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Effects of Olive Mill Vegetation Water Phenol Metabolites Transferred to Muscle through Animal Diet on Rabbit Meat Microbial Quality

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Abstract: The present study evaluated the effects of feed supplementation with olive oil by-products on the microbial quality of rabbit meat. Thirty-three New Zealand White rabbits were randomly assigned to three experimental grower–finisher diets. Each dietary group consisted of three experimental treatments: (1) a basal control diet (C), (2) a C diet supplemented with a low dosage of polyphenol (150 mg/kg, L), and (3) a C diet supplemented with a high dose of polyphenols (280 mg/kg, H). Polyphenol analyses in feed and meat were performed using the liquid-chromatography coupled to tandem mass spectrometry technique (LC-MS/MS). Higher amounts of sulphate metabolites were detected in the H group. Microbiological quality was evaluated on *Longissimus lumborum* muscles stored under aerobic conditions at 4 °C. The H diet exerted an inhibitory effect on microbial growth ($p < 0.001$), notably for *Pseudomonas* spp., when compared to C and L diets; differences among the groups were observed starting from 6 days of storage. In the H group, the *Pseudomonas* spp. population showed an increase in the latency phase and a decrease in the maximum growth rate of the fitted curves in comparison with the C and L groups. The use of dietary polyphenols could be a strategy to reduce spoilage during meat storage.

Keywords: olive oil by-products; meat storage; natural preservatives

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1. Introduction

Rabbit meat is characterized by high nutritional properties as it is lean, high in moisture, and rich in proteins and essential amino acids and as it has a favorable proportion of unsaturated fatty acids [1]. However, as a result of these characteristics, rabbit meat is a relatively perishable product that is more prone to lipid oxidation than other meats, and it can easily allow pathogenic and spoilage microorganisms to grow [2,3]. Recently, scientific researchers have focused their attention on several natural antimicrobial and antioxidant substances [4–8] that can be transferred in food directly or through an animal's diet to reduce oxidative processes in muscle food and to prolong the shelf life of meat [7,9–12]. Among natural antimicrobial substances, olive oil by-products can be considered a source of bioactive molecules that can be potentially used for muscle food preservation and nutritional quality improvement [13,14]. In particular, olive oil by-products are characterized by a high content of phenolic compounds, which have been proven to inhibit or delay the rate of growth of a wide range of Gram-positive and Gram-negative bacteria [15,16]. The high amount of polyphenols contained in olive oil by-products represents an environmental issue but, at the same time, an incredible potential source of functional compounds. Olive

oil production is very important in the Mediterranean area in terms of culture and tradition; however, it is responsible for large amounts of waste that are characterized by a great impact on the environment, mainly due to their high phytotoxicity [17]. These environmental problems have several implications in terms of the sustainability and profitability of a food system. In order to reduce these negative impacts, the 3R slogan—"reduce, reuse, recycle"—should be adopted in order to redesign the management of olive oil by-products [18,19]. In this scenario, some authors have suggested that agricultural wastes can represent an excellent source of nutrients (protein, lipids, minerals, vitamins, fiber, and polyphenols) and can help to bridge the gap between the demand and supply of feedstuffs for livestock [13,20–22]. In dietary manipulations, bioactive molecules are introduced into the muscle via feed, avoiding the addition of exogenous products after slaughter. Despite several polyphenols that are obtained from plant extracts exhibiting antimicrobial properties when directly applied into meat, this practice in foods may be limited, primarily because of their effect on organoleptic properties [23]. Regarding the use of natural antimicrobial compounds through the animal diet, only limited research has demonstrated a beneficial effect of this dietary strategy on meat in general and on rabbit meat in particular. Koné et al. [10] showed a positive effect on the microbiological quality of rabbit meat during storage by supplementing the animal diet with onion extract. Dal Bosco et al. [5] found an antimicrobial effect as a consequence of the combination of dietary and direct rabbit meat supplementation of liquorice extract. Studies on the effects of olive oil by-products used as a rabbit feed additive on the microbiological quality of meat have been limited.

