Effects of supplemental pine needles powder (*Pinus brutia*) on growth performance, breast meat composition, and antioxidant status in broilers fed linseed oil-based diets

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ABSTRACT This study was proposed to examine the effects of pine needles powder (*Pinus brutia*) supplementation on growth performance, breast meat composition, and antioxidant status in broilers fed linseed oil-based diets. For this purpose, a total of 210, Ross-308 1-day-old male broiler chicks were allocated to 5 experimental groups each containing 42 birds. Broilers were fed a linseed oil-based basal diet supplemented with 0% (control), 0.25% (P1), 0.50% (P2), 0.75% (P3), and 1% (P4) pine needles powder. During the 42-D feeding period, no significant differences were observed between experimental groups for body weight gain, feed intake, and feed conversion ratio; however, carcass yield was increased linearly with pine needles powder supplementation. No marked changes in the breast meat chemical composition were observed among experimental groups. Supplemental pine needles powder linearly decreased the malondialdehyde concentration in breast meat and liver tissues; however, 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity of breast meat samples remained unaffected. No significant variation was observed among experimental groups for superoxide dismutase enzyme activity in blood erythrocyte lysates, but blood serum total oxidation status tended to decrease with pine needles powder supplementation. In conclusion, results suggested that pine needles powder supplementation to broiler diets could be a viable option to improve the animal antioxidant status and meat oxidative stability; however, supplementation of *Pinus brutia* needles powder up to 1% into broiler diets was not sufficient to efficiently curb the fat-induced oxidation in meat. Further investigation is needed to determine the full antioxidant potential of pine needles powder supplementation in poultry by comparing different pine species, evaluating the bioavailability of their active compounds and determining most effective dietary concentration for broiler meat production without any adverse effects.

Key words: pine needles, natural antioxidant, broiler meat, polyunsaturated fats, oxidation status

2020 Poultry Science 99:479–486
http://dx.doi.org/10.3382/ps/pez542

INTRODUCTION

Poultry meat is one of the widely consumed meat sources in the world. A lot of research has been conducted to modify its nutrient profile, especially polyunsaturated fatty acid (**PUFA**) content in line with the health requirements (Kalogeropoulos et al., 2010; Wood and Enser, 2017). However, one of the major issues confronting this modification includes "lipid oxidation," as an increase in the PUFA content of meat makes it more prone to the oxidative deterioration (Ahn et al., 2007; Falowo et al., 2014). Thus, the concerns arise regarding quality and shelf life of the meat product. These days, the use of herbal supplements in animal diets has become very popular and this has shifted the researchers' attention towards natural additives for their application in animal diets as a source of functional ingredients.

Pinus brutia of family Pinaceae, commonly known as Turkish Red Pine, has the widest natural distribution area in Turkey and has been a source of traditional medicine in Turkey for years (Kızılarslan and Sevgi, 2013). Consumption of different parts of *Pinus* species such as leaves (needles), shoots, cones, and their extracts has been reported to cure asthma, wound, bronchitis, common cold, and cough in humans (Kültür, 2007; Ugulu et al., 2009; Cakilcioglu et al., 2011). Various studies also exhibited the antioxidant and anticancer effects of different pine tree products (Kwak et al., 2006; Park et al., 2008; Zeng et al., 2014). Pine needles are reported to possess a wide variety of beneficial compounds typical of medicinal plants such as essential oils (α and β-pinene), phenolic substances, vitamin C, protein, fat, and phosphorus (Pfister et al., 1998; Xie et al., 2015). Preliminary research suggested pine tree needles as a useful source of natural antioxidants in the diet, and studies showed that supplementation of

Received July 2, 2019.
Accepted September 9, 2019.
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pine needles powder (**PNP**) into broiler diets enhanced their antioxidant capacity without altering their performance (Park and Kim, 2011, Wu et al., 2015, Guo et al., 2018). However, to the best of our knowledge, literature evaluating the effectiveness of PNP supplementation in improving the oxidation resistance of PUFA-enriched broiler meat, thus its shelf life, is rather limited. Therefore, the present study aimed to investigate the effects of supplemental PNP (*Pinus brutia*) on growth performance, breast meat composition, and antioxidant status in broilers fed linseed oil-based diets.

