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Effect of *Pleurotus eryngii* Stalk Residue on the Oxidative Status and Meat Quality of Broiler Chickens

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ABSTRACT: *Pleurotus eryngii* stalk residue (PESR) is a byproduct of the edible portion of the fruiting body. The present study was conducted to evaluate the effects of PESR on the oxidative status and meat quality of broilers. Two hundred fifty 1-d-old male broilers (Arbor Acre) were evenly divided by gender and randomly allocated into control (corn-soybean meal diet) or 1.0, 5.0, 10.0, or 20.0 g/kg dried PESR groups. The results revealed that at 35 d, the dried PESR groups displayed a significantly increased water-holding capacity and decreased storage loss of breast and thigh fillets when compared to the control group. Regarding fillets color, the L^* (lightness) values were lower and the a^* (redness) and b^* (yellowness) values were higher following dried PESR supplementation. In 5.0−20.0 g/kg PESR supplementation groups, the activities of antioxidative enzymes were significantly elevated in serum, liver, spleen, and fillet tissues when compared to control group. Additionally, malondialdehyde production was slightly decreased in the PESR supplementation groups. Lower crude fat contents were observed in fillet tissues of 5.0−20.0 g/kg PESR groups when compared with the control group. In conclusion, PESR may potentially be used as an antioxidant to decrease lipid peroxidation and improve meat quality in broilers.

KEYWORDS: *Pleurotus eryngii, broiler, oxidative status, meat quality, feed additive*

INTRODUCTION

Currently, consumers are increasingly aware of the health benefits and nutritional quality of the food they consume. Oxidant stress in animals can impair the resulting meat quality.[1] Oxidative rancidity is one of the major causes of deterioration of feed or food for consumption, and it typically causes losses in the texture, consistency, flavor, appearance, and nutritional value of meat products.[2] Improved oxidative status in the living animal and increased oxidative stability of the raw product are considered to be beneficial for the processing industry and the consumer.[3] Natural antioxidants are currently receiving considerable attention in human and animal nutrition fields due to their association with food-quality characteristics.[1,4] The use of synthetic antioxidants in broilers to improve meat quality is being seriously considered; however, because some agents may exert mutagenic effects at high doses, the innocuousness of these compounds has been questioned.[5] For centuries, edible mushrooms have been used to maintain health and increase longevity; they were first used by ancient civilizations. Similar to plants, mushrooms have great potential for producing useful bioactive metabolites, such as phenolic compounds and biomolecules; they are also considered to be healthy foods because they are low in fat and calories but rich in dietary fiber.[6]

During the last few decades, *Pleurotus eryngii*, commonly called the king oyster mushroom due to its remarkable flavor and nutritional value, has rapidly become a highly valued species among consumers in North America, Europe, and Asia. *P. eryngii* is especially rich in carbohydrates (9.6% of the fresh weight), and a significant number of these carbohydrates are composed of dietary fibers (4.6% of the fresh weight; 4.1% is insoluble and 0.5% is soluble dietary fiber) and chitin (0.5% of the fresh weight).[7] Moreover, *P. eryngii* reportedly synthesizes various biologically active compounds and biomolecules, such as β-1,3-glucan, lovastatin, pleureyn, eryngin, ribonuclease, 17β-estradiol, and lectins, and it has a very low lipid content (0.8% of the fresh weight).[8,9]

Currently, *P. eryngii* is typically cultivated within a 3-D architectural structure inside an air-conditioned house. When the mushrooms' fruiting bodies mature and are ready to sell, the stalks must be cut and removed due to the formation of a residue byproduct. Our previous study demonstrated that the in vitro antioxidant activity of *P. eryngii* is similar to that of ascorbic acid, and *P. eryngii* does not seriously inhibit the viability of the chickens' peripheral blood mononuclear cells. Moreover, our results suggested that *P. eryngii* and its stalk residue may have potential as an antioxidant and feed additive in the broiler diet.[10]

To our knowledge, little information is available regarding the influence of dietary supplementation with dried *P. eryngii* stalk residue (PESR) powder in the broiler diet. Therefore, the objective of the present study was to investigate the effects of dried PESR, a byproduct of fruiting bodies, on the growth performance, lipid oxidation, and extension of meat quality of broiler chickens.

