S.K. Effects of onion extracts on growth performance, carcass characteristics and blood profiles of white mini broilers. *Asian-Australas. J. Anim. Sci.* **2015**, *28*, 247. [[CrossRef](#)]
37. Gonzalez, M.E.; Anthon, G.E.; Barrett, D.M. Onion Cells after High Pressure and Thermal Processing: Comparison of Membrane Integrity Changes Using Different Analytical Methods and Impact on Tissue Texture. *J. Food Sci.* **2010**, *75*, E426–E432. [[CrossRef](#)]
38. Pliego, A.B.; Tavakoli, M.; Khusro, A.; Seidavi, A.; Elghandour, M.M.M.Y.; Salem, A.Z.M.; Márquez-Molina, O.; Rivas-Caceres, R.R. Beneficial and adverse effects of medicinal plants as feed supplements in poultry nutrition: A review. *Anim. Biotechnol.* **2020**, 1–23. Available online: <https://www.webofscience.com/wos/woscc/full-record/WOS:000555171500001> (accessed on 9 January 2022). [[CrossRef](#)] [[PubMed](#)]
39. Abbas, R.Z.; Iqbal, Z.; Khan, M.N.; Zafar, M.A.; Zia, M.A. Anticoccidial activity of *Curcuma longa* L. in broilers. *Braz. Arch. Biol. Technol.* **2010**, *53*, 63–67. [[CrossRef](#)]
40. Mench, J.; De Jong, I.; Butterworh, A.; Raj, M.; Berg, L. *Broiler Chickens: Welfare in Practice*; 5m Books Ltd.: South Yorkshire, UK, 2021.
41. Eichner, G.; Vieira, S.L.; Torres, C.A.; Coneglian, J.L.B.; Freitas, D.M.; Oyarzabal, O.A. Litter Moisture and Footpad Dermatitis as Affected by Diets Formulated on an All-Vegetable Basis or Having the Inclusion of Poultry By-Product. *J. Appl. Poult. Res.* **2007**, *16*, 344–350. [[CrossRef](#)]
42. Collett, S.R. Nutrition and wet litter problems in poultry. *Anim. Feed Sci. Technol.* **2012**, *173*, 65–75. [[CrossRef](#)]
43. Pathare, P.B.; Opara, U.L.; Al-Said, F.A.-J. Colour Measurement and Analysis in Fresh and Processed Foods: A Review. *Food Bioprocess Technol.* **2013**, *6*, 36–60. [[CrossRef](#)]
44. Ahn, J.; Grün, I.U.; Mustapha, A. Antimicrobial and Antioxidant Activities of Natural Extracts In Vitro and in Ground Beef. *J. Food Prot.* **2004**, *67*, 148–155. [[CrossRef](#)]
45. Mancini, R.A.; Hunt, M.C. Current research in meat color. *Meat Sci.* **2005**, *71*, 100–121. [[CrossRef](#)]
46. Fernández-Ginés, J.M.; Fernández-López, J.; Sayas-Barberá, E.; Pérez-Alvarez, J.A. Meat products as functional foods: A review. *J. Food Sci.* **2005**, *70*, 37–43. [[CrossRef](#)]
47. Cui, Y.-M.; Wang, J.; Lu, W.; Zhang, H.-J.; Wu, S.-G.; Qi, G.-H. Effect of dietary supplementation with *Moringa oleifera* leaf on performance, meat quality, and oxidative stability of meat in broilers. *Poult. Sci.* **2018**, *97*, 2836–2844. [[CrossRef](#)]

48. Garcia, R.G.; de Freitas, L.W.; Schwingel, A.W.; Farias, R.M.; Caldara, F.R.; Gabriel, A.M.A.; Almeida Paz, I.C.L. Incidence and physical properties of PSE chicken meat in a commercial processing plant. *Braz. J. Poult. Sci.* **2010**, *12*, 233–237. [[CrossRef](#)]
49. Getry, J.G.; McGlone, J.J.; Miller, M.F.; Blanton, J.R., Jr. Environmental effects on pig performance, meat quality, and muscle characteristics. *J. Anim. Sci.* **2004**, *82*, 209–217. [[CrossRef](#)] [[PubMed](#)]
50. Lee, T.T.; Ciou, J.Y.; Chen, C.L.; Yu, B. Effect of *Echinacea purpurea* L. on oxidative status and meat quality in Arbor Acres broilers. *J. Sci. Food Agric.* **2013**, *93*, 166–172. [[CrossRef](#)] [[PubMed](#)]
51. Gao, Y.; Yeh, H.-Y.; Bowker, B.; Zhuang, H. Effects of different antioxidants on quality of meat patties treated with in-package cold plasma. *Innov. Food Sci. Emerg. Technol.* **2021**, *70*, 102690. [[CrossRef](#)]
52. Castaneda, M.P.; Hirschler, E.M.; Sams, A.R. Skin pigmentation evaluation in broilers fed natural and synthetic pigments. *Poult. Sci.* **2005**, *84*, 143–147. [[CrossRef](#)] [[PubMed](#)]
53. Ramiah, S.K.; Zulkifli, I.; Rahim, N.A.A.; Ebrahimi, M.; Men, G.Y. Effects of two herbal extracts and virginiamycin supplementation on growth performance, intestinal microflora population and fatty acid composition in broiler chickens. *Asian Australas. J. Anim. Sci.* **2014**, *27*, 375–382. [[CrossRef](#)]
54. Cai, R.; Yuan, Y.; Cui, L.; Wang, Z.; Yue, T. Cyclodextrin-assisted extraction of phenolic compounds: Current research and future prospects. *Trends Food Sci. Technol.* **2018**, *79*, 19–27. [[CrossRef](#)]
55. Saleh, H.; Golian, A.; Kermanshahi, H.; Mirakzehi, M.T. Antioxidant status and thigh meat quality of broiler chickens fed diet supplemented with α -tocopherolacetate, pomegranate pomace and pomegranate pomace extract. *Ital. J. Anim. Sci.* **2018**, *17*, 386–395. [[CrossRef](#)]