MATERIALS AND METHODS

All experimental procedures in this study were approved by the Animal Ethics Committee of the Ankara University (2017-2-16).

Pine Needles Powder Preparation and Chemical Analyses

Needles of *Pinus brutia* were freshly collected from pine trees in Ankara and Konya, Turkey. After that, needles were dried at 60°C for 48 h in a hot air oven, processed to powder, and sieved manually with 1-mm screen. PNP thus obtained was properly sealed in a plastic container and stored until used in the experiment.

Proximate analysis of PNP was performed by the method as described in AOAC (2000). Fatty acids from PNP were methylated (AOCS, 1997), and the obtained fatty acid methyl esters (**FAMEs**) were analyzed by gas chromatography. Mineral profile was analyzed using ICP-MS (Agilent 7500, Yamanashi-Ken, Japan), while amino acid analysis was done by the HPLC (Agilent Technologies, Waldbronn, Germany) using modified OPA derivatization. Total polyphenols were determined spectrophotometrically as described by Hrncirik and Fritsche (2004). Vitamin C concentration was determined by redox titration using iodine solution, while vitamin E was determined using HPLC-UV Agilent 1100 series. Waxy substances (C40, C42, C44, C46) were determined with gas chromatography (Agilent 6890) by the method of EEC 2568/91. Total antioxidant (**TAS**, mmol Trolox equivalent/kg) and total oxidant (**TOS**, μmol H_2O_2 equivalent/kg) levels in the PNP were measured using commercially available kits (Rel Assay Diagnostics, Gaziantep, Turkey; product no. RL 0017 and RL 0024, respectively) by Erel's colorimetric methods (Erel, 2004, 2005). Oxidative stress index (**OSI**) values were calculated as:

$$\text{OSI} = (\text{TOS}, \mu\text{mol}/\text{TAS}\mu\text{mol}) \times 100$$

Experimental Animals and Diets

A total of 210 one-day-old Ross 308 male broiler chicks were randomly assigned to 5 experimental groups (6 replicates each, 7 birds/replicate). The dietary treatments comprised of linseed oil-based basal diet without any supplement (control) and basal diet with different levels of PNP including 0.25% (P1), 0.50% (P2), 0.75% (P3), and 1% (P4). Birds were fed into 3 phases: starter (0 to 10 D), grower (11 to 24 D), and finisher (25 to 42 D); basal diet for each phase was formulated to meet or exceed the requirements of broilers as stated in breed's catalog. Ingredients of basal diet along with the chemical composition are presented in Table 1. The experiment lasted for 42 D, and broilers were fed and provided with water ad libitum.

Table 1. Ingredients and chemical composition of basal diet.

Ingredients (%)	Starter (0 to 10 D)	Grower (11 to 24 D)	Finisher (25 to 42 D)
Yellow maize	43.90	44.00	47.45
Soybean meal, 48% CP[1]	21.00	17.40	17.40
Soybean (full fat), 38% CP	29.04	32.00	28.00
Linseed oil	1.00	2.20	3.00
Limestone	1.50	1.30	1.25
Monocalcium phosphate	2.40	2.00	1.90
Methionine	0.30	0.30	0.25
Lysine	0.20	0.20	0.15
Sodium bicarbonate	0.10	0.10	0.10
Salt	0.25	0.25	0.25
Vitamin premix[2]	0.15	0.15	0.15
Mineral premix[3]	0.10	0.10	0.10
Salinomycin	0.06	0	0
Analyzed chemical composition[4]			
Crude protein (%)	22.64	21.99	20.57
Metabolizable energy[5] (kcal/kg)	3,076	3,222	3,274
Calcium (%)	1.29	1.15	1.04
Total phosphorus (%)	0.91	0.81	0.78

[1]Crude protein.
[2]Vitamin premix (1 kg) 11,000,000 IU vitamin A, 3,500,000 IU vitamin D_3, 100 g vitamin E, 3 g vitamin K_3, 3 g vitamin B_1, 6 g vitamin B_2, 15 g pantothenic acid, 1 g vitamin B_6, 20 mg vitamin B_{12}, 35 g niacin, 1.5 g folic acid, 200 mg biotin.
[3]Mineral premix (1 kg) 30 g copper, 120 g manganese, 110 g zinc, 2 g iodide, 300 mg selenium, 50 g iron.
[4]As fed basis.
[5]Calculated as described by Carpenter and Clegg (1956).