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Table 1. Ingredients and Chemical Compositions of the Experimental Diets and *P. eryngii* Stalk Residue

	grower diet (0−21d)					finisher diet (22−35d)				
	control	0.1% PESR	0.5% PESR	1.0% PESR	2.0% PESR	control	0.1% PESR	0.5% PESR	1.0% PESR	2.0% PESR
Ingredients (g/kg)										
corn	520.0	519.0	515.0	510.0	500.0	570.0	569.0	565.0	560.0	550.0
soybean meal (44%)	260.0	260.0	260.0	260.0	260.0	210.0	210.0	210.0	210.0	210.0
full-fat soybean meal (65%)	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
calcium carbonate	10.0	10.0	10.0	10.0	10.0	8.0	8.0	8.0	8.0	8.0
soybean oil	40.0	40.0	40.0	40.0	40.0	45.0	45.0	45.0	45.0	45.0
fish meal	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
dicalcium phosphate 22%	13.0	13.0	13.0	13.0	13.0	11.0	11.0	11.0	11.0	11.0
PESR powder[a]	0	1.0	5.0	10.0	20.0	0	1.0	5.0	10.0	20.0
L-lysine	0.6	0.6	0.6	0.6	0.6	0.0	0.0	0.0	0.0	0.0
DL-methionine	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
salt	2.8	2.8	2.8	2.8	2.8	2.5	2.5	2.5	2.5	2.5
vitamin premix[b]	0.6	0.6	0.6	0.6	0.6	0.5	0.5	0.5	0.5	0.5
mineral premix[c]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
total	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0
Chemical Analysis (%)										
crude protein ($N \times 6.25$)	22.0	22.0	22.0	22.1	22.1	20.0	20.0	20.0	20.1	20.1
crude fat	8.5	8.5	8.5	8.4	8.4	9.0	9.0	9.0	8.9	8.9
Calculated Analysis										
ME, MJ/kg	13.0	13.0	13.0	13.0	13.0	13.4	13.4	13.4	13.4	13.4
lysine, %	1.30	1.30	1.30	1.30	1.30	1.10	1.10	1.10	1.10	1.10
TSAA,[d] %	0.90	0.90	0.90	0.90	0.90	0.84	0.84	0.84	0.84	0.84
threonine, %	0.85	0.85	0.85	0.85	0.85	0.77	0.77	0.77	0.77	0.77
calcium, %	0.96	0.96	0.96	0.96	0.96	0.81	0.81	0.81	0.81	0.81
total phosphate, %	0.75	0.75	0.75	0.75	0.75	0.85	0.85	0.85	0.85	0.85
available phosphate, %	0.45	0.45	0.45	0.45	0.45	0.41	0.41	0.41	0.41	0.41

P. eryngii stalk residue composition[e]						
dry matter, g/kg	931.0 ± 3.0	crude ash, g/kg	56.4 ± 0.4	ether extract, g/kg	17.9 ± 0.2	P, g/kg	4.7 ± 0.1
crude protein, g/kg	86.3 ± 0.6	crude fiber, g/kg	133.2 ± 2.6	Ca, g/kg	5.6 ± 0.1	selenium content, g/kg	0.061 ± 0.003

[a]PESR = *P. eryngii* stalk residue. [b]Supplied per kg of diet: vitamin A (retinyl acetate), 3.75 mg; vitamin E (DL-α-tocopheryl acetate), 35 mg; vitamin K (menadione), 4 mg; riboflavin, 8 mg; pyridoxine, 5 mg; vitamin B_{12} (cyanocobalamin), 25 μg; calcium pantothenate, 19 mg; niacin, 50 mg; folic acid, 1.5 mg; biotin, 60 μg. [c]Supplied per kg of diet: Co ($CoCO_3$), 0.255 mg; Cu ($CuSO_4 \cdot 5H_2O$), 10.8 mg; Fe ($FeSO_4 \cdot H_2O$), 90 mg; Zn (ZnO), 68.4 mg; Mn ($MnSO_4 \cdot H_2O$), 90 mg; Se (Na_2SeO_3), 0.18 mg. [d]TSAA = total sulfur amino acid. [e]The value is expressed as the mean ± standard deviation (n = 6).

MATERIALS AND METHODS

***P. eryngii* Stalk Residue (PESR) Sample Preparation.** PESR used in the study was kindly provided by a local mushroom producer (Q-Yo Bio-Technology Farm, Changhau, Taiwan). The fresh PESR was dried in a forced hot-air dryer at 65 °C for 3 d and then ground to a powder (approximately 1 mm size) prior to its addition to the feed. The extracts were added to 100% distilled water (1:10, w/v) at 95 °C for 1 h after filtering (Advantec NO. 1, Tokyo, Japan). The filtrate was evaporated to dryness under vacuum conditions. The lyophilized extracts were rehydrated and adjusted to 1 mg/mL for subsequent analyses. The total phenolic contents were determined using Folin−Ciocalteu reagent and the contents of phenolic compounds of extracts as micrograms of the gallic acid equivalent (GAE) by using an equation that was obtained from a standard gallic acid graph according to the manufacturer's protocols.[11] Crude soluble polysaccharide assays were conducted using the phenol−sulfate method. The obtained extracts were analyzed spectrophotometrically by comparing the results to the data from the glucose standard curve at 730 nm; the β-glucan content was determined by modifying a method that was previously described.[12] An analysis of ergosterol and adenosine contents via HPLC were conducted using Waters Alliance 2690 and 996 photodiode array detectors set at wavelengths of 270 and 260 nm (Waters, Milford, MA), respectively. The separations were achieved with a 15 μL loop and a reversed-phase column (Merck LiChrospher 100 RP-18, 5 μm, 4.0 × 250 mm i.d.; Darmstadt, Germany) by isocratic elution, using 100% methanol as the mobile phase. The flow rate of the eluent was 1.0 mL/min. Trichloroacetic acid, chloroform−sulfuric acid, and glacial acetic acid−acetyl chloride were used for the qualitative detection of crude triterpenoids as previously described.[13]