Our hypothesis was that supplementing the diet of rabbits with by-products from the olive oil industry might enrich the levels of bioactive compounds in the meat that are able to influence the meat's microflora. Based on this hypothesis, the aim of the present work was to reveal the presence of phenolic compounds in meat and to investigate the effects of different dietary supplementations with olive mill wastewater by-products on rabbit meat's microbial quality during storage.

2. Materials and Methods

2.1. Animals and Diets

This study was carried out at the experimental farm of the Department of Agricultural, Food, and Environmental Sciences of the University of Perugia. The rabbits underwent a continuous photoperiod of 16 h light per day at 40 lux. Room temperature and humidity ranged from 18 to 27 °C and from 55% to 65%, respectively. Thirty-three New Zealand White male rabbits were weaned at 31 days of age, were housed in individual cages, and were randomly assigned to three dietary groups (n = 11 animals/group): the control (C) group was fed with a commercial pelleted concentrate (proximate composition as fed: crude protein, 15.8%; neutral detergent fiber, 38.2%; calcium, 1.1%; phosphorus, 0.5%; and digestible energy, 8.8 MJ/kg); the low (L) group was fed with a C diet supplemented with a commercial olive mill wastewater polyphenol extract to reach a final concentration of 150 mg/kg; and the high (H) group was fed with a C diet supplemented with the same olive mill wastewater polyphenol extract to reach a final concentration of 280 mg/kg. Feed and fresh water were always available. The study was conducted in accordance with the Legislative Decree No. 146, implementing Directive 98/58/EC from 20 July 1998 concerning the protection of animals kept for farming purposes. No clinical signs and no mortality were registered during the trial. The animals were slaughtered at 90 days of age in accordance with the European standard procedure in a commercial abattoir.

2.2. Analysis of Polyphenols in Feed and Meat

The feed and rabbit muscle (*Longissimus lumborum* muscle, LL) sample treatments were previously described in Branciari et al. [13]. Briefly, 5 g of grinded feed were mixed with 25 mL of a methanol/water 80/20 (v/v) mixture containing 20 mg/L of BHT (butylated hydroxytoluene). The extraction was repeated twice. Two aliquots of this extract were diluted

5- and 50-fold, respectively, with a mixture of Na_2EDTA 0.1 M/methanol 90/10 (*v*/*v*). Both samples were injected in a liquid chromatography-tandem mass spectrometry system (LC-MS/MS) to determine the tyrosol, hydroxytyrosol, pinoresinol, and verbascoside contents. The instrumental conditions are detailed in Branciari et al., 2017 [13]. Concerning rabbit meat, 2 g of a homogenized sample was extracted twice with a methanol/water 80/20 (*v*/*v*) mixture containing 20 mg/L of BHT (2 × 5 mL). After volume reduction and the addition of 15 mL of water, the extract was loaded onto an SPE OASIS HLB cartridge (200 mg/6 mL, Waters, Milford, MA, USA) previously conditioned with 6 mL of methanol and 6 mL of water. The cartridge was washed with 6 mL of water and elution achieved with 6 mL of methanol. After evaporation, the eluate was resuspended in 0.4 mL of a Na_2EDTA 0.1 M/methanol 90/10 (*v*/*v*) mixture and injected in the LC-MS/MS equipment for polyphenol determination as reported for the feed [13]. Finally, to measure the concentrations of polyphenol metabolites (tyrosol sulphate (T-S), hydroxytyrosol-3-sulphate (HT-3-S), and hydroxytyrosol-4-sulphate (HT-4-S)), the same purified muscle extract was injected a second time under specific LC-MS/MS conditions as reported in Branciari et al. [24].

2.3. Chemical Composition of Meat

For meat chemical composition, one rabbit per cage, with ten individuals per diet, were randomly analyzed and, for this purpose, a fragment of the LL muscle was collected after slaughtering. The chemical composition of samples was determined according to the methods of the Association of Analytical Chemists [25]. The moisture content was obtained by oven-drying the meat samples (125 °C for 2 h) (method 950.46). The fat content was gravimetrically determined using ether solvent extraction (method 960.30). The nitrogen content was determined using the Kjeldahl method (method 992.15). The protein content was obtained by multiplying the total Kjeldahl nitrogen by a coefficient factor of 6.25. The ash content was obtained using a muffle furnace at 600 °C (method 923.03).