Collection of Data and Samples

During the experiment, all birds were weighed individually at days 1, 10, 24, and 42. For each feeding phase, feed intake (**FI**) and body weight gain (**BWG**) were recorded to calculate feed conversion ratio (**FCR**). Mortalities were monitored daily, and FI and FCR were adjusted accordingly for each experimental group. At the end of the experiment, 2 birds from each replicate were randomly selected and slaughtered. Liver samples were immediately removed and stored at –18°C for thiobarbituric acid reactive substances (**TBARS**) analysis. Then, after proper evisceration hot carcasses were weighed individually.

For breast meat composition and oxidative stability determination, both right and left breast samples from each carcass were excised separately. Left breast samples were used to determine the pH after 1 and 10 D

of storage at 4°C, whereas right breast samples were further divided into 3 equal cuts (upper, middle, and lower). Upper right samples were used for proximate and fatty acid analyses; middle and lower right samples were stored at 4°C for 1 and 10 D, respectively, and were used in determining the TBARS and 2,2-diphenyl-1-picrylhydrazyl (**DPPH**) radical scavenging activity.

For the determination of the levels of TAS and TOS and superoxide dismutase (**SOD**) enzyme activity, blood samples were taken (2 birds per replicate) from the wing vein into plain and EDTA-coated tubes, respectively.

Breast Meat pH and Chemical Analyses

The pH of breast meat samples (1 and 10 D of storage at 4°C) was measured using pH meter (Mettler Toledo, SevenGoTM, pH meter SG2, puncture pH electrode LE427). Proximate composition of breast meat was determined by the methods as stated in AOAC (2000).

The fatty acid profile of breast meat samples was determined using gas chromatography (Shimadzu GC-2010, Shimadzu Co., Kyoto, Japan) equipped with a flame ionized detector (**FID**). Total lipids from 5 g of thawed meat samples were extracted using Soxhlet apparatus (AOAC, 2000), and FAMEs were prepared (Wang et al., 2011). FAMEs were then separated with a Teknokroma capillary column (TR-CN100, 100 m × 0.25 mm × 0.20 µm). Injection volume was set to 1 μL with a split ratio of 1 : 100. Helium was used as carrier gas (1 mL/min). The detector gas flows were hydrogen at 30 mL/min, makeup (helium) at 30 mL/min, and air 300 mL/min. Both injector and FID temperatures were fixed at 240°C. The temperature program was as follows: the initial temperature was held at 40°C for 2 min, then increased to 165°C for 5 min, 215°C for 8 min, and finally 240°C for 8 min at 4°C/min each. The FAMEs were determined by comparing the retention times and area percentages with a standard FAME mixture (Supelco 37 Component FAME Mix, 1 mL, CRM47885 Supelco).

Analysis of Blood Samples

Erythrocyte lysate from blood samples containing EDTA was prepared, and SOD enzyme activity was determined using Cayman's Chemicals Kit (Item No. 706,002) as per the manufacturer's protocol. For TAS and TOS assays, blood serum from plain tubes was removed using centrifugation (1,690 × g for 10 min at 4°C; SL 16R centrifuge, Thermo Scientific, Osterode am Harz, Germany) and the analyses were performed as described earlier.

TBARS Assay

Lipid oxidation of breast meat samples stored at 4°C for 1 and 10 D was measured using the distillation method (Tarladgis et al., 1960), and malondialdehyde (**MDA**) concentration in breast meat samples was expressed as mg/kg wet meat. Liver samples were also assayed for MDA concentration using Cayman's chemicals TBARS Assay Kit (Item No. 10009055) as per the manufacturer's protocol.