Broilers' Management and Sample Collection. The experiment was conducted at the National Chung Hsing University, and the experimental protocol for animal use was approved by the Animal Care and Use Committee. Two-hundred fifty 1-d-old male broilers (Arbor Acre) were randomly allotted to five treatments. Each treatment was repeated twice, and 25 broilers were assigned to each experimental pen. All of the birds were placed in a temperature-controlled house. The temperature was maintained at 34 ± 1 °C until the birds reached 7 d of age, and it was gradually decreased to 26 ± 1 °C until the birds reached 21 d of age; after this point, the broilers were maintained at room temperature (approximately 26 °C). The birds in treatment 1 were fed a diet based on corn−soybean and fishmeal (control group), and the other 4 groups were provided experimental diets based on the basal diet but containing an additional 1.0, 5.0, 10.0, or 20.0 g/kg of ground dried PESR powder at the expense of ground yellow corn (Table 1). The proximate composition was analyzed according to the AOAC[14] and showed no major deviations from the calculated values. The feed mixtures contained no anticoccidial or antibacterial supplements. During the entire experimental period (35 d), the diets were formulated to meet the requirements suggested by the NRC.[15]

The study was divided into grower (0−21 d) and finisher periods (22−35 d), and diets (in mash form) and water were provided ad libitum. At 21 and 35 d of age, the performance of the broilers was

Table 2. Analysis of the Active Ingredients of *P. eryngii* Stalk Residue[a]

ingredient	crude polysaccharide (mg/g DW[b])	β-glucan (mg/g DW)	adenosine (μg/g DW)	ergosterol (mg/g DW)	total phenolics (mg GAE[c]/g DW)	crude triterpenoid (mg/g DW)
P. eryngii stalk residue	165.0 ± 10.1	0.58 ± 0.02	58.9 ± 3.3	1.28 ± 0.30	5.00 ± 0.33	1.84 ± 0.15

[a]The value is expressed as the mean ± standard deviation (n = 6). [b]DW = Dry weight. [c]GAE = Gallic acid equivalent.

determined by measuring the body weight (BW) in each individual bird and the feed intake at the pen level; the BW gain and feed efficiency (feed/gain) were then calculated. At 35 d of age, eight birds per treatment group (four birds per replicate) were randomly selected for sampling. The birds were bled via the brachial vein, and blood was collected (6 mL) by cardiac puncture using a 10 mL Vacutainer tube. The blood was centrifuged at 2000*g* for 15 min to obtain the serum and was stored at −20 °C until it was analyzed. After the blood was collected, the birds were euthanized by cervical dislocation. The liver, spleen, and fillets (breast and thigh) were excised, perfused with 0.9% ice-cold saline, and then chopped into small pieces on ice. A 10% (w/v) homogenate was prepared in 10 mM phosphate buffer (pH 7.4) and centrifuged at 12 000*g* for 10 min at 4 °C. The supernatant was collected and stored at −80 °C to assay for antioxidant enzyme activity and malondialdehyde (MDA) determination. The muscles from the breast and thigh fillets were packed individually in sealable plastic bags and stored at 4 °C pending meat quality analysis.

Meat Quality Measurements. The breast and thigh pH values were measured 15 min after slaughter with a portable meter (HI8424, Beijing Hanna Instruments Science & Technology Co. Ltd., Beijing, China) equipped with an insertion electrode that was calibrated in buffers at pH 4 and 7 at ambient temperature. The breast and thigh fillets were excised, weighted, and placed in plastic bags; they were freely suspended using a steel wire hook and stored at 4 °C. Care was taken to minimize the contact between the muscle and the inside surface of the bag. Twenty-four hours later, the samples (breast and thigh) were wiped and weighted to evaluate the drip loss, which was expressed as a percentage of the initial muscle weight.[16] The water-holding capacity (WHC) was estimated by determining the amount of expressible juice using a modified filter paper press method.[17] A raw meat sample of breast and thigh weighing approximately 1.0 g was placed between 18 pieces of 11-cm-diameter filter paper and was pressed at 35 kg for 6 min. The amount of expressed juice was defined as the weight loss that occurred after pressing and is presented as a percentage of the initial sample weight. The meat color was measured 45 min post-mortem with a chromameter (CR-300, Minolta Co. Ltd., Osaka, Japan) to measure the CIE LAB values (L^* lightness; a^*, redness; b^*, yellowness).

