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Thermal Comfort, Growth Performance and Welfare of Olive Pulp Fed Broilers during Hot Season

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Abstract: This study evaluated the nutritional effect of dried olive pulp (OP), on broilers' thermal comfort, growth parameters and welfare in a commercial poultry farm during the hot season. A number of 108 Cobb male broilers, 19 d olds were allocated into three dietary groups: controls (CON), OP3 and OP6, based on the level of OP added to their diet (0%, 3% and 6%). The thermal comfort of broilers was assessed using the temperature–humidity index (THI). Broilers' body temperature (BT) was determined weekly. OP beneficially affected the growth performance of broilers undergoing very severe heat stress, as indicated by the increased body weight gain (BWG) recorded in OP groups compared to CON during the first week of the experiment and the higher body weight (BW) of OP fed chickens at 26 d of age ($p < 0.05$). At 26 d of age, OP6 broilers had lower BT (40.55 ± 0.06 °C) than CON (40.78 ± 0.09 °C) ($p < 0.05$). A positive dietary effect of OP in welfare parameters like feather cleanliness and panting behaviour of chickens fed 6% OP was also recorded. Using OP as feedstuff is a promising feeding strategy for alleviating the adverse effects of heat stress; it also offers the potential to recycle olive by-products, leading to an efficient waste-based circular economy.

Keywords: thermal comfort; broilers; olive pulp; growth performance; welfare; behaviour

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1. Introduction

In recent years, the poultry sector has been developing rapidly worldwide to meet the growing consumer demand for poultry meat and eggs. Chicken meat is low in saturated fat and rich in protein, vitamins and minerals [1]. Similarly, a hen's egg is the most affordable source of animal protein and constitutes a complete and highly nutritious food with significant health benefits for consumers [2]. It is estimated that animal-based food demand will grow by nearly 70% in 2050 [3] due to the expected global population rise from 7.8 to 9.9 billion in the same timeline [4]. Accordingly, worldwide consumption of eggs and poultry meat has increased twice in the last ten years and a further double is projected by the year 2050 [5]. More specifically, the consumption of eggs in the 28 countries of the European Union (EU-28) was 6836 million tonnes in 2019 and is forecast to increase by a further 8% by 2030 [6]. In addition, poultry meat consumption, which has grown rapidly in the EU over the past decade, reached 24.8 kg per capita in 2018 and is expected to continue growing at a slower pace over the next decade [7]. To cover those needs, great progress in genetic improvement in both industrially raised broilers and laying hens has been documented. However, modern broiler breeds have higher metabolic activity and produce more body heat due to their rapid growth rates [8]. Furthermore, modern broilers are particularly sensitive to high temperatures and, therefore, more susceptible to heat stress due to limited heat dissipation caused by feathering, the absence of sweat glands and the relatively high stocking densities of intensive commercial production systems [9]. Heat stress occurs when the birds cannot keep the balance between the heat that is produced in their bodies and the heat that is released from them into the environment [8,9]. This is due to a combination of factors including high ambient temperature, humidity, radiant heat and wind speed, with high ambient temperature being the most significant [8]. The chicken's normal body temperature ranges from 41 °C to 42 °C, whereas the thermo-neutral zone for maximum growth is between 18–21 °C [10]. Previous reports demonstrate that heat stress begins when the ambient temperature rises above 25 °C for poultry species and/or when an individual's heat production exceeds its heat dissipation capacity [11].

Heat stress constitutes a major issue for the avian sector that negatively affects birds' health and performances, causing significant economic costs. In 2003, annual economic losses to the poultry industry due to this condition in the United States of America reached USD 128 to USD 165 million [12]. As global temperatures rise, this number is expected to increase over the next few years. It has been suggested that there could be a loss of 25% or more of livestock production in industrialised farming systems, particularly in some tropical regions [9]. The Intergovernmental Panel on Climate Change has highlighted that the world will warm by more than 2 °C in the 21st century if no preventive measures are taken [13]. They also pointed out that climate change is already increasing the frequency and intensity of extreme weather events, such as heat waves, and will continue to do so. It is well known that when birds are kept in conditions outside their thermal comfort zone, a series of behavioural, physiological and endocrinological changes are observed in order to maintain their body temperature within normal limits [10,14]. Birds are typically reported to be less social, consume less food while drinking more water, spend less time walking and moving, and elevate their wings and breathe rapidly with their beaks open (panting). In addition, increased mortality and reduced performance are often documented, such as a reduction of feed consumption and body weight, and deterioration in the feed conversion ratio, but also in the quality of meat and eggs [14]. It has been found that feed intake decreases by 1.5% for every degree rise in temperature when the ambient temperature is between 21 °C and 30 °C and this decline will increase to 4.6% when the ambient temperature is between 32 °C and 38 °C [15]. Body temperature, gut health, appetite hormone regulation, immune response and oxidative properties are also negatively affected by heat stress [16].

