Effects of dietary propolis supplementation on fattening performance, carcass traits and meat quality in New Zealand White rabbits

Büşra YARANOĞLU[1,a,✉], Mustafa Hilmi YARANOĞLU[2,b]

[1]Balıkesir University, Faculty of Veterinary Medicine, Department of Animal Breeding and Husbandry, Balıkesir, Türkiye. [2]Balıkesir University, Research Center for Experimental Animal Breeding Department, Balıkesir, Türkiye.

[a]ORCID: 0000-0003-1260-3645; [b]ORCID: 0000-0002-9186-4861

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✉Corresponding author
busrayaranoglu@balikesir.edu.tr

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ABSTRACT

The aim of the study was to determine the effects of dietary propolis extract supplementation at different doses on the fattening performance, slaughter characteristics, meat quality, and fatty acid composition of New Zealand White rabbits. A total of 72 weaned, mixed-sex New Zealand White rabbits, aged one month, were used in the study. The rabbits were randomly assigned to three groups: one control (C) and two experimental groups (P_1 and P_2) (n = 24 per group). The control group was fed a standard ration, while the P_1 and P_2 groups received the same ration supplemented with 1000 mg/kg and 5000 mg/kg of propolis extract, respectively. The fattening period lasted for 8 weeks. Daily feed intake and feed conversion ratio were significantly higher in the P_2 group ($P<0.001$ and $P<0.01$, respectively). The dressing percentage was highest in the C group ($P<0.05$). Kidney and spleen percentages were lower in C group than experimental groups ($P<0.001$ and $P<0.05$, respectively). The pH and color parameters of the meat were within optimal ranges. The P_2 group had the highest values for expressed juice loss and cooking loss ($P<0.001$ and $P<0.05$, respectively). There were no significant differences among groups for drip loss and calculated fatty acid ratios. In conclusion, dietary supplementation of propolis extract increased feed intake, carcass yield, spleen, and kidney percentages and cooking loss; while it decreased expressed juice, and had no significant effect on fatty acid composition except for a few saturated and unsaturated fatty acids.

Introduction

In parallel with people's socio-cultural and economic development, the importance of healthy nutrition, in addition to an adequate and balanced diet, is increasing day by day. The emergence of functional foods stems from the desire to prevent disease and enhance the quality of life. Functional foods, besides meeting the basic needs of the body, provide additional benefits to human physiology and metabolic functions, enable protection from disease, and achieve a healthier life (3, 35, 52).

Every year, the world population increases rapidly and slaughters almost 70 billion animals to meet the growing demand for meat. New alternatives need to be developed to supply the need. Owing to its ability to protect against cardiovascular disease and metabolic illnesses such as diabetes and obesity, rabbit meat is recognized as a valuable functional food. Several experiments have been performed recently to improve the nutritional composition of meat by adding useful ingredients (10, 41, 44, 47). The meat of rabbits is particularly rich in polyunsaturated fatty acids (PUFA), omega-3 fatty acids, and essential amino acids, all of which are essential to human health. Saturated fatty acids (SFA) and cholesterol are significantly lower in rabbit meat when compared to beef and lamb meat. A 100-gram serving of rabbit meat fulfills the daily requirement for vitamin B_{12} and offers substantial quantities of vitamins B_2, B_3, B_5, and B_6. Due to its high protein and low fat

content, rabbit meat is considered a lean meat and serves as a healthier alternative to other red meats in carcass composition (19, 20, 25, 50, 63). Propolis has anti-inflammatory, immunomodulatory, hepatoprotective, antioxidant, antimicrobial, antiviral, hypotensive, cardioprotective, antitumoral, bacteriostatic, and bacteriocidal effects, as well as other important pharmacological properties (9). It has been used in many areas, including cancer treatments, in recent years. In the structure of propolis, there are β-amylase, fatty acids, phenolic compounds, flavonoids, their terpenes and esters. Propolis contains about 55% resin and polyphenolic fraction, 30% aromatic essential oils, 7% beeswax, 5% bee pollen, and 3% other minor components such as vitamins and some micro- and macro-minerals (49).

There are many studies in which propolis supplemented to the rations at different doses has positive effects on growth performance, carcass yield, meat quality, and composition of fatty acids in different species. Propolis has favorable effects on feed intake, growth performance, final body weight, and carcass ratio. It improves productive performance, health status, and intestinal health. Propolis is an excellent natural antibiotic and also an immune system booster (5, 7, 34, 46). Propolis facilitates the absorption of nutrients by regulating enzyme secretion, thereby increasing the digestibility of the feed (48). It is also defined that propolis supplemented to the ration increases feed consumption by increasing palatability (54, 56).