Biochemical Determinations. A spectrophotometer was used to colorimetrically assay the activities of Trolox equivalent antioxidant capacity (TEAC), catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px).[18] The procedures were conducted with assay kits that were purchased from Cayman Chemical Co. (Ann Arbor, MI). The MDA level was detected with 2-thiobarbituric acid, and the change in absorbance at 523 nm was monitored with a spectrophotometer.[19] All of the samples were measured in triplicate and at appropriate dilutions to allow the enzymatic activities to achieve the linear range of standard curves. Antioxidative enzyme activities were expressed as units (U) per milligram or mU of protein for the liver and spleen tissues and U per milliliter of serum. The protein content of the tissues was determined using the Coomassie Brilliant Blue G250 (Sigma Chemical, St. Louis, MO) assay kit.

Statistical Analysis. All of the data were evaluated by performing ANOVAs for completely randomized designs using the GLM procedure of the SAS software program.[20] Significant statistical differences among the various treatment group means were determined using Duncan's multiple range test. A significance level of 0.05 was used.

RESULTS AND DISCUSSION

Functional Components of PESR. Mushrooms are added to animal diets to enhance the oxidative status,[21] and the extracts can operate as an alternative to antibiotics to promote growth.[22,23] The active ingredients of *P. eryngii* have additionally been shown to accumulate a variety of secondary metabolites, such as phenolic compounds, polypeptides, steroids, triterpenoid saponins, and polysaccharides, which are important antioxidants and synergists and can improve the animals' oxidative statuses.[6,24] However, less information is available regarding the influence of dietary supplementation with dried PESR in broiler chickens. Analyses of the active ingredients in PESR are shown in Table 2. Dried PESR has abundant crude soluble polysaccharide (165.0 ± 10.1 mg/g dry weight). The primary polysaccharides in plants and fungi are derived from the cell wall and its metabolites. Mushroom polysaccharides primarily exist as linear and branched glucans with different types of glycosidic linkages, such as β-(1−3),(1−6)-glucans and α-(1−3)-glucans; however, some are true heteroglycans containing glucuronic acid, xylose, galactose, mannose, arabinose, or ribose. In addition to providing the requirements for biological metabolism and energy, these polysaccharides also exhibit antiviral, antibacterial, and antiparasitic functions.[8,9,24] Some polysaccharides that exhibit bioactivities are composed of monosaccharides with molecular weights ranging from 10^4 to 10^7. The complex, three-dimensional structures are formed through the clustering of β-glycoside linkages. β-1−3-D-Glucan and its derivatives possess immune-stimulating activity and various levels of free-radical-scavenging activity.[25] The activity of the mesmeric β-glucan unit was 10-fold higher than its monomeric counterpart at the same concentration level. Polysaccharide contents are influenced by different cultivation conditions. For example, the polysaccharide content of *Lentinus edodes* is 72 ± 4 mg/g dry weight when the general commercial cultivation medium is used; however, the polysaccharide content can be increased to 410 ± 72 mg/g dry weight by switching to the whey and permeate-based culture medium.[26] Phenolic compounds are widely found as secondary metabolites in plants and mushrooms, and the contents can be used as a critical index for determining the antioxidant capacity.[24,26] The total phenolic contents of the five analyzed mushrooms ranged from 7.14 mg GAE/g extracts (*P. eryngii*) to 37.33 mg GAE/g extracts (*Agaricus bisporus*).[6] The active compounds react directly with free radicals, such as hydroxyl ($^{\bullet}OH$) radicals, superoxide anion ($O_2^{\bullet-}$) radicals, and hydrogen peroxide (H_2O_2, as oxygen in the non-free-radical state), to minimize cellular damage and inhibit or delay lipid oxidation.[3] However, some low molecular weight phenolic compounds may be absorbed by the small intestine and subsequently reach to the plasma and target organs. Although the levels of phenolic compounds are low or reduced in circulation, display reduced net absorption levels, and are relatively rapidly excreted and have short half-lives, the consumption of phenolic compounds has also been associated with an enhanced oxidative status in animals and hu-