DPPH Radical Scavenging Activity

For DPPH radical scavenging activity, breast meat samples (1 and 10 D of storage at 4°C) were extracted according to the method of Jung et al. (2010) and DPPH radical scavenging activity was determined using the protocol of Brand-Williams et al. (1995) with some modifications. Briefly, 1 mL of the extracted sample was diluted with 4 mL of distilled water and then mixed with 5 mL of methanolic DPPH solution (0.2 mM). After vortex, the mixture was left at room temperature for 60 min. The absorbance of the sample was measured at 517 nm using a UV-vis spectrophotometer (Shimadzu, Model UV-1208). 5 mL of distilled water and 5 mL of DPPH solution served as control. The DPPH radical scavenging activity (%) was calculated as:

$$\text{Radical scavenging activity}, \% = [1-(\text{absorbance of sample}/\text{absorbance of control})] \times 100$$

Statistical Analysis

Statistical analysis was conducted using SPSS version 23.0 for Windows software (SPSS Inc., Chicago, IL). Normal distribution was tested using the Kolmogorov–Smirnov test. One-way ANOVA was used to determine the effects of PNP supplementation on different parameters. Comparisons among means were done by the Tukey test. Polynomial contrasts were used to determine the linear, quadratic, and cubic effects of PNP supplementation on different parameters. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Characteristics of PNP (Pinus brutia)

Chemical analyses data exhibited PNP as a good source of crude protein, crude fiber, vitamin C and E, total polyphenols, alpha-linolenic acid, and minerals (Table 2, 3).

Animal Performance and Carcass Yield

As shown in Table 4, PNP supplementation into broiler diets had no effect on BWG, FI, and FCR when compared to the control group ($P > 0.05$). A linear increase in carcass yield of experimental groups ($P < 0.05$) was observed with the increase in PNP levels (Table 5).

Table 2. Chemical characteristics of pine needles powder (*Pinus brutia*).

Item	Content
Dry matter (%)	94.64
Crude protein (%)	9.45
Ether extract (%)	6.73
Crude ash (%)	3.05
Crude fiber (%)	27.8
Vitamin E (mg/kg)	71
Vitamin C (mg/kg)	124.2
Wax contents (mg/kg)	1,608
Total polyphenols (g/kg)	91
TAS (mmol/kg)	5.16
TOS (µmol/kg)	27.38
OSI	0.53

TAS, total antioxidant status; TOS, total oxidant status; OSI, oxidative stress index.

Table 3. Fatty acid, amino acid, and mineral profiles of pine needles powder (*Pinus brutia*).

Amino acids	g/100g	Fatty acids	%[1]	Minerals	mg/kg
Aspartate	0.760	Myristic	4.04	Calcium	6,271
Glutamate	0.974	Palmitic	12.75	Phosphorus	895
Asparagine	0.011	Stearic	3.06	Magnesium	1,251
Serine	0.468	Palmitoleic	0.60	Manganese	17.34
Histidine	0.187	Oleic	20.43	Iron	155.2
Glycine	0.494	Linoleic	17.09	Copper	2.32
Threonine	0.427	ALA	30.01	Zinc	15.20
Citrulline	0.032	Arachidonic	10.11	Selenium	0.04
Arginine	0.662	Gadoleic	0.56	Molybdenum	0.48
Alanine	0.483	Behenic	0.62	Cobalt	0.14
Tyrosine	0.250	Lignoceric	0.33	Chromium	0.77
Cysteine	0.030	Margaric	0.35	Lead	0.75
Valine	0.438	Heptadecenoic	0.05	Cadmium	0.02
Methionine	0.068			Arsenic	1.02
Tryptophan	0.028			Mercury	0.04
Phenylalanine	0.442				
Isoleucine	0.339				
Ornithine	0.029				
Leucine	0.677				
Lysine	0.531				
Hydroxyproline	0.027				
Sarcosine	0.009				
Proline	0.540				
Total	*7.91*				

[1]Percentage of total fatty acids.
ALA, alpha-linolenic acid.

Breast Meat pH and Chemical Composition

When compared to the control group, pH of breast meat samples (1 D of storage at 4°C) from PNP supplemental groups (except P3 group) was significantly low ($P < 0.05$). Within supplemental groups, however, no differences were observed. On day 10 of storage, no significant differences were present between experimental groups for breast meat pH (Table 5).

No significant differences were observed between experimental groups for breast meat proximate composition including dry matter, protein, fat, and ash contents. Also, when compared to the control group no significant differences were observed for fatty acid composition (Table 6).

Blood Sample Analyses

No significant differences were observed between experimental groups for TAS, TOS, and OSI values ($P > 0.05$). SOD levels in blood erythrocyte lysates were also not affected by PNP supplementation ($P > 0.05$) (Table 7).