The study aimed to determine the effects of dietary supplementation with varying doses of propolis extract in New Zealand White rabbits on fattening performance, carcass traits, meat quality, and fatty acid profile.

Materials and Methods

Experimental Design and Animals: A total of 72 weaned, mixed-sex New Zealand White rabbits at the age of 1 month were used in the study. The rabbits were bred under the same conditions at the Department of Experimental Animal Breeding at Balıkesir University. The study consisted of three groups: one control (C) and two experimental groups (P_1 and P_2). There were 12 male and 12 female rabbits for each group. Rabbits were housed in individual cages (Techniplast). The dimensions of the cages were 71.3 × 71.6 × 47.6 cm (width × depth × height). Each rabbit had an individual feeder and drinker. There was plastic bedding material in the cages. The optimum temperature in the cages was 19±2°C and the humidity was around 50%. A lighting schedule of 12 hours light and 12 hours dark was applied. At the beginning of the study, each rabbit was randomly assigned to one of the experimental groups using simple random sampling. Rabbits were weighed at the start of the experiment to ensure that initial body weights were balanced across groups. Sex was taken into consideration while creating the study groups. The fattening period was determined to be 8 weeks. Rabbits in the control group were fed a ration appropriate for their energy and protein requirements (Table 1). Propolis ethanolic extract was added to the control diet at 1000 mg/kg for rabbits in experimental group P_1 and 5000 mg/kg for rabbits in experimental group P_2.

Table 1. Ingrediants and chemical composition of diets.

	Dietary Treatment		
	C	**P_1**	**P_2**
Ingrediants (%)			
Barley	20.75	20.75	20.75
Corn	18.00	18.00	18.00
Wheat Bran	14.00	14.00	14.00
Soybean meal	18.50	18.50	18.50
Yeast	0.20	0.20	0.20
Alfalfa flour	25.00	25.00	25.00
Methionine	0.15	0.15	0.15
Phytase	0.10	0.10	0.10
L-lysine	0.20	0.20	0.20
By-pass fat	1.50	1.50	1.50
Vitamin and mineral mixture[1]	1.60	1.60	1.60
Chemical composition (%)			
Dry matter	94.20	94.35	94.60
Crude protein	19.35	18.06	18.96
NDF	31.45	31.45	31.45
ADF	14.55	14.81	13.98
ADL	1.02	1.16	2.52
Ether extract	4.45	4.98	4.27
Starch	19.50	20.38	19.30
Ash	9.68	9.16	9.52
Digestible energy (kcal/kg)	2850	2875	2810

NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber; ADL: Acid Detergent Lignin C**:** Standard diet; P_1: Standard diet +1000 mg/kg ethanolic extract of propolis;
P_2: Standard diet +5000 mg/kg ethanolic extract of propolis
[1]Supplied per kg diet: 13,000 IU vitamin A; 1200 IU vitamin D_3; 75 mg vitamin E; 2 mg vitamin B_1; 6 mg vitamin B_2; 3 mg vitamin B_6; 0.02 mg vitamin B_{12}; 0.3 mg Co; 8 mg Cu; 27 mg Fe; 19 mg Mn; 44 mg Zn; 0.07 mg Se.

Preparing the Ethanolic Extract of Propolis: Propolis extraction was prepared following the method described by Blonska et al. (14) and Tatlı Seven et al. (56). A homogeneous mixture was obtained by mixing 1 unit of raw propolis with 9 units of 70% alcohol and kept in a dark room for 1 week. This mixture was then filtered with filter paper, evaporated at 45°C, and used in the study. The propolis extract was added to the total diet and thoroughly mixed carefully until a homogeneous mixture was obtained using a feed mixer.

Evaluation of Fattening Performance: The rabbits were weighed at the beginning of the trial, following weaning and initial weights were recorded. Rabbits were weighed weekly throughout the experiment, and daily weight gain (DWG) was calculated based on these measurements. The daily feed intake (DFI) was determined. The feed conversion ratio (FCR) was calculated by dividing DFI by the DWG. Data on fattening performance were compiled for the entire fattening duration. The fattening period was 8 weeks. At the end of fattening, the rabbits were weighed and subsequently slaughtered.

Determination of Slaughter and Carcass Characteristics: Carcasses of rabbits were obtained according to the reference dissection method of Blasco and Quhayoun (12). Hot carcass was weighed. Chilled carcass weight was determined after the 24-hour rest period at 4°C. The chilled carcass weight was divided by the slaughter weight to find the dressing percentage. The weights of the spleen, kidneys, liver, thymus, trachea, esophagus, heart, and lungs were measured, and their proportions relative to the slaughter weight were calculated. The reference carcass weight (RCW) was determined by removing the weights of the internal organs, dissectable fat (kidney, inguinal, and scapular fat), and the head from the chilled carcass weight.