Table 3. Effects of Dietary *P. eryngii* Stalk Residue Supplementation on Meat Quality in Broiler after 35 d[a]

	treatment[d]						statistical significance	
	control	0.1% PESR	0.5% PESR	1.0% PESR	2.0% PESR	SEM[c]	*p*-value	control vs PESR
				Breast				
pH	6.15 c	6.25 b	6.29 ab	6.37 a	6.38 a	0.05	^{f}	^{g}
drip loss (%)	4.13 a	4.04 b	4.00 b	3.87 c	3.85 c	0.04	^{f}	^{g}
WHC (%)[b]	66.2 c	68.9 b	70.3 ab	72.6 a	73.0 a	0.99	^{e}	^{f}
*L** (lightness)	59.0 a	58.1 b	57.9 b	54.5 c	53.9 c	0.51	^{f}	^{g}
*a** (redness)	4.44 c	4.66 b	4.70 b	4.79 a	4.81 a	0.06	^{f}	^{g}
*b** (yellowness)	4.42 c	4.59 b	4.70 b	5.10 a	5.21 a	0.08	^{f}	^{g}
				Thigh				
pH	6.22 c	6.28 b	6.29 b	6.40 a	6.41 a	0.06	^{f}	^{f}
drip loss (%)	4.99 a	4.78 b	4.71 b	4.49 c	4.40 c	0.04	^{f}	^{g}
WHC (%)[2]	67.9 c	70.1 b	73.2 a	74.2 a	74.8 a	0.97	^{f}	^{f}
*L** (lightness)	59.8 a	58.9 ab	57.6 b	55.2 c	54.9 c	0.44	^{f}	^{g}
*a** (redness)	5.12 c	5.21 bc	5.33 b	5.59 a	5.65 a	0.07	^{f}	^{f}
*b** (yellowness)	5.76 c	5.90 bc	6.11 b	6.50 a	6.55 a	0.10	^{f}	^{f}

[a]Results are provided as the means of eight samples corresponding to eight birds for the control group (corn−soybean meal diet) and 1.0, 5.0, 10.0, and 20.0 g/kg dried *P. eryngii* stalk residue (PESR) supplemented groups. [b]WHC = water-holding capacity. [c]SEM = standard error of the mean. [d]Means within the same rows without the same letter are significantly different ($p < 0.05$). [e]$p < 0.05$. [f]$p < 0.01$. [g]$p < 0.001$.

Table 4. Effects of Dietary *P. eryngii* Stalk Residue Supplementation on Antioxidant Enzymes and MDA Levels in the Serum, Liver, Spleen, and Meat in Broilers after 35 d[a]

	treatment[d]						statistical significance	
	control	0.1% PESR	0.5% PESR	1.0% PESR	2.0% PESR	SEM[c]	*p*-value	control vs PESR
				Serum				
TEAC[b] (U/mL)	10.86 b	10.92 b	14.56 a	17.77 a	18.97 a	0.34	^{f}	^{g}
CAT[b] (U/mL)	4.23 c	5.11 b	6.89 a	7.21 a	7.43 a	0.15	^{f}	^{g}
SOD[2] (U/mL)	140.8 b	158.2 b	169.4 a	175.7 a	178.8 a	5.88	^{f}	^{g}
MDA[b] (nmol/mL)	5.33 a	4.93 b	4.35 c	4.22 c	4.10 c	0.07	^{f}	^{g}
				Liver				
CAT (U/mg of protein)	15.96 c	16.86 b	19.92 a	20.34 a	21.23 a	0.44	^{f}	^{g}
SOD (U/mg of protein)	101.2 c	115.6 b	125.9 ab	138.0 a	143.4 a	6.11	^{f}	^{g}
MDA (nmol/mg of protein)	0.91 a	0.81 b	0.69 c	0.69 c	0.67 c	0.004	^{f}	^{g}
				Spleen				
CAT (U/mg of protein)	11.21 c	13.91 b	15.36 ab	16.24 a	17.00 a	0.42	^{f}	^{g}
SOD (U/mg of protein)	32.16 c	38.12 b	44.57 a	45.72 a	45.99 a	2.32	^{f}	^{g}
MDA (nmol/mg of protein)	0.57 a	0.50 b	0.40 c	0.37 c	0.36 c	0.004	^{f}	^{g}
				Breast				
GSH-Px[b] (mU/mg of protein)	7.71 b	8.25 b	10.56 a	11.27 a	12.17 a	0.38	^{e}	^{f}
CAT (U/mg of protein)	0.46 c	0.59 b	0.76 a	0.77 a	0.87 a	0.006	^{f}	^{g}
SOD (U/mg of protein)	35.24 c	43.22 b	49.72 a	52.29 a	56.17 a	2.67	^{f}	^{g}
MDA (nmol/mg of protein)	12.29 a	10.10 b	8.77 c	8.66 c	8.17 c	0.11	^{f}	^{g}
				Thigh				
GSH-Px (mU/mg of protein)	6.68 b	7.37 b	9.85 a	10.23 a	11.79 a	0.40	^{e}	^{f}
CAT (U/mg of protein)	0.44 c	0.62 b	0.81 a	0.81 a	0.89 a	0.005	^{f}	^{g}
SOD (U/mg of protein)	28.19 c	35.66 b	41.17 a	42.32 a	44.12 a	2.19	^{f}	^{g}
MDA (nmol/mg of protein)	11.65 a	9.22 b	8.44 c	8.39 c	8.13 c	0.09	^{f}	^{g}