Antioxidant Status of Meat and Liver

MDA levels in breast meat samples stored at different time intervals are presented in Table 7. For 1-D storage at 4°C, lowest MDA levels were observed in the P2 group; however, they were not significantly different when compared to the control group ($P > 0.05$). More prominent results were observed at the 10th day of storage where P2 supplemental group resulted in a significant decrease in MDA levels in breast meat when compared to the control group ($P < 0.05$). An increase in PNP supplementation produced a linear decrease in breast meat MDA levels at both storage times ($P < 0.05$). MDA levels in liver tissues also decreased linearly with an increase in PNP supplementation ($P < 0.05$).

No significant effect of PNP supplementation was observed on DPPH radical scavenging activity of breast meat at both storage times (Table 7). P2 supplemental group showed the numerically highest activity for scavenging the DPPH radical at both storage times.

DISCUSSION

In the present experiment, supplementation of PNP (*Pinus brutia*) up to 1% into broiler diets showed no negative effects on birds' health. Among experimental groups, changes in BWG, FI, and FCR were statistically insignificant. These findings were in agreement with some previously available studies showing no significant effects of PNP supplementation on broiler performance parameters (Kim, 2011; Kim et al., 2012; Park and Kim, 2013; Wu et al., 2015). Guo et al. (2018) reported that in Guangxi-Tiejiaoma broilers FCR was negatively affected when fed diets containing high levels of PNP (3 and 5%); however, 1% PNP supplementation had no effect on FCR when compared to the control group. This negative effect on FCR could be attributed to increased fiber and wax content or other anti-nutrients present in PNP when supplemented at higher levels.

The present study showed that supplementation of PNP decreased the pH of breast meat samples (1-D storage at 4°C); however, no change in pH was observed for meats stored up to 10 D. Kim et al. (2012) also reported a significant decrease in pH of thigh meat of broilers when supplemented with PNP (1 and 2%), and this decrease was explained by the presence of bioactive compounds in the meat especially polyphenols and flavonoids from the PNP. Park and Kim (2011) also observed similar results for pH of thigh meat when broiler

Table 4. Effect of supplemental pine needles powder on BWG, FI, and FCR.

	Dietary treatments						Significance (*P*-value)			
Item	Control	P1 $_{0.25\%}$	P2 $_{0.50\%}$	P3 $_{0.75\%}$	P4 $_{1\%}$	SEM[1]	*Combine*	*Linear*	*Quadratic*	*Cubic*
Day 0 to 10										
BWG (g)	200.98	207.39	195.68	201.74	199.87	2.184	0.591	0.622	0.948	0.524
FI (g)	240.22	239.17	237.27	241.20	242.56	2.130	0.958	0.679	0.578	0.915
FCR	1.20	1.16	1.21	1.20	1.21	0.009	0.241	0.234	0.588	0.321
Day 11 to 24										
BWG (g)	987.15	980.05	969.56	1001.80	983.21	7.240	0.744	0.796	0.756	0.380
FI (g)	1355.54	1348.34	1350.65	1344.19	1334.75	7.698	0.945	0.436	0.848	0.830
FCR	1.37	1.38	1.39	1.34	1.36	0.009	0.415	0.283	0.540	0.415
Day 25 to 42										
BWG (g)	2210.96	2206.69	2237.59	2193.44	2198.35	10.279	0.720	0.613	0.530	0.855
FI (g)	3582.81	3563.60	3541.87	3609.19	3597.40	15.803	0.708	0.523	0.454	0.513
FCR	1.62^{a,b}	1.62^{a,b}	1.58^{b}	1.65^{a}	1.64^{a}	0.007	0.020	0.133	0.077	0.292
Day 0 to 42										
BWG (g)	3399.10	3394.13	3402.84	3396.99	3381.42	13.070	0.991	0.745	0.763	0.815
FI (g)	5178.58	5151.10	5129.78	5194.58	5174.72	20.392	0.888	0.816	0.578	0.555
FCR	1.52	1.52	1.51	1.53	1.53	0.004	0.329	0.365	0.158	0.558

BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio.
[1]Standard error of mean.
Different superscript letters (a, b) within the same row indicate significant differences between experimental groups ($P < 0.05$).

Table 5. Effect of supplemental pine needles powder on carcass yield and pH of breast meat.