The carcasses were sectioned between the 7^{th} and 8^{th} thoracic vertebrae and the 6^{th} and 7^{th} lumbar vertebrae, dividing them into three parts: fore, middle, and hind. The percentages of these parts were found by dividing the weights of each part by the RCW. The percentages of dissectable fat on the carcass, hind legs percentage, and forelegs percentage were estimated by dividing the weights of these parts by the RCW in the same manner.

The longissimus thoracis et lumborum (LTL) muscle was removed from both sides of the carcass, and fresh samples were immediately used for expressed juice, cooking loss, and drip loss analyses. All analyses, except fatty acid composition (FAC) analysis, were conducted on the day of the slaughter. For FAC determination, samples were packed in plastic bags and chilled at 18°C until analysis (26).

Evaluation of Meat Quality Parameters: pH was assessed utilizing a portable glass electrode pH meter (Mettler Toledo-Seven2Go pH meter) for both LTL and *biceps femoris* (BF) muscles. Calibration of the pH meter was executed with a series of calibration standards, encompassing pH 4.01/7.00/9.21. All pH measurements were conducted in triplicate, each at distinct sites on the sample. pH assessments were performed at three time points: immediately after slaughter, and then at 45 minutes and 24 hours post-slaughter. Lightness (L*), redness (a*), and yellowness (b*) parameters were determined with a colorimeter (Konica Minolta, CR 400). The color of LTL and BF muscles was determined at 2 times: immediately after slaughter and 24 hours later.

For determining the expressed juice (EJ), meat samples (5 g each) were divided into five equal pieces. These pieces were placed between filter papers, the weight of which had been previously determined. A weight of 2250 g was applied to these samples for 5 minutes. Following this time frame, the filter papers were weighed once again after the meat samples were taken out. The amount of EJ was calculated as a percentage. This was done by subtracting the initial weight of the filter paper from its final weight and then dividing this difference by the initial weight of the samples. This method of determining the EJ was explained by Beriain et al. (9) and is based on the method proposed by Grau and Hamm (29).

For assessing the cooking loss (CL), samples of meat from each animal were initially weighed approximately 50 g each and subsequently cooked for 45 min at 80°C until the internal temperature of the meat reached 70°C, monitored using a portable meat thermometer. Internal heat measurement was done every 5 minutes to detect the desired temperature. Meat samples were cooked in a bain-marie. Following the cooking process, samples were reweighed, and the CL was calculated as the ratio of the difference between the weights before and after cooking to the initial weight (32).

For assessing the drip loss (DL), meat samples were weighed to determine the initial weight. Subsequently, the samples were put into plastic bags and stored at 4°C for 24 and 48 hours. Meat samples were weighed again at 24 h and 48 h to quantify DL at the respective time points. DL was calculated as the ratio of the difference between the weights before and after resting weights to the initial weight (30).

For the determination of fatty acid composition, meat samples were extracted following the method proposed by Blight and Dyer (13). These samples were then placed in gas chromatography-mass spectrometry (GC-MS) vials, exposing the fatty acid methyl esters. The analysis was performed using an HP Agilent 6890/5972 GC-MS device. An HP-88 capillary column was used, which had a length of 100 m, an internal diameter of 0.25 mm, and a film thickness of 0.20 µm. The internal standards for the fatty acids were the FAME Mix C4-C24 (Supelco, 18919-1AMP). The injector temperature was set at 250°C, while the detector temperature was set at 270°C. The injection split ratio was 1:50, and the total injection volume was 1 µl.

Statistical Analysis: According to the results of the power analysis performed to calculate the sample size in the study, at a minimum significance level of 5%, when the power of the test was 95%, the effect size was 0.9, and the sample size required to find a statistically significant

difference between the groups using ANOVA in independent samples was calculated as a total of at least 24 rabbits. For this reason, 24 animals were used for each group. SPSS 25 was used to analyse the data. ANOVA was used to assess the impact of various doses of dietary propolis extract supplementation on fattening, slaughter, and meat quality characteristics. The Tukey test was used to compare significant differences among groups. Treatment differences were considered significant at $P<0.05$.

Results

Growth and fattening performance data obtained at different doses of dietary propolis supplementation were shown in Table 2. There were no significant differences between the groups in terms of final live weight (FLW) and DWG ($P>0.05$). DFI was at the highest level for the P_2 group ($P<0.001$). The feed conversion ratio of P_2 group was higher than that of the C group ($P<0.01$).