In a global effort to combat heat stress and its detrimental effects on the health and welfare of chickens and the financial costs to poultry farms, the scientific community has focused on the research and implementation of different strategic approaches. The development of heat-tolerant breed lines, proper poultry house management as well as various nutritional interventions have been employed for mitigating this problem [14,16]. One of the nutritional practices used to help chickens better cope with heat stress is to incorporate phytogenic feed additives with antioxidant activity, such as polyphenols, into their diet [10,14,15]. Polyphenols have great potential as novel feed additives to improve poultry productivity in heat-stressed poultry, as they are natural antioxidants capable of reducing oxidative stress that is abundant in a variety of plants [15].

Olive trees (*Oleaeuropea* L.), widely cultivated in Mediterranean countries for their edible fruit and olive oil, are rich in phenolic substances, such as oleuropein, which have important pharmaceutical properties [17–21]. Olive pulp (OP), which is produced when olive oil is extracted, represents one such feed additive of plant origin. It has been documented that the olive oil industry generates huge amounts of secondary products such as leaves, pits, effluent from olive mills and solid residues such as pomace and olive cakes, which have a negative impact on the environment because they are phytotoxic and rich in organic matter [22]. In compliance with worldwide demands for sustainable livestock farming systems, great efforts have been made in the latest years for using olive by-products as feedstuff, which has made it possible to potentially recycle them, thus leading to an efficient circular waste-based economy [23,24]. In addition, the dependence of animal production on seeds consumed by humans and the costs of waste disposal is reduced by using alternative feedstuffs in animal diets [23]. Taking into consideration that feed cost represents approximately 70% of the total cost of the production in poultry industry, seeking newer sources of raw materials from agricultural and industrial by-products and evaluating them as feed ingredients to reduce these costs is of great importance for the viability of the enterprises [25,26]. Finally, this practice is fully in line with the EU's Green Deal strategy to improve the efficient use of resources by moving towards a clean and circular economy to mitigate climate change, reverse the loss of biodiversity and reduce pollution [27].

Olive pulp is the remainder left after the drying of olive cake (the raw material obtained when olive oil is extracted). It contains increased levels of residual oil and essential fatty acids (73% oleic acid, 13% palmitic acid, and 7% linoleic acid) [28]. It also has beneficial oleuropeoside compounds such as oleuropein, phenolic compounds such as tyrosol [29] and a lot of bio-active ingredients with antibacterial, antioxidant and antifungal functions [30,31]. Consequently, the nutritional and chemical composition of OP makes it a valuable and low-cost nutrient for livestock [32,33]. The effect of feeding various levels of OP (2–20%) on the growth performance of broilers has been investigated in a number of studies with promising but not always consistent results [29,34–43]. Furthermore, recent reports have shown that feeding OP to broilers at levels of 3% and 6% beneficially affects birds' welfare parameters such as foot pad dermatitis (FPD) and feather cleanliness [44]. On the other hand, the review of the literature shows that there are currently no data available on the dietary impact of OP on the thermal comfort of broilers. In contrast, there are a few studies on the nutritional effects of various by-products such as olive oil [45–47] and olive leaves [48,49] on heat stress in chickens. Recent nutritional studies in broilers reared at high ambient temperatures have demonstrated the antioxidant effect of olive oil [45,46], improved birds' immune system [46], improved hormonal indicators and chick performance and the increased activity of genes responsible for the birds' thermal resistance [47]. Similarly, the addition of olive leaf extract to broiler water or feed has been shown to alleviate the negative impact of heat stress and improve performance and antioxidant status in heat-tressed chickens [48,49]. In view of the foregoing, the purpose of the current study was to evaluate the dietary impact of dried OP on the thermal comfort and growth performance of broilers during the hot season. In addition, some welfare and quality behavioural traits of the birds were also evaluated. The OP inclusion rates used in the present study were selected on the basis of the outcomes of our previous research work in which we obtained very good results in terms of feather cleanliness and (FPD) [44].

2. Materials and Methods

2.1. Ethics Statement

The Research Ethics Committee of the Hellenic Agricultural Organisation-DIMITRA approved the experimental protocol of the trial and the animal care procedures used (22072/26.04.2021).