[a]Results are provided as the means of eight samples corresponding to eight birds for the control group (corn−soybean meal diet) and 1.0, 5.0, 10.0, and 20.0 g/kg dried *P. eryngii* stalk residue (PESR) supplemented groups. [b]TEAC, CAT, SOD, MDA, and GSH-Px represent the total antioxidant capacity, catalase, superoxide dismutase, malondialdehyde, and glutathione peroxydase, respectively. [c]SEM = standard error of the mean. [d]Means within the same rows without the same letter are significantly different ($p < 0.05$). [e]$p < 0.05$. [f]$p < 0.01$. [g]$p < 0.001$.

mans.[21,27,28] In this study, we did not assay the accumulation of the bioactive compounds in tissues; however, PESR included active ingredients that, when added to the diet, may reduce oxidant levels and improve the quality of meats.

Growth Performances and Meat Quality. The growth performances of the broilers reflected body weight gains of 1702, 1712, 1770, 1767, and 1768 g/bird in the control, 1.0, 5.0, 10.0, and 20.0 g/kg PESR groups, respectively. At 0−35 d of age, the 5.0 g/kg PESR supplemental group had a higher body weight gain compared with the other groups. The broilers' feed intakes did not differ significantly among the groups and periods ($p > 0.05$), and the 5.0 g/kg PESR and control groups gained 2763.3 and 2752.8 g/bird from 0 to 35 d of age, respectively. The feed efficiency of the broilers in the 5.0 g/kg PESR supplemented group was higher than that of the control group at 22−35 d (1.71 vs 1.77) and 0−35 d of age (1.57 vs

1.63). Previous studies have shown that the inclusion of *A. bisporus* mushrooms in the diet conferred growth promoting activity and delayed lipid oxidation in poultry diet. [21] Moreover, additional studies have demonstrated that the combined use of Chinese herbal and mushroom extracts can act as an alternative to antibiotic growth promoters.[22,23] The relative weight (g/100 g BW) of the digestive organs, such as the gizzard, duodenum, jejunum−ileum, and cecum, did not significantly differ among the groups at 21 and 35 d of age (data not shown). Table 3 presents the effects of PESR supplementation on the pH, drip loss, WHC, and meat color in broiler breast and thigh fillets after 35 d. The pH value increased with the addition of dietary PESR. The drip loss of the breast and thigh muscles was significantly lower with the addition of PESR, and the WHC increased ($p < 0.05$) following this addition. The meat color in the broiler fillets, as expressed by L^* (lightness), a^* (redness), and b^* (yellowness), was significantly influenced ($p < 0.05$) by dietary supplementation with PESR powder. The highest L^* values and the lowest a^* and b^* values were obtained in the control group. The L^* value of the meat color of the fillets was lower in the broilers that were fed the 10.0 and 20.0 g/kg PESR diet in comparison with those fed the 1.0 and 5.0 g/kg PESR diets. Higher a^* and b^* values ($p < 0.05$) for the meat color of the fillets were detected in the 10.0 and 20.0 g/kg PESR diets when compared to the control group. The pH value directly reflects the muscle acid content and affects the drip loss, WHC, and color. The average pH value 15 min after slaughter was between 6.3 and 6.6 for the broiler breast meat (which was considered normal meat).[27] In the current study, the breast and thigh fillets had pH values of approximately 6.3, and only the control group samples displayed a lower pH value (approximately 6.2). These results indicated that no quality problems existed in the meats from each group. A higher drip loss and lower WHC could induce liquid outflow and the loss of soluble nutrients and flavor; these processes could lead to the formation of dry, hard, tasteless muscles and decreased meat quality. in broiler meat, L^* is used to estimate the incidence of paleness; a pale, soft, and exudative condition; or both.[29] In a previous study, the L^* value of an apparently normal breast was 55, and apparently pale breasts had CIE L^* values of 60; the authors stated that high L^* and low ultimate pH values (<5.7) were indicative of pale broiler breast meat with a low WHC.[27] A previous study demonstrated that increased L^* and decreased a^* values may be associated with increased metmyoglobin formation of the muscles.[30] Antioxidants are substances that can slow the oxidation rate of autoxidizable materials. The presence of natural antioxidant compounds may retard metmyoglobin formation in meatballs and result in a decreased L^* value.[30] Added oregano (30 g/kg) an antioxidants conferred a higher b^* value than in a nonsupplemented group in chicken muscles.[31] PESR contains abundant secondary metabolites (antioxidants), such as phenolic compounds, and may be insusceptible to prooxidant substances that can react with oxymyoglobin; therefore, PESR may decrease autoxidation in metmyoglobin formation, causing increased color stability in meats.