Slaughter and carcass characteristics determined at different doses of dietary propolis supplementation groups were presented in Table 3. There was no significant difference in carcass weight among the groups (hot, chilled, and reference). While the dressing percentage was highest in the control group ($P<0.05$), no significant difference was observed between the P_1 and P_2 groups ($P>0.05$). In terms of spleen and kidney percentage, higher values were observed in the experimental groups compared to the C group ($P<0.05$; $P<0.001$). When carcass parts and dissectable fat percentages were evaluated, no significant difference was found between groups ($P>0.05$).

Table 2. Effects of dietary supplementation of propolis extract on growth and fattening performance of New Zealand White rabbits

Traits	Dietary Treatments			
	C (n=24)	**P_1 (n=24)**	**P_2 (n=24)**	**P**
Initial live weight (30th day) (g)	937.15±27.21	929.86±25.95	938.96±11.92	-
Final live weight (86th day) (g)	2599.67±41.89	2588.96±65.26	2605.62±68.88	-
Daily weight gain (g/day)	29.36±0.77	29.59±1.09	31.74±1.08	-
Daily feed intake (g/day)	99.70^{b}±1.79	104.43^{b}±3,31	124.93^{a}±4.74	***
Feed conversion ratio (FCR)	3.43^{b}±0.08	3.62^{ab}±0.15	3.98^{a}±0.13	**

X±SEM, C: Standard diet; P_1: Standard diet+1000 mg/kg ethanolic extract of propolis. P_2: Standard diet+5000 mg/kg ethanolic extract of propolis. -: P>0.05; **: P<0.01; ***: P<0.001. Values with various superscripts in the same row differ significantly (P<0.05)

Table 3. Effects of dietary supplementation of propolis extract on slaughter and carcass characteristics of New Zealand White rabbits

Traits	Dietary Treatments			
	C (n=24)	**P_1 (n=24)**	**P_2 (n=24)**	**P**
Hot carcass (HC), g	1677.85±25.87	1615.46±41.78	1609.79±46.33	-
Chilled carcass (CC), g	1604.13±24.78	1545.02±42.53	1565.65±45.43	-
Reference carcass (RC), g	1287.90±18.61	1213.52±31.68	1246.56±37.91	-
Dressing percentage (DP), %	61.76^{a}±0.53	60.28^{b}±0.49	60.07^{b}±0.40	*
% SW				
Head	7.12±0.84	5.36±0.10	5.67±0.39	-
Kidney	0.57^{b}±0.01	0.71^{a}±0.03	0.77^{a}±0.03	***
Liver	2.46±0.08	2.54±0.07	2.54±0.08	-
Spleen	0.04^{b}±0.00	0.06^{a}±0.00	0.06^{a}±0.00	*
LH	0.93±0.02	0.92±0.02	0.95±0.02	-
% RCW				
Dissectible fat	5.76±0.40	6.36±0.52	5.17±0.25	-
Hind leg	30.59±0.19	30.78±0.18	30.63±0.19	-
Fore leg	13.60±0.23	13.11±0.20	13.36±0.15	-
Fore part	28.79±0.29	28.16±0.23	28.79±0.23	-
Mid part	34.71±0.26	34.49±0.34	33.78±0.31	-
Hind part	36.50±0.20	37.08±0.20	37.16±0.31	-

(X±SEM), C: Standard diet; P_1: Standard diet+1000 mg/kg ethanolic extract of propolis; P_2: Standard diet +5000 mg/kg ethanolic extract of propolis. SW: Slaughter weight; RCW: Reference carcass weight. -: P>0.05; *: P<0.05; ***: P<0.001 a, b: Values with various superscripts in the same row differ significantly (P<0.05).

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The data obtained with respect to the meat quality characteristics were shown in Table 4. The initial pH (pH_0) values measured in LTL and BF muscles were higher in the P_2 group than in the P_1 and C groups (P<0.001). There were no significant differences between groups for pH values at 24 h. In the study, the L* value measured in the LTL 24 h after slaughter was higher in the P_2 group than in the C group (P<0.05). The L* value measured in the BF muscle at 24 h after slaughter was found to be highest in the P_2 group (P<0.01). The b* value measured in the BF muscle at 24 hours after slaughter was found to be highest in the C group (P<0.05). The P_2 group had the highest EJ and CL values (P<0.05). There was no significant difference between the C and the P_1 groups. No significant differences were found in DLbetween the research groups (P>0.05).

Fatty acid compositions determined at different doses of dietary propolis supplementation groups wee presented in Table 5. It was found that C16.0 had the highest amount, followed by C18:1, C18:2 ω6, and C16:1 fatty acids. The P_2 group had the lowest values for C10:0 and C12:0 (P<0.001) and also had the highest value for C17:1 (P<0.01). For C17:0, the C group had lower values than the P_1 group (P<0.05). For C15:0, the P_1 group had higher levels than the P_2 group (P<0.01). For C16:0, C and P_2 groups showed higher levels than the P_1 group (P<0.05). The calculated fatty acid rates of the study groups were shown in Table 6. There wasn't any significant difference between groups (P>0.05).