2.2. Animals, Diets and Experimental Design

This study was performed in a closed and environmentally controlled commercial poultry farm in Greece (40.29963156500821, 22.558737078563748) during the summer months (from 29 June until 20 July 2021) and lasted 22 days. Overall, 108 Cobb male broilers, 19 d old, with starting body weight (BW) of 951.16 ± 4.17 g, were used in the trial. The broilers used in this study were chosen from a commercial flock that entered the poultry house at the age of 1 d old. Before starting the experiment, an adjustment period of 18 days was preceded to allow chickens to adapt to the new poultry house environment and to limit the stress factors associated with their transportation from the hatchery to the poultry farm. Furthermore, this period was necessary for the broilers to achieve a uniform body weight. The 19 d old chicks were placed in 9 consecutive floor pens of 1 m^2 (12 birds/pen) that were bedded with rice hulls and equipped with nipple drinkers and a bell feeder. Mash feed and fresh water were provided ad libitum, throughout the experiment. The stocking density in each pen (33 kg/m^2) met the requirements of EU Directive 2007/43/EC [50]. The poultry house was equipped with tunnel and longitudinal fans as well as windows and pad cooling. Environmental conditions such as temperature, relative humidity, lighting and ventilation were automatically controlled and followed the guidelines of Cobb management [51].

The experimental design, the OP and the diets used in this study are described in detail by Dedousi et al. [44]. In brief, chickens were randomly distributed into 3 groups, CON, OP3 and OP6, based on the inclusion level of OP in the diet (0%, 3% and 6%, respectively) with 36 broilers/group, 3 replicates (pens)/group and 12 broilers/replicate (pen). Chickens 19 and 20 d of age were fed a grower diet, followed by a finisher 1 and a finisher 2 diet applied from 21 to 32 d of age and from 33 to 40 d of age, respectively. Thus, 9 isonitrogenous and isocaloric rations were formulated, 1 per feeding period/dietary treatment (Table S1) where dried OP mainly replaced wheat and some sunflower oil in the CON diet. The dried OP that was used in this study was a commercial product in flour form [44] and its nutrient and fatty acid composition is presented in Table S2.

2.3. Temperature–Humidity Index

Temperature–humidity index (THI) was used to assess the broilers' thermal comfort and it was determined using the equation reported by Marai et al. [52]:

$$THI = Tdb - \{(0.31 - 0.31\ RH)\ (Tdb - 14.4)\}$$

where THI is the temperature–humidity index, Tdb is the dry bulb temperature in Celsius and RH is the relative humidity percentage (RH)/100.

The thermal environmental parameters (Tdb and RH) were recorded three times daily inside the poultry house during the whole experimental period by using 9 thermometers–hygrometers (Therma-hygrometer®, PC810-145, Electronic Temperature Instruments, Worthing, West Sussex, UK) that were placed just above the head level of birds at each pen. From the recorded data, an average value for Tdb and RH was estimated and used for the THI equation. From the daily THI values obtained an average THI value on a weekly basis was calculated and classified as follows:

THI: <27.8 = absence of heat stress; THI: 27.8 to <28.9 = moderate heat stress; THI: 28.9 to <30.0 = severe heat stress; and THI: 30.0 and more = very severe heat stress [53].

The average temperature and humidity (mean ± SD) throughout the experiment was 26.58 ± 2.68 °C and 72.19 ± 13.66%, respectively.

2.4. Production Traits and Body Temperature

Broilers' BW and body temperature (BT) were measured at 19, 26, 33 and 40 d of age. Body weight gain (BWG) and feed consumption (FC) per bird were estimated weekly and throughout the experiment (19–40 d of age). Similarly, taking into consideration FC and BWG, the feed conversion ratio (FCR) per chicken was determined on a weekly basis and throughout the study. Mortality records were kept daily.

The cloacal temperature values in degrees Celsius °C were recorded as an index of the core BT [54]. Each BT measurement was performed at the same time (16:00 p.m.) by using a digital clinical thermometer (±0.1 °C accuracy; KRUUSE digital thermometer®, model VT-801SLEW, DK-5550, Langeskov, Denmark). After the birds had been gently caught and restrained, the cloacal temperature of each chicken was measured by inserting the thermometer at a depth of approximately 3 cm into the cloaca and tilting it to ensure direct contact with the cloacal wall. The thermometer was left until it reached a steady reading, followed by a repeated beep. To prevent possible cross-infection between birds, the thermometer was wiped with fresh, clean cotton wool moistened with methylated alcohol between each measurement.

2.5. Welfare and Behaviour Traits

At 26, 33 and 40 d of age, broilers from every dietary treatment were individually assessed and scored for feather cleanliness, FPD, hock burn and behavioural characteristics, using the Welfare Quality (2009) protocol [55]. Firstly, qualitative observations of behavioural characteristics (panting, moving/walking and lifting up winds) were made for avoiding handling stress that could lead to confounding data. Quality behaviour traits were estimated by individual visual observations (no recording) that lasted approximately

2 min for each pen. The number of birds in each dietary treatment expressing a specific type of behaviour was recorded, divided by the total live birds in that group and multiplied by 100. Therefore, the data % was estimated. A similar method was employed to assess the welfare characteristics for each scoring category/welfare characteristic. The visual observations were performed by the same person and at the same time (15:30 p.m.) at each time point (26, 33 and 40 d of age).