	Dietary treatment						Significance (*P*-value)			
Item	Control	P1 $_{0.25\%}$	P2 $_{0.50\%}$	P3 $_{0.75\%}$	P4 $_{1\%}$	SEM[1]	*Combine*	*Linear*	*Quadratic*	*Cubic*
Carcass yield (%)	72.73	72.83	73.23	73.88	73.44	0.137	0.058	0.010	0.457	0.137
pH d-1	6.04^{a}	5.84^{b}	5.90^{b}	5.94^{a,b}	5.92^{b}	0.015	<0.001	0.086	0.002	<0.001
pH d-10	6.06	6.09	6.15	6.17	6.12	0.015	0.194	0.075	0.137	0.404

[1]Standard error of mean.
Different superscript letters (a, b) within the same row indicate significant differences between experimental groups ($P < 0.05$).

diets were supplemented with 0.3, 0.6, and 0.9% PNP. In their experiment, supplementing broiler diets with 0.6 and 0.9% PNP had resulted in a significant decrease in pH of thigh meat as compared to the control and 0.3% supplemental group. In another experiment with 1, 3, and 5% PNP supplementation, no difference was observed between groups regarding the pH of fresh breast and thigh meats (Xiong et al., 2011).

In agreement with our study, Xiong et al. (2011) reported no significant effects of PNP supplementation (1, 3, and 5%) on crude fat and ash contents of breast meat; however, they stated a significant increase in protein content of breast meat. Limited data are available regarding the effects of PNP supplementation on proximate composition of broiler breast meat. For thigh meat of broilers, some researchers (Kim, 2011; Park and Kim, 2013) reported no effect of PNP supplementation on proximate profile (moisture, protein, fat, ash), while some stated a significant decrease in the fat content of thigh meat (Xiong et al., 2011; Kim et al., 2012).

Research showed that the dietary antioxidants, rich in phenolic compounds, have the potential of improving the fatty acid composition of broiler meat especially the PUFA content (Jung et al., 2010; Saleh et al., 2017). The present study showed no clear effect of PNP supplementation on fatty acid composition of breast meat. In an experiment with broilers (0.3, 0.5, 0.9% PNP), Park and Kim (2011) reported that 0.9% PNP supplementation had significantly increased oleic acid in thigh meat while linolenic acid was decreased when compared to the control group. Vitina et al. (2011) and Polis et al. (2013) stated that supplementation of spruce and pine needles extractives had positive effects on MUFA (including oleic acid) and PUFA (including eicosapentaenoic and docosahexaenoic acids), while concentrations of saturated fatty acids (including myristic, palmitic, and stearic) were reduced when compared to the control group. These effects on fatty acid profile might be due to the high concentration of active substances in extracts than needles. No further data are available regarding the effects of PNP on meat fatty acid profile.

SOD is considered the main element of the first level defense against oxidation (Surai, 1999); it dismutases the main free radical produced in the cells, the superoxide radical (Halliwell, 2012). Also, through this prevention of free radical production, SOD may discourage the production of MDA caused by lipid peroxidation (Liu et al., 2011; Guo et al., 2018). Thus, SOD and MDA are important biomarkers evaluating the oxidative damage in animals. The present study exhibited no significant changes in SOD enzyme activity in

Table 6. Effect of supplemental pine needles powder on chemical composition of breast meat.