Table 4. Effects of dietary supplementation of propolis extract on meat quality parameters of New Zealand White rabbits

	Dietary Treatments			
Traits	**C (n=24)**	**P_1 (n=24)**	**P_2 (n=24)**	**P**
Longissimus thoracis et lumborum muscle				
pH 0. hour	6.51^{b}±0.04	6.56^{b}±0.03	6.68^{a}±0.02	**
pH 45 minute	6.23±0.04	6.28±0.02	6.34±0.03	-
pH 24. hour	5.81±0.02	5.81±0.02	5.75±0.02	-
*L** 0. hour	42.40±1.37	40.26±0.75	40.80±0.69	-
*a** 0. hour	2.86±0.23	2.60±0.20	2.61±0.18	-
*b** 0. hour	2.04±0.20	2.16±0.16	2.24±0.13	-
*L** 24. hour	48.78^{b}±0.78	49.14^{ab}±0.69	51.20^{a}±0.70	*
*a** 24. hour	3.90±0.26	3.85±0.25	4.38±0.22	-
*b** 24. hour	4.12±0.26	3.57±0.20	4.04±0.17	-
***Biceps Femoris* Muscle**				
pH 0. hour	6.47^{b}±0.04	6.52^{b}±0.03	6.66^{a}±0.02	**
pH 45 minute	6.24±0.05	6.25±0.02	6.30±0.03	-
pH 24. hour	5.87±0.02	5.89±0.01	5.86±0.01	-
*L** 0. hour	48.25±0.85	48.43±0.84	49.11±0.85	-
*a** 0. hour	2.94±0.22	3.31±0.24	3.31±0.17	-
*b** 0. hour	1.92±0.51	2.90±0.11	3.58±0.19	-
*L** 24. hour	52.21^{b}±0.53	51.78^{b}±0.64	54.52^{a}±0.58	**
*a** 24. hour	3.78±0.24	3.28±0.25	3.29±0.19	-
*b** 24. hour	3.96^{a}±0.27	3.16^{b}±0.16	3.21^{b}±0.18	*
Expressed juice (%)	6.41^{c}±0.40	8.66^{b}±0.49	11.70^{a}±0.70	***
Cooking loss (%)	31.55^{b}±0.39	31.28^{b}±0.41	33.47^{a}±0.75	*
Drip loss 48. hour (%)	1.67±0.27	1.38±0.07	1.16±0.10	-
Drip loss 72. hour (%)	2.79±0.42	2.52±0.16	2.14±0.21	-

(X±SEM), C: Standard diet; P_1: Standard diet +1000 mg/kg ethanolic extract of propolis; P_2: Standard diet +5000 mg/kg ethanolic extract of propolis. -: P>0.05; *: P<0.05; **: P<0.01; ***: P<0.001. a, b, c: Values with various superscripts in the same row differ significantly (P<0.05)

Table 5. Effects of dietary supplementation of propolis extract on fatty acid composition of New Zealand White rabbits

	Dietary Treatments			
Traits	**C (n=24)**	**P_1 (n=24)**	**P_2 (n=24)**	**P**
SFA				
C10.0	0.296^{a}±0.023	0.264^{a}±0.026	0.137^{b}±0.025	***
C12.0	0.303^{a}±0.023	0.264^{a}±0.018	0.151^{b}±0.019	***
C13.0	0.098±0,031	0.180±0.044	0.102±0.021	-
C14.0	3.588±0.168	3.697±0.162	4.067±0.122	-
C15.0	0.987^{ab}±0.064	1.173^{a}±0.082	0.870^{b}±0.051	**
C16.0	34.518^{a}±0.417	32.822^{b}±0.424	34.151^{a}±0.461	*
C17.0	0.771^{b}±0.054	0.860^{a}±0.063	0.616^{ab}±0.054	*
C18.0	6.335±0.304	5.960±0.274	5.880±0.246	-
C20.0	0.055±0.003	0.047±0.002	0.050±0.001	-
C21.0	0.072±0.018	0.057±0.023	0.085±0.022	-
C22.0	0.097±0.009	0.085±0.011	0.105±0.008	-
C23.0	0.019±0.004	0.020±0.002	0.013±0.001	-
C24.0	0.047±0.005	0.053±0.004	0.057±0.004	-
MUFA				
C14.1	0.450±0.036	0.515±0.052	0.585±0.043	-
C15.1	0.589±0.062	0.675±0.101	0.817±0.070	-
C16.1	5.958±0.383	6.044±0.352	6.800±0.408	-
C17.1	0.357^{b}±0.029	0.299^{b} ±0.036	0.549^{a}±0.063	**
C18.1	24.133±0.387	24.016±0.371	23.373±0.367	-
C20.1	0.077±0.051	0.012±0.002	0.038±0.028	-
C22.1 ω9	0.020±0.009	0.028±0.013	0.011±0.004	-
C24.1	0.096±0.008	0.098±0.009	0.105±0.009	-
PUFA				
C18.2 ω6	17.836±0.491	19.361±0.630	18.137±0.490	-
C18.3 ω3	1.090±0.098	1.202±0.055	1.074±0.046	-
C18.3 ω6	0.143±0.047	0.085±0.003	0.086±0.004	-
C20.2	0.107±0.009	0.116±0.008	0.107±0.007	-
C20.3 ω3	0.017±0.001	0.020±0.001	0.018±0.002	-
C20.3 ω6	0.153±0.016	0.160±0.017	0.162±0.014	-
C20.4 ω6	1.512±0.136	1.638±0.151	1.615±0.152	-
C20.5 ω3	0.222±0.019	0.224±0.018	0.211±0.018	-
C22.2	0.008±0.002	0.004±0.000	0.006±0.001	-
C22.6 ω3	0.047±0.030	0.021±0.002	0.022±0.002	-