Biochemical Determinations. Table 4 shows the effects of dietary PESR supplementation on the levels of antioxidant enzymes and MDA in the serum, liver, spleen, and meats in broilers after 35 d. The serum TEAC values of 35-d-old male broilers were significantly higher in the 5.0, 10.0, and 20.0 g/kg PESR groups compared to the 1.0 g/kg and control groups ($p < 0.05$). The groups that were supplemented with higher amounts (e.g., the 20.0 g/kg PESR group) were not significantly different than the 5.0 g/kg PESR group. Moreover, the antioxidative enzymatic activities of CAT and SOD displayed the same trend as the serum TEAC values in the liver, spleen, breast, and thigh tissues of 35-d-old broilers. The breast and thigh tissues of the control and 5.0 g/kg PESR groups showed significantly decreased GSH-Px activity compared with the other groups ($p < 0.05$). Minimal research has been conducted regarding the effect of PESR on oxidative status in broiler chickens. Antioxidant enzymes are synthesized and regulated endogenously, and these are an important index of the oxidative status of animal tissues.[3,21] SOD catalyzes the dismutation of a superoxide anion (O^{2-}) into hydrogen peroxide and prevents the generation of free radicals. CAT converts H_2O_2 into H_2O, and TEAC measures the capacity of a compound to capture ABTS [2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)], a radical cation. When soybean isoflavone (ISF), a high-antioxidant product from soybean, was added to the broiler diet, antioxidant enzymatic activities (especially SOD and CAT) increased in both the plasma and breast muscle.[3] In addition, the pH value and WHC of the breast fillets in the ISF groups also improved; the authors suggested that the improved oxidative status of male broilers by ISF supplementation may protect skeletal muscle cells against metabolic byproducts such as lactic acid, which may affect the oxidant stability of post-mortem muscles.[3] Therefore, the authors reported that ISF should improve meat quality and oxidative status by decreasing lipid peroxidation in broilers. Moreover, the dietary addition of vitamins C and E may enhance the oxidative status of plasma and liver tissues and may reduce oxidative stress in broilers.[32] Our previous study demonstrated that the in vitro antioxidant activity of *P. eryngii* is similar to that of vitamin C and suggested that *P. eryngii* and its stalk residue may have potential as an antioxidant and feed additive in broiler diet.[10] The dietary mushroom-supplemented (*A. bisporus*) group displayed elevated GSH-Px and reduced MDA formation in the liver, thigh, and breast tissues when compared with the nonsupplemented group.[21] The authors suggested that the cells may utilize the mushrooms' antioxidant properties, thus sparing the intracellular antioxidant systems, such as GSH-Px.[21,28] The inclusion of 30 g/kg of grape pomace concentrate increased the oxidative status (ABTS method) in the serum of broilers; in addition, the secondary metabolites found in the aforementioned extracts affect the gene expression of antioxidant enzymes in liver tissues.[33,34] Therefore, in this study, elevated antioxidant enzymatic activities may passively spare glutathione by decreasing cellular oxidative stress; this process resulted in reduced MDA formation in the serum and meats of the PESR groups as well as improve meat quality of broiler chickens.

The extent of lipid peroxidation by reactive oxygen can be monitored by evaluating the MDA levels.[19] Dietary supplementation with 5.0, 10.0, and 20.0 g/kg PESR resulted in lower MDA values in the serum, liver, spleen, and breast and thigh fillets when compared to the 1.0 g/kg PESR and control groups ($p < 0.05$). Plant-derived antioxidants, such as ascorbic acid and α-tocopherol, have been shown to reduce MDA production in chickens' pectoralis muscles and reduce drip loss because the improved oxidative status protects against a stress-induced increase in lipid oxidation.[31] Moreover, the authors also indicated that the improved WHC and pH values in the post-mortem muscle were attributed to the increased oxidative status of the chickens. Supplementation with natural anti-