Item	Dietary treatments					SEM[1]	Significance (*P*-value)			
	Control	P1 0.25%	P2 0.50%	P3 0.75%	P4 1%		*Combine*	*Linear*	*Quadratic*	*Cubic*
Dry matter (%)	26.63	26.37	26.46	26.24	26.64	0.089	0.595	0.890	0.196	0.686
Crude protein (%)	24.20	24.05	24.08	23.94	24.21	0.084	0.856	0.912	0.359	0.709
Crude fat (%)	1.28	1.26	1.27	1.24	1.24	0.020	0.967	0.506	0.893	0.967
Crude ash (%)	1.13	1.10	1.12	1.10	1.14	0.010	0.701	0.727	0.280	0.851
Fatty acids[2]										
Myristic acid	0.347	0.408	0.352	0.362	0.422	0.010	0.063	0.150	0.453	0.021
Myristoleic acid	0.036	0.047	0.040	0.032	0.038	0.002	0.207	0.416	0.491	0.030
Palmitic acid	18.032	18.733	18.014	17.780	16.990	0.259	0.329	0.101	0.251	0.636
Palmitoleic acid	1.192	1.060	1.193	0.979	1.318	0.045	0.142	0.583	0.110	0.357
Stearic acid	10.464	10.281	10.552	10.815	10.063	0.142	0.538	0.791	0.342	0.151
Oleic acid	24.318^{a,b}	23.207^{b}	25.083^{a}	23.588^{a,b}	24.679^{a,b}	0.191	0.007	0.374	0.482	0.747
Linoleic acid	30.826	31.471	30.804	31.994	31.829	0.219	0.280	0.105	0.897	0.978
GLA	0.199^{b}	0.273^{a}	0.161^{b}	0.199^{b}	0.212^{b}	0.008	<0.001	0.303	0.609	0.001
ALA	13.270	13.317	12.748	13.010	13.178	0.257	0.961	0.794	0.630	0.782
DGLA	0.714	0.618	0.561	0.676	0.711	0.0338	0.564	0.831	0.132	0.620
Erucic acid	0.495	0.439	0.375	0.450	0.439	0.018	0.337	0.426	0.129	0.540
Arachidonic acid	0.049	0.069	0.063	0.049	0.049	0.003	0.171	0.390	0.093	0.089
EPA	0.056	0.083	0.052	0.070	0.074	0.004	0.090	0.416	0.921	0.119
SFA	28.846	29.417	28.920	28.954	27.478	0.351	0.494	0.205	0.233	0.860
MUFA	26.040^{a,b}	24.754^{b}	26.692^{a}	25.051^{a,b}	26.473^{a}	0.210	0.006	0.393	0.255	0.906
PUFA	45.113	45.829	44.388	45.993	46.051	0.371	0.577	0.444	0.583	0.819
USA	71.154	70.582	71.080	71.046	72.523	0.351	0.493	0.205	0.232	0.860

GLA, gamma linolenic acid; ALA, alpha-linolenic acid; DGLA, dihomo-gamma-linolenic acid; EPA, eicosapentaenoic acid.
SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated acids; USA, unsaturated fatty acids.
[1]Standard error of mean.
[2]Percentage of total fatty acids.
Different superscript letters (a, b) within the same row indicate significant differences between experimental groups ($P < 0.05$).

Table 7. Effect of supplemental pine needles powder on SOD enzyme activity, MDA levels, and DPPH radical scavenging activity.

Item	Days of storage	Dietary treatments					SEM[1]	Significance (*P*-value)			
		Control	P1 0.25%	P2 0.50%	P3 0.75%	P4 1%		*Combine*	*Linear*	*Quadratic*	*Cubic*
TAS (mmol/L)		1.64	1.64	1.65	1.75	1.82	0.043	0.618	0.146	0.541	0.903
TOS (µmol/L)		4.62	4.48	4.49	3.99	3.90	0.222	0.810	0.245	0.842	0.878
OSI		0.29	0.27	0.28	0.23	0.22	0.015	0.508	0.102	0.748	0.850
SOD (U/mL)		403.22	396.72	411.68	386.15	355.43	18.048	0.933	0.463	0.611	0.833
MDA liver[2]		1.184^{a}	0.997^{a,b}	1.064^{a,b}	0.927^{a,b}	0.834^{b}	0.034	0.013	0.001	0.328	0.242
MDA meat[3]	1	0.684^{a,b}	0.709^{a}	0.600^{b}	0.642^{a,b}	0.632^{ab}	0.011	0.013	0.022	0.356	0.266
	10	1.558^{a}	1.518^{a}	1.015^{b}	1.321^{a}	1.424^{a}	0.040	<0.001	0.049	<0.001	0.267
DPPH (%)	1	25.289	26.804	31.058	24.088	30.168	1.073	0.170	0.347	0.065	0.056
	10	43.926	43.625	46.653	38.712	40.915	0.985	0.101	0.150	0.092	0.052

TAS, total antioxidant status; TOS, total oxidant status; OSI, oxidative stress index; SOD, superoxide dismutase; MDA, malondialdehyde; DPPH, 2,2-diphenyl-picrylhydrazyl.
[1]Standard error of mean.
[2]µM MDA/mg liver tissue.
[3]mg MDA/kg breast meat.
Different superscript letters (a, b) within the same row indicate significant differences between experimental groups ($P < 0.05$).