Afterward, every bird was gently restrained by one person, and its chest was inspected to assess feather cleanliness using a 3-point scale: Score 0—completely clean feathers; Score 1—slight feather soiling; Score 2—moderate feather soiling; and Score 3—severe feather soiling. Then, the percentage of birds with each score was determined. Both feet were inspected for FPD (swelling-bubble foot) or hock burn and scored as follows: (a) FPD: 0—no evidence of FPD; Score 1—minimal evidence of FPD, Score 2—evidence of FPD. (b) Hock burn: 0—no evidence of hock burn; Score 1—minimal evidence of hock burn, Score 2—evidence of hock burn. Afterward, the percentage of birds scored in each category was assessed.

The order of all broilers' parameters evaluated was: behavioural traits, BT, BW and, finally, welfare parameters estimation.

2.6. Statistical Analysis

Jeffreys's Amazing Statistics Program JASP (v 0.16.3; JASP Team, 2022) software was used for data analysis [56]. The broilers were the experimental units used for all parameters except FCR which was the only parameter that the replicates were the experimental unit. Prior to the onset of this study, the minimum required number of replications was calculated using F tests, ANOVA: fixed effects, omnibus one-way procedure at the G*Power 3.1 software using FCR of chickens at market age as a parameter of evaluation. The desired power was set at 0.8 and the error rate probability at 0.05; the desired detectable difference in FCR was considered to be 0.07 kg feed/kg gain (means: 1.93, 2.00 and 2.07), and the standard deviation of 0.04 kg feed/kg gain [57]. The results of the analysis revealed that a minimum sample size of 9 replicates (3 per group, Power = 0.839) was required (Figure S1). The chi-square test was used to assess the significance of the differences in the percentages of welfare and quality behavioural characteristics among groups. The Shapiro–Wilk test was used to test the normality of the data for the analysis of broiler growth performance (BW, BWG, FC and FCR) and BT. Levene's test was employed for evaluating the homogeneity of variance. A one-way ANOVA was used for the comparison of the average values of the evaluated parameters among groups. Tukey's test was used for post hoc analysis. If the distribution was not normal, Kruskal–Wallis and Mann–Whitney non-parametric tests were used for comparisons at a significance level of $p \leq 0.05$.

3. Results

3.1. Temperature–Humidity Index

Figure 1 displays the THI values for broilers calculated from 19 d of age (d19; onset of the experiment) until 40 d of age (d40; end of the experiment), as well as THI values (mean $\pm$ SE) for each week of the study. As shown in Figure 1, during the first week of the experiment (d19–d26), broilers were under very severe heat stress (average THI value = 32.04 $\pm$ 0.64). In the second week of the trial (d27–d33), the average THI value dropped to 27.86 $\pm$ 0.53 indicating that broilers were under moderate heat stress. Finally, in the last week (d34–d40) of the experiment, the average THI value had fallen to 26.36 $\pm$ 0.86 denoting the absence of heat stress. The average THI value in the first week of the trial was significantly higher than that observed in the second and third weeks ($p < 0.05$). However, the differences in mean THI values between the second and third weeks of the experiment were similar ($p > 0.05$).

Figure 1. Temperature–humidity index (THI) calculated from 19 d of age (d19; onset of the experiment) until 40 d of age (d40; end of the experiment) and THI values (mean $\pm$ SE) for each week of the study. [a, b] Different superscripts denote significant differences ($p < 0.05$).

3.2. Growth Performance and Body Temperature

The addition of OP in chickens' feed significantly affected ($p < 0.05$) their BW and BWG (Table 1). At 26 d of age, broilers fed the CON diets presented significantly lower BW compared to OP-fed broilers ($p < 0.05$). However, at 33 and 40 d of age, similar BW was recorded in birds of all groups ($p > 0.05$). From 19 to 26 d of age, significantly higher BWG was observed in the OP6 group compared to the other two dietary treatments ($p < 0.05$). No significant dietary effect of OP on FC and FCR was detected both at weekly intervals as well as over the entire experimental period ($p > 0.05$).

At 26 d of age, OP-fed broilers had lower BT compared to CON birds (Table 1), but significant differences were only observed between CON and OP6 groups ($p < 0.05$). At 33 and 40 d of age, similar BT was recorded in birds of all groups ($p > 0.05$). Throughout the study, no deaths were recorded among the groups.

Table 1. Growth parameters and body temperature of broilers fed control and different OP diets. Data are presented as mean ± SE.

