Antimicrobial Activity in Vitro and Growth Performance of Broilers Supplemented with Dried Pleurotus Eryngii Stalk Residue

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***Abstract*—A study was conducted to evaluate the effects of *Pleurotus eryngii* stalk residue (PESR) on antimicrobial activity *in vitro* and growth performance assessment of broiler chickens. The result showed that PESR in *in vitro* antimicrobial activity assay by pepsin-digested showed approximately 100 mg extracts of PESR performed a significant antimicrobial effect to pathogenic bacterium *Sal. typhimurium* E29 and *E. coli* O157:H7 strains. All tested concentration were no effect to the beneficial bacterial *Lactobacillus reuteri* Pg4 strain. Five hundred 1-day-old broilers were evenly divided by gender and randomly allocated into 5 groups, including the control group (corn-soybean meal diet), 0.5, 1.0 and 2.0% dried PESR groups. The results indicated no significant effects among treatments in the weight gain of grower and finisher periods. In finisher, the feed efficiency of broilers in dried PESR supplemented groups significantly improved than the control group. One percentage of PESR group was significantly increased over the control group in the entire period. It appears that PESR had antimicrobial capability in *in vitro*, and it could be used as a feed additive to improve the feed efficiency of broilers.**

***Index Terms*—broiler, *pleurotus eryngii*, growth performance, antimicrobial activity.**

I. Introduction

Today's poultry producers are confronted with numerous challenges to prevent diseases and maintain health without use of sub-therapeutic antibiotics. As animal welfare and food/feed safety concerns continue to increase, researchers will continue to seek better alternatives to current methods of molting laying hens [1]. In recent years, there has been increasing interest in the use of natural antioxidants such as edible mushrooms, which are considered good sources of functional ingredients because these natural antioxidants aid in avoiding undesirable health problems that may arise from use of synthetic antioxidants such as butylated hydroxytoluene (BHT) or butylated hydroxyanisole (BHA) [2], [3].

Edible mushrooms have been used to increase longevity and maintain health for countries. They have some advantages such as poor in fat and in calories but rich in vitamins B, D as well as sometimes vitamins A and C [4]. Moreover, there are over 160 species of the mushrooms with proven medicinal value, particularly immune enhancing and/or boosting properties. Otherwise, edible mushrooms could improve the growth performance as well as without residue and resistance of drug [5], [6]. Most of the mushrooms are cultivated by a 3D-architecture in Asia. When harvested, the by-products of mushrooms of stalk residue could be as a greenly organic feeds. Although some references were mentioned in health of human [4], it still less actually apply in animal feed. *Pleurotus eryngii*, commonly called the king oyster mushroom due to its nutritional value and remarkable flavor, has rapidly become a highly valued species among consumers in Asia, North America, and Europe in the last few decades [7]. In this study, the by-products of edible mushrooms, *Pleurotus eryngiig* stalk residue (PESR), is to determine *in vitro* antimicrobial activity and growth performance assessment of broiler chickens.

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II. Materials and Methods

A. Material and Extraction Procedures

Pleurotus eryngiig stalk residue used in the present study was provided by Q-YO BIO-TECHNOLOGY FARM in Taiwan. PESR were dried out by 65℃ for 3 days and ground to fine powder (ca. 1mm size). Extract with distilled water (1:10, w/v) under 95℃ for 3 h after filtering (Advantec NO.1, Japan). The filtrate evaporated to dryness under vacuum. The further lyophilized extracts were added aqueous and adjusted to 1 mg/ml for following analyses. 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. [8]. The HPLC (Hitachi, Japan) consists of a pump (L-7100), a column oven (655A-52), a UV-VIS detector (L-4200) (330 nm) and Mightysil RP-18 GP 5 m 150 x 4.6mm (Kanto, Tokyo, Japan) was used to determine the contents of ergosterol and adenosine. Total phenolic contents were determined using a Folin-Ciocalteu reagent according to the method [9]. The results of the chemical analysis are shown in Table I. Proximate composition of PESR was analyzed according to the AOAC [10].

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B. Antimicrobial Activity Assay

For determination of inhibitory activity, strains of *Salmonella typhimurium* E29, *E. coli* DH5α and *Lactobacillus reuteri* Pg4 [11] were cultured for 20 h with the XLD (Xylose Lysine Desoxycholate agar, Difco 27885), Coliform (Merck 110426, Darmstadt, Germany) and MRS (de Man Rogosa and Sharpe agar, Difco 288130) medium, respectively. A standard inoculum of logarithmic phase cells was added to each tube at a final concentration approximately of 10^8 or 10^9 cfu/ml (*Salmonella* 10^9 cfu/ml, *E. coli* and *Lactobacillus* 10^8 cfu/ml). Each treatment of lyophilized powders was then dissolved in 500 µl PBS, cultured on plates, and colony-forming units were counted to determine bacterial growth inhibition effects.