(X±SEM) C: Standard diet; P_1: Standard diet+1000 mg/kg ethanolic extract of propolis; P_2: Standard diet+5000 mg/kg ethanolic extract of propolis. SFA: Saturated Fatty Acids, MUFA: Monounsaturated Fatty Acids, PUFA: Polyunsaturated Fatty Acids -: P>0.05; *: P<0.05; **: P<0.01; ***: P<0.001. a, b: Values with various superscripts in the same row differ significantly (P<0.05).

Table 6. Effects of dietary supplementation of propolis extract on calculated fatty acid rates of New Zealand White rabbits

	Dietary Treatments			
Traits	**C (n=24)**	**P_1 (n=24)**	**P_2 (n=24)**	**P**
Saturated Fatty Acids (ΣSFA)	47.184±0.489	45.482±0.485	46.285±0.508	-
Monounsaturated Fatty Acids (ΣMUFA)	31.680±0.679	31.688±0.613	32.277±0.635	-
Polyunsaturated Fatty Acids (ΣPUFA)	20.981±0.564	22.695±0.624	21.309±0.644	-
Total Unsaturated Fatty Acids (TUFA)	52.662±0.493	54.382±0.480	53.586±0.508	-
ΣDesired Fatty Acids (DFA)	58.996±0,380	60.342±0.405	59.466±0,419	-
Nutritive Value (NV)	0.886±0.014	0.917±0.018	0.861±0.016	-
ΣPUFA/SFA	0.446±0.013	0.501±0.017	0.464±0.016	-
ΣMUFA/SFA	0.675±0.019	0.699±0.017	0.701±0.017	-
ΣTUFA/SFA	1.121±0.021	1.200±0.023	1.165±0.022	-
Σω6 / Σω3	1.554±0.221	1.308±0.119	1.400±0.097	-
Atherogenic Index (AI)	1.420±0.030	1.372±0.023	1.437±0.030	-
Thrombogenic Index (TI)	8.749±0.364	8.944±0.207	8.487±0.239	-

(X±SEM) C**:** Standard diet; P_1: Standard diet +1000 mg/kg ethanolic extract of propolis; P_2: Standard diet +5000 mg/kg ethanolic extract of propolis -: P>0.05. Desired Fatty Acids (DFA): C18:0+ΣTUFA. Nutritive Value (NV): (C18:0 + C18:1) / C16:0.
Atherogenic Index (AI): (C12:0 + 4*C14:0 + C16:0) / (ΣMUFA + Σω3+ Σω6) Thrombogenic Index (TI): (C14:0+C16:0+C18:0) / (0.5*ΣMUFA) + (0.5* Σω6) + (3*Σω3) + (Σω3/Σω6)