	CON	OP3	OP6
Body weight (g)			
19 d	941.94 ± 7.96	963.75 ± 6.82	947.78 ± 6.47
26 d	1473.75 ± 17.65 ^{a}	1522.36 ± 13.69 ^{b}	1533.06 ± 20.25 ^{b}
33 d	2226.11 ± 33.05	2244.44 ± 24.20	2224.31 ± 29.81
40 d	3091.67 ± 46.70	3145.42 ± 35.65	3071.39 ± 46.50
Body weight gained (g)			
19–26 d	531.81 ± 13.87 ^{a}	558.61 ± 13.48 ^{a}	585.28 ± 17.25 ^{b}
27–33 d	752.36 ± 23.05	722.08 ± 17.10	691.25 ± 18.99
34–40 d	865.56 ± 20.52	900.97 ± 17.12	847.08 ± 21.34
Total period (19–40 d)	716.57 ± 17.44	727.22 ± 16.32	707.87 ± 15.15
Feed consumption (g)			
19–26 d	861.53 ± 16.02	910.00 ± 12.89	916.11 ± 27.61
27–33 d	1250.14 ± 86.84	1187.64 ± 36.43	1196.39 ± 4.84
34–40 d	1499.72 ± 18.44	1535.92 ± 54.27	1534.72 ± 38.00
Total period (19–40 d)	1203.80 ± 96.42	1211.19 ± 92.56	1215.74 ± 90.45
FCR			
19–26 d	1.66 ± 0.05	1.66 ± 0.04	1.61 ± 0.05
27–33 d	1.71 ± 0.06	1.68 ± 0.04	1.80 ± 0.07
34–40 d	1.78 ± 0.05	1.73 ± 0.04	1.85 ± 0.05
Total period (19–40 d)	1.72 ± 0.03	1.69 ± 0.02	1.75 ± 0.03
Body temperature (°C)			
19 d	41.16 ± 0.05	41.22 ± 0.12	41.26 ± 0.12
26 d	40.78 ± 0.09 ^{a}	40.63 ± 0.05 ^{ab}	40.55 ± 0.06 ^{b}
33 d	40.91 ± 0.08	40.83 ± 0.11	40.80 ± 0.08
40 d	41.62 ± 0.11	41.77 ± 0.15	41.57 ± 0.08

$^{a, b}$ Means within a raw at a given age with different superscripts are significantly different ($p < 0.05$).

3.3. Welfare and Behaviour Traits

The data in Table 2 show the impact of OP on feather cleanliness in broilers as assessed at 26, 33 and 40 d of age. At 33 d of age, 16.67% of OP6 boilers had completely clean feathers (Score 0), whereas no birds from the other two groups were evaluated with Score 0 for feather cleanliness ($p < 0.05$). In addition, CON presented a higher percentage of broilers with moderate feather soiling (Score 2) than OP at that period of time, but significant differences were observed between CON and OP3 groups ($p < 0.05$). The evaluation of chickens for FPD and hock burn revealed no significant differences among groups ($p > 0.05$).

Data obtained from quality behaviour characteristics estimation are presented in Table 3. No significant differences ($p > 0.05$) for panting, moving and lifting up winds were noticed among groups during the evaluation performed at 26 and 33 d of birds' age. However, at 40 d of age, the percentage of broilers presenting panting behaviour was significantly higher in CON than OP6 group ($p < 0.05$).

Table 2. Broiler percentages recorded in CON, OP3 and OP6 groups and scored for feather cleanliness, foot pad dermatitis and hock burn at f 26, 33 and 40 d of age (Welfare Quality (2009) protocol [55]).

	26 d			33 d			40 d		
Score	**CON**	**OP3**	**OP6**	**CON**	**OP3**	**OP6**	**CON**	**OP3**	**OP6**
					Feather cleanliness				
0	81.67	77.78	62.78	0.00 [a]	0.00 [a]	16.67 [b]			
1	18.33	22.22	34.44	50.00 [a]	75.00 [b]	55.56 [ab]	72.22	78.33	77.78
2	0.00	0.00	2.78	50.00 [a]	25.00 [b]	27.78 [ab]	27.78	21.67	22.22
3	-	-	-	-	-	-	-	-	-
					Foot pad dermatitis				
0	88.89	86.11	100.00	63.89	66.67	77.78	27.78	22.22	19.44
1	11.11	13.89	0.00	36.11	33.33	22.22	72.22	72.22	77.78
2	-	-	-	-	-	-	0.00	5.56	2.78
					Hock burn				
0	100.00	100.00	100.00	61.11	52.78	69.44	-	-	-
1	-	-	-	38.89	47.22	30.56	100.00	100.00	100.00
2	-	-	-	-	-	-	-	-	-

[a, b] Means within a raw at a given age (26 d, 33 d, 40 d) for each scoring category 0–3 with different superscripts are significantly different ($p < 0.05$).

Table 3. Broiler percentages of recorded in CON, OP3 and OP6 groups and scored for panting, moving/walking and lifting up winds at the age of 26, 33 and 40 d of age.