C. Broilers and Experimental Design

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. Four hundreds 1-day-old *Arbor Acre* broilers were randomly allotted to 4 treatments. For each treatment, 4 replicates were conducted, and 25 broilers were assigned to each experimental pen. Broilers in treatment 1 were fed a diet based on corn-soybean and fishmeal (the control diet), and in treatments groups were added 3 dietary concentrations of dried *Pleurotus eryngii* stalk residue (0.5%, 1.0% and 2.0%), respectively. For the entire experimental period (5 weeks), all of the diets were formulated to meet the requirements suggested by the NRC [12]. The study was divided into the grower (0- 21 days) and finisher period (22-35 days), and diets in mash form and the water were provided *ad libitum*. On days 21 and 35, the performance of the chicken broilers was assessed by measuring the feed intake and body weight (BW) of the birds, and the BW gain and feed conversion ratio (F/G) were recorded.

D. Statistical Analysis

The data were subjected to standard ANOVA procedures for completely randomized designs. The GLM procedure of the SAS software package [13] was employed, and a single degree of freedom was used to separate treatments. Linear and quadratic effects were analyzed by orthogonal comparison. Significant differences among treatments were determined by conducting a Duncan's multiple range test. A threshold value of $P < 0.05$ was established to denote statistical significance.

III. Results and Discussion

A. Antimicrobial Activity Assay

In vitro antimicrobial activity assay by pepsin-digested showed approximately 100 mg extracts of PESR performed a significant antimicrobial effect to pathogenic bacterium *Sal. typhimurium* E29, *E. coli* O157:H7 and an laboratory *E. coli* DH5α strain (Fig. 1). However, lower concentration (20 mg) showed that the growth was inhibited by *Sal. typhimurium* E29 and *E. coli* DH5α strains. All tested concentration were no negative effect to the beneficial bacterial for *Lactobacillus reuteri* Pg4 strain. The dried PESR had abundant active ingredients/functional components, including the crude soluble polysaccharide (165.0 mg/g DW), adenosine (58.9 µg/g DW), ergosterol (1.28 mg/g DW) and total phenolic compounds (5.0 mg gallic acid equivalent (GAE)/g DW), respectively (Table I).

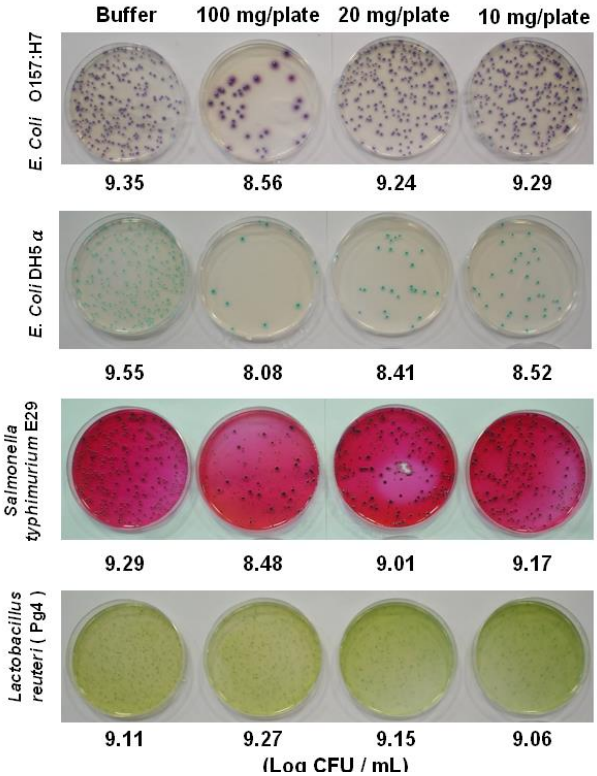

Figure 1. I*n vitro* antimicrobial activity capacity of *Pleurotus eryngii* stalk residue

B. Growth Performance

Analysis of the chemical composition of *Pleurotus eryngii* residue was showed in Table II. The dry matter and crude protein were 93.1% 8.6% in *Pleurotus eryngii* residue. The ingredients and chemical composition of the experimental diets were presented in Table III and were formulated to meet the requirements by the NRC (1994). The growth performances of the broilers reflected body weight gains of 1702, 1724, 1721 and 1752 g/bird in the control, 0.5, 1.0 and 2.0% PESR groups (Table IV), respectively. At 0-35 d of age, the 0.5% 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 0.5% PESR and control groups gained 2723 and 2752 g/bird from 0-35 d of age, respectively. The feed efficiency of the broilers in the 0.5% 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 (1.56 vs. 1.62). Previous studies have shown that the inclusion of *Agaricus bisporus* mushrooms in the diet conferred growth promoting activity and delayed lipid oxidation in poultry diet. Moreover, additional studies

have demonstrated that the combined use of Chinese herbal and mushroom extracts can act as an alternative to antibiotic growth promoters [14]-[16]. In conclusion, it appears that PESR had antimicrobial capability in *in vitro*, and it could be used as a feed additive to improve the feed efficiency of broilers.