2.4. Microbial Analysis of Meat

Sampling for microbiological determination was performed on the cranial part of LL muscles of 10 carcasses/group. Five 2-cm-thick slices were aseptically obtained from each muscle, and each slice was packaged and stored as reported in Ranucci et al. [26]: packaged in a polystyrene tray wrapped with oxygen-permeable polyethylene film and stored in a refrigerated display case at 4 + 1 °C under cool white fluorescent lighting (Lumos® LED up light 4000 K, 908 lx; OSRAM spa, Milan, Italy). Immediately after packaging and after 3, 6, 9, and 12 days of storage (T0, T3, T6, T9, and T12, respectively), 10 g of each sample was aseptically removed and placed in a stomacher bag with 90 mL of buffered peptone water (Oxoid Ltd., Basingstoke, UK). After homogenization for 120 s (Stomacher 400 circulator, Seward Ltd., Norfolk, UK), decimal serial dilutions were made and the following determinations were performed in duplicate. Total viable count (TVC) and psychrotrophic counts (TPC) were carried out on plate count agar (PCA) (Biokar Diagnostics, Beauvais, France) and incubated at 30 °C or 6.5 °C for 2 and 10 days, respectively. *Enterobacteriaceae* count was performed on Violet Red Bile Glucose Agar (VRBG Oxoid, Basingstoke, UK), incubated aerobically at 37 °C for 24 h. Lactic acid bacteria (LAB) count on de Man, Rogosa, and Sharpe agar (MRS, Oxoid) was anaerobically incubated for 48 h at 37 °C; *Pseudomonas* spp. count on Pseudomonas Agar Base (Oxoid) with CFC Pseudomonas Supplement (Oxoid) was incubated for 48 h at 25 °C. The results were quantified as colony-forming units (CFU) per gram of sample and converted into Log values to obtain Log10 CFU/g meat prior to statistical analysis according to Gill et al. [27]; according to Cullere et al., a value of 0.70 Log CFU/g was used when no colonies were detected [28].

2.5. Statistical Analysis

The data were analyzed using the GLM procedure of SAS [29] and expressed as least square means ± standard error of the mean. A mixed model with repeated measures considering diet (C, L, and H) and time (T0, T3, T6, T9, and T12) as fixed factors was

used. The rabbit was considered a random factor. The differences between means were determined using the Tukey test and considered significant when $p < 0.05$. The effects of dietary supplementation on the growth of the target microorganisms were evaluated with the DMFit tool of the free predictive microbiology software Combase (https://www.combase.cc/index.php/en/DMFit, accesed on 25 January 2021), allowing for the definition of growth parameters such as Lag phase duration (Λ, 1/h) and maximum growth rate (μmax, 1/h) by means of the Baranyi and Roberts model [30]. The fitted results were analyzed by one-way ANOVA (with the dietary group as a fixed variable) and Tukey's test ($p < 0.05$).

3. Results and Discussion

Polyphenolic molecules and their amount in feed are shown in Table 1.

Table 1. Levels of selected polyphenols in experimental feeds.

Feed	Hydroxytyrosol (mg/kg)	Tyrosol (mg/kg)	Verbascoside (mg/kg)	Pinoresinol (mg/kg)	Sum (mg/kg)
C	0.4	2.6	2.5	0.3	5.9
L	97	18	35	0.4	150
H	182	30	70	0.4	283

C = basal control diet; L = diet supplemented with 150 mg/olive mill wastewater polyphenol extract; H = C diet supplemented with 280 mg/kg of olive mill wastewater polyphenol extract.