	26 d			33 d			40 d		
Quality Behaviour Traits	**CON**	**OP3**	**OP6**	**CON**	**OP3**	**OP6**	**CON**	**OP3**	**OP6**
Panting	0	0	0	25	25	16.67	86.11 [a]	75.00 [ab]	61.11 [b]
Moving/walking	72.22	66.67	66.67	33.33	36.11	41.67	33.33	13.89	33.33
Lifting up winds	0	0	5.55	0	0	0	0	0	0

[a, b] Means within a raw at a given age for each type of behaviour with different superscripts are significantly different ($p < 0.05$).

4. Discussion

The current work provides, for the first time, research evidence on the dietary impact of OP on thermal comfort in broilers during the hot season. At the same time, the dietary effects of OP on broiler growth performance, and some welfare and quality behavioural traits of the birds were also investigated. The results of the present study revealed a beneficial dietary impact of OP on the growth performance of broilers subjected to very severe heat stress, as indicated by the higher BWG recorded in OP groups compared to CON during the first week of the experiment (d19–d26) and the higher BW documented in OP fed chickens at 26 d of age. These findings, in combination with the lowest BT recorded in the OP groups at 26 d of age compared to the CON, are very important as they suggest the thermo-protective role of OP in broilers exposed to high temperatures, favoring their thermal comfort. The higher BWG observed in OP-fed broilers could be attributed to the numerically higher feed intake recorded in OP birds compared to CON from 19 to 26 d of age. In support of our findings, previous research work has shown that heat stress reduces feed consumption and impairs growth performance in poultry due to a variety of physiological changes manifesting in birds' bodies, such as oxidative stress, acid–base imbalance and suppressed immune system [9,14–16,58,59].

The amelioration of BW and BWG of very severely heat-stressed broilers fed OP, documented in this study, as well as their lower BT compared to controls, could be linked to the antioxidant properties of OP bioactive compounds such as polyphenols. Polyphenols are natural antioxidants that can reduce oxidative stress [15]. They enhance the mechanism for removing free radicals and inhibit the mechanism for forming free radicals [60]. Former investigations have demonstrated that polyphenols' antioxidant properties improve broilers' BWG and the quality of their meat and ameliorate the negative effects of heat stress [61,62] by reducing reactive oxygen species (ROS) production, thus reducing oxidative stress [63]. In addition, the high concentration (70.77%) of oleic acid in OP used in the present investigation (Table S2) may also have contributed to this positive finding. It was shown that oleic acid decreases oxidative stress and the inflammatory response through the activation of peroxisome proliferator-activated receptors in animals [64]. Consistent with our findings, Shakeri et al. [62] observed that dietary polyphenols reduced BT in heat-stressed broilers. The beneficial effects of polyphenols in the regulation of heat shock protein expression [15] may facilitate chickens to decrease their BT.

Similar feeding studies in broilers and layers confirm the beneficial effect of OP supplementation on oxidative stress in birds. According to Fotou et al. [43], the inclusion of 5% OP in the feed of broilers significantly increased plasma α-tocopherol concentration compared to the control group. Improved oxidative stability of meat was observed in broilers fed with 5% OP [39] and polyphenol-rich olive cake at the dose of 165 g/kg diet [65]. Furthermore, Safwat et al. [66] indicated that supplementing laying hens' diet with 7% olive cake for 12 weeks significantly increased the antioxidant parameters like total antioxidant capacity, glutathione peroxidase superoxide dismutase and significantly decreased the oxidative parameters namely malondialdehyde and nitric oxide in hens' serum.

Since this is the first report investigating thermal comfort in broilers fed OP, it is difficult to compare our findings with other studies. However, the dietary effect of various olive oil by-products in chickens exhibiting heat stress has been evaluated in a limited number of studies, with promising results in terms of birds' health and performance. A recent investigation revealed that the addition of 15 mL of olive leaf extract per liter of water reduced the negative effects of heat stress and ameliorated the performance of broilers in a hot humid climate [48]. According to Agah et al. [49], the inclusion of olive leaf extract in the diet of broilers at the dose of 200 or 400 mg/kg of feed improved the antioxidant status of the birds and liver function and reduced the stressor index in heat-stressed birds. These authors also noticed a slight improvement in broiler performance when reared under heat-stress conditions. According to Mujahid et al. [45], the addition of 6.7% olive oil to the diet of broiler chickens reared under thermo-neutral or heat-stress conditions reduced mitochondrial ROS production and decreased oxidative damage in the birds. Recent feedings trials performed in broilers reared at high ambient temperatures revealed the antioxidant effect of olive oil and the improvement of the birds' immune system [46], the improvement of hormonal indicators and chickens' performances as well as the increased activity of heat tolerance genes in chickens [47].