Discussion and Conclusion

In the study, an increase in feed intake was observed as the amount of dietary supplemented propolis increased among the groups, but this increase did not affect DWG and FLW. Furthermore, propolis extract showed neither a positive nor negative effect on carcass characteristics and fatty acid composition. It is suggested that dietary propolis supplementation could make the feed more palatable (41, 49). It has been reported that propolis increased the digestibility and absorption of feed by regulating the enzyme activities of saccharase, amylase, and phosphatase (60). In contradiction to the findings of the research, Al-Homidan et al. (5) and Piza et al. (45) revealed that low and high levels of propolis in the diet did not alter feed intake, DWG, and FCR. In California×New Zealand crossbred rabbits supplemented with propolis, final body weight and DWG were higher in the propolis-supplemented group, and FCR was lower than in the control group (53). Waly et al. (60) reported that increasing propolis levels increased live weight and total live weight gain. Total feed intake and FCR were decreased compared to the control group in the study. The lack of differences in FLW among the groups indicated that animals began fattening at similar ages and weights, suggesting a uniform adaptation to the fattening process (4). As the amount of propolis increases in the diet, DFI increases, but also FCR increases. This means that more feed intake is required for 1 kg of live weight gain. On the other hand, no significant difference was observed between the groups in terms of DWG and FLW. One possible explanation is that the additional benefit from the propolis-supplemented diet may have been utilized for other physiological processes, such as antioxidant activity, immune system activation, and changes in intestinal microflora, rather than contributing directly to weight gain. A second reason could be that the digestible energy rate of the feed changed with the supplementation of propolis, and the FCR increased even though DFI increased (36). The FCR increases numerically. These factors may also explain the lower dressing percentage observed in the propolis-supplemented groups. It was observed that the propolis extract did not have a positive or negative effect on the carcass characteristics in the study. Contrary to the findings of this study, Attia et al. (8), Sierra-Galicia et al. (53) and Waly et al. (60) found that supplementing propolis and various additives to the rabbits' rations resulted in higher dressing percentages than the control group. In similar studies, Yalçın et al. (65) and Onbaşılar and Yalçın (43) reported average feed intakes of approximately 3.63–3.92 kg and 3.38–3.49 kg per kilogram of live weight gain, respectively, in New Zealand White rabbits. Additionally, Onbaşılar and Onbaşılar (42) reported FCR ranging from 3.13 to 3.56 in the same breed.

Propolis is known to be a potent immunomodulator that enhances the immune system in animals (20, 49). Once in the body, it activates the relevant cells in the spleen by stimulating the production of lymphocytes. Therefore, spleen enlargement may occur due to increased lymphocyte activity as a result of long-term dietary

supplementation with propolis (7, 23). Waly et al. (60) reported that a higher amount of propolis increased the spleen ratio in fattening rabbits. Sherif and El-Saadany (51) observed a similar situation in broiler chickens. On the contrary, Al-Homidan et al. (5) found no significant difference in the spleen-to-thymus ratio in rabbits fed different doses of propolis.

The kidney may play a significant role in the metabolism of dietary propolis. Hashem et al. (30) reported in their study that different feed additives, including propolis, increased the lung and kidney rates determined in the carcass because the catabolites of these substances could have been eliminated in these organs. In another study, red propolis was detected to be protective material of the renal system; it minimizes oxidative stress and saves the endothelium in a rat model of acute kidney injury (17). The kidney percentage observed in the control group of the present study was consistent with the average value of 0.59 g/100 g slaughter weight reported by Yalçın et al. (64) for New Zealand White rabbits. Similarly, Onbaşılar and Yalçın (43) reported kidney percentages ranging from 0.60-0.64 g/100 g slaughter weight. It was also demonstrated that green propolis reduced mortality and protected kidneys and lungs against the inflammatory response in male Wistar rats (55). There was no significant difference between groups in liver and LH organs. The results were in agreement with Attia et al. (7) and Waly et al. (60).

pH is a fundamental parameter of meat quality, influencing the conversion of muscle to meat. The decrease in pH to levels of 5.6-5.8 in 24 hours after slaughter was crucial for the microbiological and physicochemical content of the meat (11, 57). Consistent with previous findings, pH measured at 60 minutes post-slaughter was higher in the experimental groups but balanced out by 24 hours (40). In young animals the optimal final pH values were 6.10 for biceps femoris muscle and 5.87 for longissimus lumborum muscle. In pH analyses, the time to reach the final pH is between 12 and 24 hours. The short time to reach the final pH is an important factor that negatively affects meat color; it suffers from a watery, pale color, and soft lean condition known as pale, soft, and exudative (PSE) meat. Inversely, a much slower rate of pH decline to levels much higher than the isoelectric point, in the range of 6.6–6.9, leads to the converse condition in which meat is dry, dark in color, and firm. This condition is known as dark, firm, and dry (DFD) (24, 36). Contrary to the research findings, a review study that included the effects of dietary antioxidants on meat quality characteristics in rabbits reported that additives added to the diet did not affect the physical and chemical properties of the meat (1).

Meat color significantly influences consumer preferences (16, 29). In this regard, it is very important that the propolis-supplemented rations increased the L* value in meat in terms of consumer preference for meat. The lack of significant changes in the a* value of both muscle groups measured in the study could be explained by the similar myoglobin and iron ratio in the relevant muscles (21). In contrast to the results, it has been revealed that different additives added to the diet did not change the color values of the meat (10, 15).