The measured polyphenols were selected based on their representativity in olive mill waste. In the experimental feeds, the most abundant compounds were hydroxytyrosol and verbascoside, followed by tyrosol and pinoresinol (Table 1). As expected, the H diet showed the highest total polyphenol content, followed by the L diet. The presence of low concentrations of polyphenols, mainly tyrosol and verbascoside, in the C feed is related to their widespread diffusion in various plant species [31,32]. The proximate composition of the LL muscle was not affected by diet. The average values of moisture, protein, lipids, and ash were 73.12%, 23.79%, 0.87%, and 1.32%, respectively. This similarity in the chemical composition of meat in the control and treated groups was also highlighted by Branciari et al. [13] in chickens fed with olive mill wastewater and by Dal Bosco et al. [20] in meat of rabbits fed with olive pomace. Dietary supplementation with olive by-products does not seem to affect the proximate composition of the meat.

For the presence of phenolic compounds in rabbit meat, only the following metabolites were detected at the following concentrations: hydroxytyrosol-3-sulphate (HT-3-S) and hydroxytyrosol-4-sulphate (HT-4-S) at 0.16 and 0.40 μg/kg, respectively, in the "C" group; at 0.60 and 1.26 μg/kg, respectively, in the "L" group; and at 1.11 and 2.52 μg/kg, respectively, in the "H" group (Table 2). Tyrosol-sulphate (T-S) was detected in the meat of all groups at concentrations below 0.2 μg/kg. The presence of polyphenol sulphate metabolites was previously documented in muscle and cheese as well as in biological fluids [13,24,33–35]. LC-MS/MS quantification of these compounds in rabbit meat (Table 2 and Figure 1) showed significant differences among the three groups. Significantly higher levels of HT-4-S, HT-3-S, and T-S were always detected in the H group. The contents of HT-4-S and HT-3-S increased about two-fold in rabbits fed with the H diet compared with the L one. Moreover, hydroxytyrosol sulphates were about three times higher in the muscles of animals fed with the L diet compared to rabbits fed with the C diet. This study shows that olive-derived phenolic supplementation mainly produces two hydroxytyrosol metabolites (3- and 4-sulphate isomers), confirming that these molecules are absorbed in the gastrointestinal tract [34,35]. These results agree with previous data [36] and confirm that these molecules are endogenously generated by phase II metabolism starting from tyramine and dopamine.

Table 2. Effect of dietary supplementation on the presence of metabolite compounds in meat.

Groups	Tyrosol Sulphate (µg/kg)	Hydroxytyrosol-4-Sulphate (µg/kg)	Hydroxytyrosol-3-Sulphate (µg/kg)
C	0.03 [a]	0.40 [a]	0.16 [a]
L	0.01 [a]	1.26 [b]	0.60 [b]
H	0.10 [b]	2.52 [c]	1.11 [c]
SEM	0.024	0.510	0.234
p-Value	<0.001	<0.001	<0.001

C = basal control diet; a, b, and c, different letters in the same row denote a significant difference; L = diet supplemented with 150 mg/olive mill wastewater polyphenol extract; H = C diet supplemented with 280 mg/kg of olive mill wastewater polyphenol extract.