According to the THI values obtained from 27 to 33 d of age, broilers experienced moderate heat stress. During this period the thermo-protective role of OP was not as pronounced as in the previous week, as evidenced by the similarity of growth parameters and broiler BT among groups at 33 d of age. Moreover, it appears that CON birds, in order to overcome their retarded growth during the previous week and to meet their maintenance and growth needs, consumed a numerically greater amount of feed compared to OP chickens during 27 to 33 d of life. In support of our finding, previous research has shown that compensating broilers also display an increase in feed intake in relation to body weight and a degree of associated digestive adjustment [67]. The increased feed intake recorded in CON broilers resulted in similar BWG in chicks from 27 to 33 d of age in all groups and similar BW at 33 d of age. Consequently, feed efficiency during the finishing 1 period did not differ among treatments.

This study showed that the addition of OP to the broilers' diet at inclusion rates of 3% and 6% had no effect on the overall growth parameters as indicated by final BW at 40 d of age, BWG, FC and FCR throughout the trial (19–40 d). These results are in line with those observed in our former report [44]. Similar overall production traits among treatments have also been documented by other authors who investigated the dietary impact of OP in broilers' feed at different inclusion rates like 2%, 4%, 6% and 8% [38], 2.5%, 5% and 7.5% [34], 5% [40,41], 6% [35], and 5% and 10% [29,36,37,68]. In contrast to the aforementioned researchers, Fotou et al. [43] observed reduced slaughter BW, higher FC and FCR in chickens fed growing and finishing diets supplemented with 2.5% and 5% OP flour, respectively, compared with controls. In addition, reduced feed intake [35,41,68], poorer FCR [35,39–41], lower BWG [34,35] and final BW [34,35,41] of broilers consuming diets containing OP at doses of 8% [39,40], 3% and 9% [35], 10% [34,41], 15% [41] and 20% [68] compared to controls have been reported in former studies. The reduced growth performance of OP-fed broilers observed in previous reports has been linked to the high crude fiber content of OP, which has a negative impact on nutrient utilisation [34,41,43]. On the other hand, Saleh et al. [42] found elevated BWG in chickens 35 d old that received rations with 4% OP in comparison to control birds.

The differences in the production traits of OP-fed broilers documented in the present investigation with those observed in other relative studies may be due to several factors, namely: differentiations in the incorporation rate of OP, in the composition of OP and the rations used, as well as in the broiler hybrid and age. The crude fiber content of olive by-products used also plays an important role. The chemical composition of dried olive remnants is highly varied due to different olive varieties and oil extraction methods or the subsequent processing out such as drying or destining [69]. The crude fiber content of dried OP has been shown to decrease with destoning, allowing higher doses of dried OPs to be included in feed rations without adversely affecting broiler growth performance [29].

Broilers consuming diets containing 6% OP showed better feather cleanliness scores compared to the other two treatments as evaluated at 33 d of age. This finding is important for birds' health and welfare. Plumage cleanliness plays a significant role in thermoregulation, so feathers that become wet or dirty by litter can miss their protective function, negatively affecting bird welfare [70]. Feather cleanliness assessment is also a good index of management quality and litter moisture, as dirty feathers can provide information about the broilers' housing conditions [71]. A beneficial effect of dried OP on broilers' feather cleanliness when incorporated in their diets at rates of 3% and 6% have also been recorded by Dedousi et al. [44]. According to the aforementioned authors, the favorable impact of OP on feather cleanliness could probably be associated with the increased fiber content of the OP diets compared to the control, which may have improved litter quality rather than the birds' intestinal health. The later researchers also observed a beneficial dietary impact of OP on broilers' foot pad dermatitis (FPD), as evaluated at 34 and 41 d of age, attributed mainly to the positive effect on skin health and the anti-inflammatory functions of OP's bioactive ingredients such as polyunsaturated fatty acids and polyphenol. However, such an effect was not recorded in the present document. An observed difference between the two trials is that the percentage of broilers with no evidence of FPD (Score 0) as evaluated at 34 and 41 d of age in all dietary groups was higher in our previous study [44] than in the present trial. This difference could be associated with the different hybrids used (Ross vs. Cobb) and the different seasons in that trials were carried out (winter vs. summer months). An association has been shown between season and the prevalence of FPD in broilers [72] mostly related to the relative humidity outside, which has an effect on the relative humidity inside and litter quality [73,74]. Moreover, during hot months birds consume more water [75,76] due to high ambient temperature resulting in high water excretion and wet litter problems [77]. It is generally acknowledged that environmental conditions like litter moisture are the major risk factors that influence the occurrence and severity of FPD [78]; however, former studies have shown that the susceptibility to the development of FPD varies between cross-breeds or between commercially available high growth broiler genotypes at that time [79–82]. In line with our previous findings the addition of OP in both inclusion rates (3% and 6%) did not affect the incidence and severity of hock burns [44].