TABLE I. ANALYSIS OF THE ACTIVE INGREDIENTS OF *PLEUROTUS ERYNGII* RESIDUE1

Ingredients	Total phenolics (mg GAE/g DW)	Crude polysaccharide (mg/g DW)	Adenosine (μg/g DW)	Ergosterol (mg/g dry weight)
Pleurotus eryngii residue	5.00 ±0.33	165.0 ±10.1	58.9 ±3.3	1.28 ±0.30

[1] The value is expressed as mean ± standard deviation (n=5).
[2] DW= Dry weight.
[3] GAE = Gallic Acid Equivalent.

TABLE II. ANALYSIS OF THE CHEMICAL COMPOSITION OF *PLEUROTUS ERYNGII* RESIDUE1

Item	*Pleurotus eryngii* residue (%)
Dry matter	93.1
Crude protein	8.6
Crude ash	5.6
Neutral detergent fiber	54.0
Acid detergent fiber	40.2
Ether extract	1.79
Ca	0.56
P	0.47

[1] The value is expressed as mean ± standard deviation (n=5).

TABLE III. INGREDIENTS AND CHEMICAL COMPOSITION OF THE EXPERIMENTAL DIETS (G/KG)

Ingredients	Grower diet (0-21d)	Finisher diet (22-35d)
Corn	520/515/510/500	570/565/560/550
Soybean meal (44%)	260.0	210.0
Full-fat soybean meal (65%)	100.0	100.0
Calcium carbonate	10.0	8.0
Soybean oil	40.0	45.0
Fish meal	50.0	50.0
Dicalcium phosphate 22%	13.0	11.0
PER powder[1]	0/5/10/20	0/5/10/20
L-Lysine	0.6	0.0
DL-Methionine	2.0	2.0
Salt	2.8	2.5
Vitamin premix[2]	0.6	0.5
Mineral premix[3]	1.0	1.0
Total	1000.0	1000.0
Calculated value		
Crude protein, %	22.0	20.0
Crude fat, %	8.5	9.0
ME, kcal/kg	3100.0	3200.0
Lysine, %	1.30	1.10
TSAA, %	0.89	0.84
Threonine, %	0.84	0.76
Calcium, %	0.95	0.80
Total phosphate, %	0.74	0.84
Available phosphate, %	0.45	0.40

[1] PER: *Pleurotus eryngii* residue.
[2] Supplied per Kg of diet: Vit. A (retinyl acetate) 3.75 mg; Vit. E (DL-α-tocopheryl acetate) 35 mg; Vit. K (menadione) 4 mg; Riboflavin 8 mg; Pyridoxine 5 mg; Vit B_{12} (cyanocobalamin) 25 mcg; Ca-pantothenate 19 mg; Niacin 50 mg; Folic acid 1.5 mg; Biotin 60 mcg.
[3] Supplied per Kg of diet: Co ($CoCO_3$) 0.255 mg; Cu ($CuSO_4 \bullet 5H_2O$) 10.8 mg; Fe ($FeSO_4 \bullet H_2O$) 90 mg; Zn (ZnO) 68.4 mg; Mn ($MnSO_4 \bullet H_2O$) 90 mg; Se (Na_2SeO_3) 0.18 mg.

TABLE IV. EFFECT OF DIFFERENT LEVELS OF *PLEUROTUS ERYNGII* STALK ON GROWTH PERFORMANCES OF BROILER CHICKS

Item	Treatments[1]				SEM[2]
	Control	0.5%PER	1.0%PER	2.0%PER	
0~21 d					
Body average weight, g/bird	835.0	856.3	838.1	855.2	14.8
Body weight gain, g	788.4	810.4	792.3	808.9	14.6
Feed intake[3], g/bird	1122.8	1119.1	1103.4	1142.0	21.0
Feed/gain	1.43	1.42	1.39	1.41	0.02
21~35 d					
Body average weight, g/bird	1748.8	1770.7	1767.0	1798.3	17.6
Bodyweight gain, g/bird	913.8	914.4	928.9	943.1	17.21
Feed intake, g/bird	1630.1	1629.9	1579.3	1626.1	27.9
Feed/gain	1.77	1.71	1.72	1.72	0.02
0~35 d					
Body average weight, g/bird	1748.8	1770.7	1767.0	1798.3	17.6
Body weight gain,g/bird	1702.2	1724.8	1721.2	1752.0	17.5
Feed intake, g/bird	2752.8	2723.3	2682.8	2768.1	35.6
Feed/gain/bird	1.62	1.58	1.56	1.58	0.04

[1] Results are given as means of 4 samples corresponding to 100 birds for the control group (corn-soybean meal diet) and 0.5, 1.0, and 2.0% dried *Pleurotus eryngii* stalk (PER) supplement groups.
[2] SEM= standard error of the mean.
[3] Results are given as means of 4 pens for 25 chicks each pen.
[4] NS= $P > 0.05$.
[a,b] Means within same rows in same time without same superscript are significantly different ($P<0.05$).

IV. CONCLUSION

PESR had antimicrobial capability in *in vitro*, and it could be used as a feed additive to improve the feed efficiency of broilers.

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