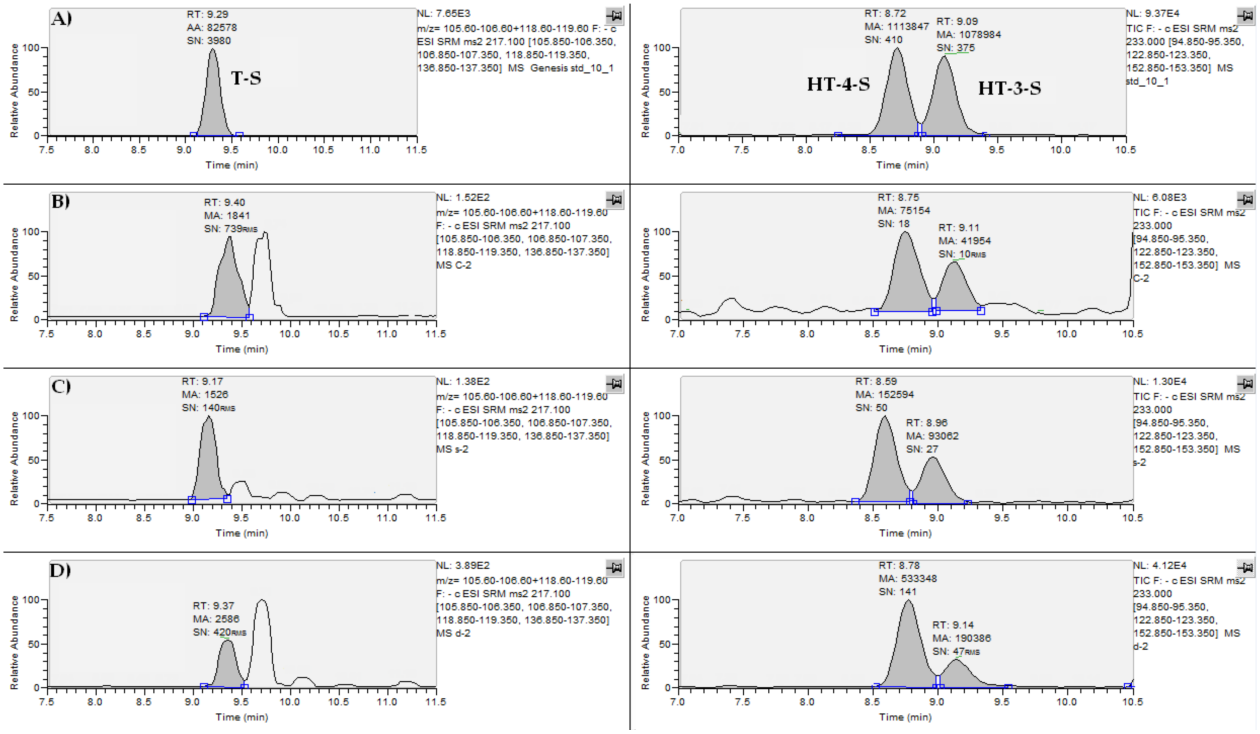

Figure 1. LC-MS/MS chromatograms of standard solutions of T-S, HT-4-S, and HT-3-S (10 ng/mL) (**A**) and rabbit muscle samples belonging to groups "C" (**B**), "L" (**C**), and "H" (**D**).

The results of microflora evolution on refrigerated LL muscles are presented in Tables 3 and 4. Immediately after slaughter (T0), the higher microbial populations recorded on rabbit meat were TVC, TPC, and LAB count, followed by *Pseudomonas* spp. and *Enterobacteriaceae* counts. These results are in agreement with other studies on rabbit meat [28]. The initial microbial load of meat depends on the physiological status of the animal at slaughter and on the hygienic conditions during slaughter, handling, and production processes [37]. The meat tested in this study showed optimal initial microbiological quality, probably derived from good hygiene standards and handling practices and from proper postmortem meat acidification. Pereira and Ferreira [3] have emphasized the importance of a low microbial count on rabbit meat shelf-life, assuming that growth parameters are affected by initial contamination. Microbial analyses indicated that TVC, TPC, *Pseudomonas* spp. count, LAB count, and *Enterobacteriaceae* count increased significantly over time ($p < 0.001$). The evolution of cell count is the result of a selective action exerted by ecophysiological factors such as temperature, pH, and packaging atmosphere [38]; comparable results were also reported in other studies on rabbit meat storage under conditions comparable to those of this study [10,28]. The initial (T0) TVC ranged between 2.79 and 2.94 log CFU/g (Table 4). TVC increased to values of 8.22, 8.19, and 7.92 log CFU/g for C, L, and H, respectively, within 9 days and remained steady for up to 12 days. The TPC starting levels were 2.27, 2.05, and