For technological and economic reasons, it is desirable to keep the water in the meat as much as possible (22). The main factor influencing meat's EJ is the immobilization of water from the myofibrillar system tissues. In this regard, it can be stated that the supplementation of propolis to the diet decreased the membrane stability of the meat, resulting in increased water loss (36). In the study, it was seen that the EJ of the meat decreased as the amount of propolis in the ration increased. While some of the studies on this topic were similar to the results of the study (2, 37), some of them reported that additives added to the ration did not affect EJ (38, 41).

The amount of cooking loss can describe the potential for loss of nutritional value of meat during the cooking process. The low cooking loss value of broiler meat can indicate good meat quality (22). When meat is exposed to heat, connective tissue changes and protein denaturation cause CL (33). Similarly, Kone et al. (38) reported higher CL values in the groups supplemented with plant extracts and essential oil products than in the control group. North et al. (41) did not find an important difference between the quercetin-supplemented experimental groups and the control group. The results of this study in terms of CL and drip loss were similar to the results of Meineri et al. (40).

It is essential to determine the FAC because each fatty acid found in meat has a different melting point, which affects the taste and consumption of meat. FAC of meat used in human nutrition is also very important in relation to chronic and cardiovascular diseases (62, 63). Rabbit meat has high levels of unsaturated fatty acids (UFA). Although the high PUFA content improves the nutritional value of the meat, it also accelerates oxidation in the meat, causing rancidity and undesirable color changes. This shortens the shelf life of the products, decreases the nutritional value, and poses a risk to human health. In this regard, antioxidant feed additives should be added to the ration to prevent oxidation and ensure its storage for a longer period (6, 28). In crossbred Red Angus and Nellore bulls, C18:2 ω6 fatty acids were determined to be higher and C22:6 ω3 fatty acids were determined to be lower doses in the experimental groups supplemented with propolis extract compared to the control group (59). Itavo et al. (34) reported for Texel and Suffolk crossbred lambs that C18:0 fatty acid was at lower doses and

C18:1ω9 and C18:2ω6 were at higher doses in the propolis extract-supplemented ration compared to the control group.

SFA, PUFA, PUFA/SFA, and Σω6/Σω3 ratios are essential for evaluating the nutritional value of meat fats. Unlike the study findings, Da Silva et al. (18) reported higher UFA levels and lower SFA levels for Texel lambs with added propolis extract in the ration.

The ratio between PUFA and SFA, also the Σω6/Σω3 ratio, are considered two important indexes for nutritional evaluation of fat (50, 63). In terms of a healthy diet, it is very important to increase the PUFA ratio in the diet, especially ω3 PUFA. It has been recommended that the ideal Σω6/Σω3 ratio should be greater than 0.40 and less than 4.00 (50, 63). In the study, rabbit meat was in the optimal range for the Σω6/Σω3 ratio. In the study, the ratio of PUFA/SFA varied from 0.44 to 0.50%. Wołoszyn et al. (61), Mapiye et al. (39), and Santos-Silva et al. (50) revealed that the ratio of PUFA/SFA should be more than 0.45 in the diet. Fats presenting low PUFA/SFA are considered unfavourable because they may induce an increase in cholesterolemia (50, 63). The ratio of AI and TI in the diet should be lower to avoid cardiovascular diseases (50, 63). Ulbrich and Southage (58) stated that the AI should not overlap 0.5. In the research, the value of AI was found to be higher than the recommended ranges. The reason for this may be due to higher ratios of C12:0, C14:0, and C16:0 fatty acids in rabbit meat samples. The recommended ranges for AI fit all meat animals, including lamb, beef, and chicken (63).

In conclusion, dietary propolis supplementation increased daily feed intake but did not significantly affect FLW, DWG, or carcass weights. Higher spleen percentages in the carcass of the experimental groups suggest a potential immunomodulatory benefit of propolis. The observed increase in EJ may be due to compromised cell membrane integrity caused by propolis. Future studies should explore the digestion and nutrient absorption effects of propolis, as well as multidisciplinary investigations evaluating immune system parameters in various animal species supplemented with different doses of propolis.

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Ethical Statement

The research was done under the consent of Animal Experiments Local Ethics Committee of Balıkesir University (2018/2-5).

Conflicts of Interest

The authors declared that there is no conflict of interest.

Author Contributions

Experimental design was planned by BY and MHY. Animal fattening period was carried out by MHY. Slaughtering and meat quality analysis were performed by BY and MHY. Data analysis was conducted by BY. All authors discussed the findings and contributed to the preparation of the final manuscript.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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