Table 5. Effects of Dietary *P. eryngii* Stalk Residue Supplementation on Proximate Composition of Meats in Broilers after 35 d[a]

	treatment[c]						statistical significance	
	control	0.1% PESR	0.5% PESR	1.0% PESR	2.0% PESR	SEM[b]	*p*-value	control vs PESR
				Breast				
% moisture	71.3	71.9	70.0	70.8	70.6	1.02	NS[d]	NS
% crude protein	23.9	24.0	24.6	24.7	23.8	0.69	NS	NS
% crude fat	1.72 a	1.63 ab	1.52 b	1.48 b	1.46 b	0.02	[e]	[f]
				Thigh				
% moisture	74.5	73.8	74.2	74.6	73.8	1.55	NS	NS
% crude protein	20.4	20.2	20.4	20.7	20.5	0.66	NS	NS
% crude fat	3.66 a	3.21 b	3.02 c	2.98 c	2.95 c	0.04	[e]	[f]

[a]Results are provided as the means of eight samples corresponding to eight birds for the control group (corn−soybean meal diet) and 1.0, 5.0, 10.0, and 20.0 g/kg dried *P. eryngii* stalk residue (PESR) supplemented groups. [b]SEM = standard error of the mean. [c]Means within the same rows without the same superscript are significantly different ($p < 0.05$). [d]NS = $p > 0.05$. [e]$p < 0.01$. [f]$p < 0.001$.

oxidants originating from tea catechins (100−300 mg/kg) and rosemary−sage extracts (500 mg/kg) was shown to decrease lipid oxidation in broiler muscles.[35,36] In the present study, the MDA concentration was slightly reduced in the serum, liver, spleen, and fillet tissues of the PESR-supplemented birds when compared to the control birds. Following the addition of PESR powder to the birds' diets, the MDA values displayed similar patterns to those of the antioxidant enzymes. In the current study, following the addition of 5.0−20.0 g/kg dietary PESR to the broiler diet, the enzymatic activities of CAT and SOD were improved, and the production of MDA was decreased. This result suggests that dietary PESR supplementation in birds may decrease lipid peroxidation and enhance oxidative status in broilers.

Proximate Composition and Ammonia N Concentration of Gastrointestinal Contents. The proximate composition of the broiler breast and thigh muscles of the birds that received PESR supplementation for 35 d is shown in Table 5. No significant differences in the moisture and CP contents were observed among the treatments. The crude fat content of the breast and thigh muscles was significantly decreased ($p < 0.05$) in all of the dietary PESR-supplemented groups when compared with the control group. The dietary antioxidant supplementation with garlic powder and α-tocopherol resulted in a lower crude fat content in the chicken thigh muscle, suggesting that the activities of hydroxymethylglutaryl-co-enzyme A reductase, cholesterol 7α-hydroxylase, and fatty acid synthetase were reduced.[4] These results also suggest that this supplementation scheme affected fatty acid synthesis, causing decreased fat accumulation in the tissue.[37] Additionally, high levels of fibrous material, such as garlic husks, can mediate increased bile acid excretion and decreased serum lipid levels.[38] The present study may reflect similar results; some components/active ingredients may inhibit enzymatic activities and reduce the fibrous quality of PESR and fat accumulation in broiler meats. The ammonia N concentrations in the jejunum−ileum and ceca of the 21- and 35-d-old broilers were not significantly different among the treatments (data not shown). A high ammonia N concentration may increase the incidence of diarrhea and negatively affect the growth and differentiation of intestinal epithelial cells.[39] The dried PESR that was supplemented in the broilers' diets in the current study did not increase the ammonia N concentration of the gastrointestinal contents when compared with that of the control group.

To our knowledge, few previous studies have tested the protective role of stalk residue from mushroom-enriched diets against oxidative status and meat quality in birds. Future studies are required to determine whether PESR can be used as an alternative to growth promoters in healthy and/or challenged broilers and to elucidate the mechanisms for the potentially enhanced performances and oxidative statuses in broilers.

In conclusion, the results reported herein imply that PESR, a byproduct of fruiting bodies, contains a variety of secondary metabolites that can decrease lipid peroxidation in broilers. Moreover, the inclusion of 5.0 g of dietary PESR per kg of diet was more effective (based on the higher body weight gain and feed efficiency) than the control or other PESR-supplemented groups. Therefore, these results demonstrate that PESR has promising antioxidant potential to enhance oxidative status and meat quality in male broiler chickens.

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Notes

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ABBREVIATIONS USED

PESR, *P. eryngii* stalk residue; BW, body weight; TEAC, trolox equivalent antioxidant capacity; CAS, catalase; SOD, superoxide dismutase; MDA, malondialdehyde; DW, dry weight; GAE, gallic acid equivalent; GSH-Px, glutathione peroxydase.

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