2.23 log CFU/g for C, L, and H, respectively; this population reached final values of 7.71, 7.41, and 7.22 on day 9. LAB counts were initially present at 2.05, 2.08, and 2.04 log CFU/g in C, L, and H, respectively. The final LAB counts exceeded 6 log CFU/g. The initial levels of *Enterobacteriaceae* were 1.12, 1.03, and 1.07 log CFU/g for C, L, and H, respectively, and increased to approximately 4 log CFU/g after 9 days. The *Pseudomonas* spp. counts changed from 1.17, 1.21, and 1.29 log CFU/g for C, L, and H, respectively, to 7.46, 7.38, and 6.39 log CFU/g during the 12-day storage period. No effect of treatment was observed on TVC, LAB count, and *Enterobacteriaceae* count. These results are in contrast with studies showing that the addition of oregano essential oil to rabbit diets results in TVC reduction starting on the eighth day of storage, while the effect of supplementation was evident from the start of the storage period for the *Enterobacteriaceae* and LAB counts [9]. Kone et al. [10] found that the addition of polyphenolic compounds through diet supplementation with onion and cranberry extracts had a significant effect on rabbit thigh TVC at the beginning of storage to day 6. The same authors notice that no TVC difference was observed at the end of the aerobic storage period. In agreement with the present study, Castrica et al. [39], considering rabbit fed a Goji berry supplemented diet, found no effect on TVC and *Enterobacteriaceae* count compared to the control group. Nevertheless, comparing the microbial status of the three dietary treatments, it emerged that, in group H, the growth of *Pseudomonas* spp. was partly inhibited ($p < 0.001$)) and a declining trend ($p = 0.078$) was also noticed for TPC. This could be explained by the fact that, in the total psychrotrophic count of rabbit meat at the end of the storage period (day 7), the microbial population is probably represented mostly by *Pseudomonas* spp., as reported by other authors [40]. The inhibition of *Pseudomonas* spp. growth could be observed after 6 days of storage, when the counts on rabbit LL were approximately 1 log CFU/g, lower than in the C and L samples. Several studies demonstrated the inhibitory effect of olive phenolic compounds on the growth of *Pseudomonas* spp. [7,15,41] in vitro and in different matrices. The inhibitory effect of the olive phenolic compound added to feed on *Pseudomonas* spp. growth in meat during storage has not been reported in the literature. However, in rabbits fed oregano supplementation, an inhibition of *Pseudomonas* spp. was reported in meat from day 8 of storage [9]. Similarly, Kone et al. [10] reported an effective inhibition of *Pseudomonas* spp. total growth in meat from rabbits fed onion extract. The results from ComBase application to the microbial population are reported in Table 4; the DMFit program was used to calculate the growth parameters for each microbial population. The complete Baranyi–Roberts model highlights differences in the latency phase (Lag) between H groups compared to the C and L groups for *Pseudomonas* spp., and the reported values were 60.85, 65.37, and 74.01 h in the C, L, and H groups, respectively. No effect on Lag was observed for the other microbial populations. The duration of Lag is determined by changes made by bacteria in response to stressor factors [42]. Lag optimization and elongation may therefore represent a strategy adopted by bacterial communities to deal with environmental stress and may correspond, in this study, to hurdles present in the muscle, probably also represented by polyphenolic metabolites in the H samples. In the same microbial population (*Pseudomonas* spp.), an increase in μmax values (maximum growth rate) of the fitted curve were reported only in the H and C groups while no difference was observed for the other bacteria population. In particular, the values were 0.044, 0.045, and 0.035 Log CFU/h in the C, L, and H samples, respectively. The effect of diet on bacterial growth rate in the C and H samples is shown by the difference found between the final values reported on day 12 (Table 4). The use of 280 mg/kg of polyphenolic supplementation decreased the maximum growth rate to a greater extent than the lower dose (150 mg/kg). Studies in the literature show the high antimicrobial activity against Gram-negative and Gram-positive bacteria of hydroxytyrosol, one of the most effective phenolic compounds [15,43,44]. The antimicrobial effects of olive phenols in general and of hydroxytyrosol in particular are the results of a mechanism of action against microbial cells as they are able to penetrate the structurally different cell membranes of both Gram-negative and Gram-positive bacteria. Phenolic compounds are known to cause cell peptidoglycan disruption, to damage the cell membrane, or to cause both. This activity is also probably maintained by hydroxytyrosol

metabolites. The effect of polyphenols could be enhanced by a packaging system such as MAP and vacuum packaging; Cullere et al. [28] found that MAP and vacuum packaging had a greater efficacy compared to air-permeable bags in limiting total microbial growth, especially for *Enterobacteriaceae* and *Pseudomonadaceae*.