experimental groups. In an experiment, supplementation of 3% *Pinus yunnanensis* needles powder to broilers had significantly increased serum SOD levels as compared to the control, while 1 and 5% PNP supplemental group showed no effect (Guo et al., 2018). In agreement to our findings, Wu et al. (2015) also stated no change in SOD enzyme activity in serum and liver samples when broilers were supplemented with *Pinus ponderosa* needles powder (containing 0.3 and 0.6% non-fermented PNP in the starter and grower phases, respectively); however, when fermented PNP was added in the diet, a significant increase in SOD activity was observed. Fermentation of PNP might have increased the availability of bioactive compounds to broilers, thus increasing the SOD activity.

TBARS is the most common and practical analysis used to determine the lipid oxidation as MDA concentration. The supplementation of *Pinus brutia* leaves powder in broiler diets resulted in a linear decrease in MDA levels in breast meat stored at 4°C for 1 and 10 D. P2 supplemental group seemed more effective in decreasing the meat MDA concentration, and this effect was more prominent in meat samples stored for the 10-D period. The present study also showed a linear decrease in the liver MDA levels with PNP supplementation. Park and Kim (2011) also observed a

linear decrease in MDA levels in thigh meat of broilers supplemented with 0.3, 0.6, and 0.9% PNP. In another experiment, Park and Kim (2013) observed similar results regarding MDA values in thigh meat of broilers supplemented with 0.5 and 1% PNP. Supplementation of 5% *Pinus yunnanensis* needles powder had significantly decreased the serum MDA levels as compared to the control group (Guo et al., 2018). Wu et al. (2015) also observed a significant decrease in blood serum and liver MDA levels of broilers fed diets supplemented with fermented PNP. However, no effect was observed with non-fermented groups.

DPPH radical scavenging activity is also an important indicator of antioxidant potential of foods. In an experiment, higher DPPH-radical scavenging activity in the thigh meat of broilers was observed in 1 and 2% PNP supplemental groups as compared to the control group (Kim et al., 2012). Similar results were obtained by Park and Kim (2011) regarding DPPH-radical scavenging activity in the thigh meat of broilers supplemented with 0.3, 0.6, and 0.9% PNP. The present study showed no significant effect of PNP supplementation on DPPH activity of breast meat. However, at both storage points, P2 supplemental group showed numerically higher scavenging activity. The reason for this is unknown, but the same group also presented with lowest polyunsaturated fat content. Thus, it is plausible that a precise concentration of an antioxidant is required to combat the PUFA-induced oxidation in meat. Among other oxidative damage biomarkers, the TOS, TAS, and OSI levels in the blood serum of experimental groups also showed that, although not significant, but with the increase in the PNP supplementation in the broilers diets there was a decreasing trend in the oxidative damage, thus describing the enhanced antioxidant capacity of broilers with PNP supplementation.

CONCLUSION

Early studies reported the prominent antioxidant potential of PNP supplements to broiler diets, but its efficacy in PUFA-enriched meat was not investigated. In the present study, a linear decrease in the MDA concentration in the breast meat and liver tissues of supplemental groups with an increasing trend in the antioxidant status of the birds showed the potential of PNP to reduce the lipid oxidation. However, for PUFA-enriched breast meat, the dietary supplementation of PNP up to 1% was inadequate in order to produce a prominent antioxidant effect and was dependent on the PUFA levels in the meat. Regarding performance parameters, no improvement was recorded; however, a linear increase in carcass yield was observed with PNP addition. Effect of PNP supplementation on the chemical composition of breast meat was not clear and needs more research. In conclusion, the current study suggests that PNP can be a viable option as a natural antioxidant for broiler diets. However, more research is needed to further examining the different antioxidant systems in the animal body and also determining maximum dietary supplementation for PNP into broiler diets without toxicity effects.

ACKNOWLEDGMENTS

The authors thanks to Dr. Zuhal Dilaver, Landscape Architecture Department, Faculty of Agriculture, Ankara University, for her assistance in identification of plant material.

This research was supported by the Scientific and Technological Research Council of Turkey, TÜBITAK (117O701).

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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