The assessment of quality behaviour characteristics revealed a significantly lower percentage of broilers presenting panting behaviour in the OP6 group (61.11%) compared to CON (86.11%) at 40 d of age. This finding further suggests the thermo-protective role of

OP which seems to beneficially affect birds' thermal comfort, especially at the rate of 6%. Panting is considered as a "cooling behaviour" that birds present under high temperatures, in an effort to reduce body heat and restore normothermia [75]. Unlike mammals, birds have no sweat glands but have developed some behavioural adjustments to heat, including increased breathing, panting and wing flapping [76]. Thus, to maximise latent heat loss through the evaporation of water from the airways, broilers suffering from environmental hyperthermia increase their respiratory rate (thermal tachypnea/polypnea or panting) [75]. Chickens redistribute blood flow to their skin during panting to further assist radiant heat loss. Increased peripheral blood flow decreases excessive heat production, and increased panting helps to facilitate evaporative cooling [83]. However, excessive panting is associated with various situations that negatively affect birds' health and performance like dehydration, and increased CO_2 expiration which leads to hypocapnia and, ultimately, to respiratory alkalosis, an acid–base imbalance [8,75] consisting of a great threat to growing broilers [84]. Fast panting also leads to hyperthermia and increased oxidative stress due to the increased production of ROS, which has a negative effect on the birds' defense system [59].

In our study, panting behavior increased over time in all diet groups. This finding is consistent with previous reports showing a significant effect of broiler age on panting behaviour [85,86]. According to McLean et al. [85], increased panting was observed in 5- and 6-week-old broilers compared to younger birds. Similarly, in the study by Akter et al. [86], the number of chickens panting was higher on days 49, 51 and 54 than on days 43 and 44 of life, indicating that panting increased with age. In our trial, the higher percentage of panting broilers observed in week 3 of the experiment coincided with an increase in mean atmospheric relative humidity of more than 80% (Figure S2). Thus, despite the lower THI in week 3, the very high humidity levels may have led to an increase in panting in the broilers. However, it is also possible that increased panting itself may have contributed to the higher atmospheric relative humidity by increasing water loss through evaporation and possibly increasing water intake through drinking to replace the lost water. Therefore, it is not easy to disentangle cause and effect.

To the best of our knowledge, there are no studies investigating the effects of supplementing OP on panting behaviour in broilers exposed to high ambient temperatures. The thermoregulatory mechanism of OP may be related to its bioactive compounds such as polyphenols and flavonoids which have potent antioxidant and anti-inflammatory activity. Flavonoids are strong antioxidants, acting as free radical scavengers and inhibitors of lipid peroxidation; they also show a variety of physiological actions, including antihypertensive and vasodilator effects, which improve oxygenation [87]. This may suggest that OP can enhance non-evaporation cooling mechanisms (including conduction, convection and radiation) reducing the need for evaporative heat loss through panting. Previous studies have shown that dietary polyphenols increase hematocrit values in heat-stressed broilers [62]. Thus, it could be supported that the reduced panting rate in OP-fed broilers may also reflect improved oxygenation levels through its effects on the hematopoietic system. Moreover, polyphenols may stimulate the expression of stress response proteins like heat shock proteins and antioxidant enzymes, which can repress ROS [62]. Consequently, OP could decrease endogenous heat production, resulting in less panting behaviour. Similar to our results, reduced panting behaviour has also been reported by others when broiler or quail diets were supplemented with polyphenols [62,88] or other antioxidants such as propolis [87], a combination of ascorbic acid and betaine [89] and phytogenic additives [90]. The promising results observed in this study with regard to the positive nutritional effect of OP on the thermal comfort and welfare of broilers are of great importance for the poultry sector. They could provide potential solutions easily adopted by the broiler industry to alleviate the adverse effects of heat stress through dietary manipulation. However, further research could provide evidence of the mechanisms involved in the thermo-protective role of OP, as well as the possible effects of OP bioactive compounds on the heat tolerance genes of broilers.

5. Conclusions

The present study revealed that the supplementation of OP in broiler feed beneficially affects their thermal comfort. This is supported by the ameliorated growth parameters observed in OP-fed broilers compared to controls, especially at the higher dose (6%), under conditions of very severe heat stress. This positive effect suggestive of the thermo-protective role of OP could be attributed to the antioxidant properties of OP bioactive compounds like polyphenols and oleic acid. Moreover, a positive dietary effect of OP in feather cleanliness and the panting behaviour of chickens was also recorded. These findings further indicate the beneficial impact of OP on broilers' welfare when incorporated into their diets at the rate of 6%.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su151410932/s1, Table S1: formulation and nutrient composition of diets containing olive pulp (OP) compared with the control diet (CON); Table S2: nutrients and fatty acid composition of olive pulp (OP) used in the study; Figure S1: sample size determination using FCR as parameter of interest with desired power 0.8, error rate probability 0.05 and desired detectable difference 0.07 kg feed/kg gain with SD 0.04 kg feed/kg gain. Figure S2: temperature and humidity average daily values from day 19 d of age (d19; onset of the experiment) until day 40 d of age (d40; end of the experiment).

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