Table 3. Microbial quality of rabbit *Longissimus lumborum* stored at 4 °C under aerobic conditions for 12 days (Log CFU/g).

		Days of Storage					SEM	*p*-Value		
		0	3	6	9	12		T	D	TXD
Total viable count (TVC)	C	2.81 ^{a}	3.05 ^{a}	5.21 ^{b}	8.22 ^{c}	8.29 ^{c}	0.187	<0.001	0.520	0.973
	L	2.79 ^{a}	3.11 ^{a}	5.18 ^{b}	8.19 ^{c}	8.20 ^{c}				
	H	2.94 ^{a}	2.97 ^{a}	5.09 ^{b}	7.92 ^{c}	8.04 ^{c}				
Total psychotrophic count (TPC)	C	2.27 ^{a}	2.50 ^{a}	5.01 ^{b}	7.71 ^{c}	7.81 ^{c}	0.240	<0.001	0.078	0.829
	L	2.05 ^{a}	2.68 ^{a}	4.90 ^{b}	7.41 ^{c}	7.69 ^{c}				
	H	2.23 ^{a}	2.38 ^{a}	4.70 ^{b}	7.22 ^{c}	7.17 ^{c}				
Pseudomonas spp.	C	1.17 ^{a}	1.97 ^{b}	4.81 ^{cW}	7.34 ^{dW}	7.46 ^{dW}	0.205	<0.001	<0.001	0.010
	L	1.21 ^{a}	1.87 ^{b}	4.71 ^{cW}	7.23 ^{dW}	7.38 ^{dW}				
	H	1.29 ^{a}	1.74 ^{a}	3.75 ^{bX}	6.09 ^{cX}	6.39 ^{cX}				
Lactic acid bacteria (LAB)	C	2.05 ^{a}	2.23 ^{a}	4.54 ^{b}	6.38 ^{c}	6.67 ^{c}	0.193	<0.001	0.224	0.977
	L	2.08 ^{a}	2.29 ^{a}	4.27 ^{b}	6.21 ^{c}	6.42 ^{c}				
	H	2.04 ^{a}	2.19 ^{a}	4.19 ^{b}	6.01 ^{c}	6.39 ^{c}				
Enterobacteriaceae	C	1.12 ^{a}	1.75 ^{a}	2.85 ^{b}	4.06 ^{c}	4.23 ^{c}	0.215	<0.001	0.904	0.998
	L	1.03 ^{a}	1.63 ^{a}	2.66 ^{b}	4.02 ^{c}	4.37 ^{c}				
	H	1.07 ^{a}	1.74 ^{a}	2.81 ^{b}	3.98 ^{c}	4.18 ^{c}				

SEM = standard error of the mean; a, b, c, and d, different letters in the same row denote a significant difference; W and X, different letters in the same column denote a significant difference; T = days of storage; D = diet; C = basal control diet; L = diet supplemented with 150 mg/kg of olive mill wastewater polyphenol extract; H = C diet supplemented with 280 mg/kg olive mill wastewater polyphenol extract.

Table 4. Output parameters estimated by the DMFit program for each microbial population in the three dietary groups.

Microorganism and Parameters	C	L	H
Total viable count (TVC)			
Initial value (Log CFU/g)	2.91 ± 0.11	2.92 ± 0.16	2.94 ± 0.13
Λ (h)	100.34 ± 10.93	101.32 ± 17.78	101.85 ± 10.78
μmax (Log CFU/g/h)	0.0529 ± 0.014	0.0531 ± 0.021	0.0509 ± 0.010
Final value (Log CFU/g)	8.32 ± 0.13	8.25 ± 0.20	8.03 ± 0.21
R^2	0.997	0.994	0.995
SE of Fit	0.137	0.208	0.189
Total psychrotrophic count (TPC)			
Initial value (Log CFU/g)	2.28 ± 0.12	2.09 ± 0.16	2.27 ± 0.09
Λ (h)	84.59 ± 10.00	82.26 ± 10.02	95.58 ± 13.64
μmax (Log CFU/g/h)	0.0459 ± 0.003	0.0379 ± 0.004	0.0503 ± 0.013
Final value (Log CFU/g)	7.94 ± 0.11	7.67 ± 0.16	7.22 ± 0.08
R^2	0.999	0.996	0.998
SE of Fit	0.0149	0.167	0.0949
Pseudomonas spp			
Initial value (Log CFU/g)	1.18 ± 0.09	1.22 ± 0.05	1.33 ± 0.10
Λ (h)	60.85 ± 4.61 ^{a}	65.37 ± 12.74 ^{a,b}	74.01 ± 7.40 ^{b}
μmax (Log CFU/g/h)	0.0443 ± 0.002 ^{a}	0.0447 ± 0.01 ^{a,b}	0.0354 ± 0.003 ^{b}
Final value (Log CFU/g)	7.50 ± 0.08 ^{a}	7.41 ± 0.05 ^{a}	6.42 ± 0.11 ^{b}
R^2	0.999	0.997	0.998
SE of Fit	0.0912	0.052	0.11

Table 4. *Cont.*

Microorganism and Parameters	C	L	H
Lactic acid bacteria (LAB)			
Initial value (Log CFU/g)	2.02 ± 0.15	2.07 ± 0.13	1.99 ± 0.12
Λ (h)	77.52 ± 12.40	79.05 ± 18.56	76.36 ± 10.09
μmax (Log CFU/g/h)	0.0373 ± 0.006	0.0338 ± 0.005	0.0316 ± 0.003
Final value (Log CFU/g)	6.50 ± 0.13	6.41 ± 0.17	6.36 ± 0.12
R^2	0.995	0.997	0.996
SE of Fit	0.15	0.022	0.127
Enterobacteriaceae			
Initial value (Log CFU/g)	1.13 ± 0.10	1.05 ± 0.14	1.08 ± 0.08
Λ (h)	42.32 ± 11.00	48.36 ± 15.53	37.94 ± 10.45
μmax (Log CFU/g/h)	0.0176 ± 0.002	0.0179 ± 0.002	0.0169 ± 0.001
Final value (Log CFU/g)	4.26 ± 0.01	4.41 ± 0.16	4.21 ± 0.09
R^2	0.995	0.991	0.996
SE of Fit	0.0976	0.141	0.0895

μmax = specific maximum growth rate; Λ = lag phase; SE = standard error of fit; R^2 = adjusted R-square statistics of the fit; C = basal control diet; L = diet supplemented with 150 mg/kg of olive mill wastewater polyphenol extract; H = C diet supplemented with 280 mg/kg of olive mill wastewater polyphenol extract; a, b, c, and d, different letters in the same row denote significant difference.

4. Conclusions

In agreement with the hypothesis formulated for this study, olive mill wastewater polyphenol sulphate metabolites were detected in the meat of rabbits fed with the phenolic extract supplement, and as expected, their content was greater in the group supplemented with the highest dosage. Although the mechanism of action of these potentially bioactive compounds against microbial growth is still partially undefined, as hypothesized, an influence on meat microflora has been highlighted. The observed antimicrobial activity varies among microbial populations and phenolic integration levels. The feeding strategy adopted in this investigation reduced *Pseudomonas* spp. growth only for the group fed with the H diet. This inhibitory effect can be attributed to an elongation of the Lag phase and a decrease in the maximum growth rate of the microbial growth curve. The results confirm that the use of polyphenols in animal feed could be a valuable strategy to reduce spoilage microorganisms in meat during storage, improving meat microbial quality. Further studies should consider the most effective dosage of dietary supplementation and the most suitable packaging methods to enhance the antimicrobial effect.

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