

# Effect of Supplementing Ground Leaf of Misai (*Orthosiphon stamineus*) in Diet on Growth Performance of Broiler Chickens

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## ABSTRAK

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Penggunaan herbal untuk pakan ternak merupakan salah satu cara dalam mengatasi kerugian penggunaan bahan kimia buatan yang berlebihan. Penelitian ini mengevaluasi respon ayam pedaging yang diberi pakan tambahan serbuk daun misai kucing (*Orthosiphon stamineus*). Dalam penelitian ini digunakan 160 ekor ayam broiler jantan umur satu hari yang diberi air *ad libitum* dan pakan sampai 20 hari. Perlakuan diberikan pada broiler jantan umur 21 hari. Data dikumpulkan dan dievaluasi setelah dilakukan terminasi ayam broiler jantan umur 42 hari. Terlihat bahwa suplementasi serbuk daun *O. stamineus* dengan konsentrasi 8 g/kg pada pakan ayam pedaging menghasilkan performa pertumbuhan yang sebanding dengan suplementasi tetrasiklin dan vitamin E. Di samping itu pakan mengandung 8 g/kg *O. stamineus* memiliki efek penurunan enzim darah. Pada ayam pedaging yang menerima suplementasi tetrasiklin, bagaimanapun, terlihat adanya aktivitas enzim serum yang signifikan. Hasil penelitian juga menunjukkan bahwa konsentrasi 8 g/kg serbuk daun *O. stamineus* dalam pakan setara dengan 200 mg/kg suplementasi vitamin E. Oleh karena itu, serbuk daun *O. stamineus* dapat mendorong produksi ayam broiler organik, yang aman, sebagai suplemen pakan yang berkelanjutan.

**Kata Kunci:** Ayam Pedaging, Pakan Tambahan, Serbuk Daun, *Orthosiphon stamineus*

## ABSTRACT

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The use of herbs in animal nutrition is one of the important approaches in overcoming the disadvantages of excessive use of artificial chemicals in animal nutrition. The present study was done to evaluate response of broilers feeding on a diet supplemented with the ground leaf of misai (*Orthosiphon stamineus*). The birds in this, study were 160 one-day-old male broiler chickens, given *ad libitum* water and feed for up to 20 days. Treatments were given to 21-day-old male broiler chickens. Data was collected and evaluated after slaughtering 42-day- male broiler chickens. It was shown that supplementing broiler diets with *O. stamineus* powdered leaf at a rate of 8 g/kg resulted in growth performance comparable to tetracycline and Vitamin E supplementation. It was also shown that supplementing the diet with 8 g/kg *O. stamineus* had a blood enzyme-lowering effect. In broilers receiving tetracycline supplementation, however, significant serum enzyme activity was observed. Results also showed that 8 g/kg of ground *O. stamineus* leaf in the diet was equivalent to 200 mg/kg Vitamin E supplementation. Therefore, *O. stamineus* leaf powder can promote organic, safe, and sustainable broiler chicken production, and as diet supplement.

**Key Words:** Broiler, Diet Supplementation, Ground Leaf, *Orthosiphon stamineus*

## INTRODUCTION

The use of antibiotics in raising broilers has been limited by the European Union's withdrawal of glycopeptides avoparcin and bacitracin, the macrolides spiramycin and tylosin streptogramin virginiamycin as prophylactic antibiotics and growth promoters between 1995 and 1999 (Muaz et al. 2018). Medicinal herbs have the potential to alternate the use of synthetic antibiotics and antioxidative growth promoters in broiler production. Medicinal herbs are rich in phenolic

compounds, flavonoids and aromatic compounds that exhibit natural antibiotic and antioxidative potential to improve the growth performance of livestock by enhancing the immune status of the animals (Kırkpınar et al. 2011; Kiczorowska et al. 2016; Wang et al. 2019; Bai et al. 2019). In this regard, one promising medicinal plant is *Orthosiphon stamineus*, which exhibits a wide range of biological properties, including antibacterial and antioxidant traits (Malahubban et al. 2013a).

The use of appropriate additives in the diet to prevent lipid oxidation provides major benefits to

animals and consumers. Lipid oxidation, for example, can produce pathological alterations in the mucosal membrane of the gastrointestinal tract, impede enzyme action, and raise cholesterol and peroxide levels in the serum, all of which can contribute to atherosclerosis. Furthermore, lipid oxidation can result in the formation of malondialdehyde, a powerful mutagen and carcinogen (Vandemoortele et al. 2021). According to Khajali & Wideman (2016), broiler chickens are sensitive to lipid peroxidation and the formation of reactive oxygen species due to an excessive accumulation of fat in their bodies, primarily polyunsaturated fatty acids reactive oxygen species (ROS). Then, in broiler chickens, ROS production was linked to the development of pulmonary hypertension syndrome (PHS).

Furthermore, pathogenic bacteria such as *E. coli*, *Salmonella* sp., *Clostridium* sp., and *Campylobacter* sp. can cause disease in chickens. These pathogenic bacteria compete for nutrition with the host microorganisms in the small intestine. Due to the deconjugating effects of bile acids, colonisation may also impair fat and fat-soluble vitamin digestion. Rinttilä & Apajalahti (2013) found that competition lowered growth performance and increased illness incidence.

In the present study, *O. stamineus* ground leaf was added as a supplement to the diet of broiler chickens evaluated for its antibacterial and antioxidant potential, with tetracycline and Vitamin E serving as the respective positive controls. The benefits of the diet additive were assessed in terms of growth performance, carcass characteristics, serum biochemistry and lipid peroxidation in serum and liver of broiler chickens. Moreover, the effect of *O. stamineus* ground leaf supplementation in the diet was investigated on the population growth of *Escherichia coli* and *Lactobacillus* spp of the intestine in broilers.

## MATERIALS AND METHODS

### Preparation of *Orthosiphon stamineus* used as animal feed additive

Fresh *Orthosiphon stamineus* (OS) samples were collected from the Herbal Farm at Universiti Putra Malaysia. Gene Bank Centre, Faculty of Agriculture, Universiti Putra Malaysia, verified the plant sample. Under nursery settings, four-five nodes stem cuttings were obtained from mature OS plants and planted in black polythene bags containing a mixture of soil, sand, and peat moss (2:1:1). Ten weeks after planting, the first crop OS plant was harvested. OS shoots were trimmed to roughly 30 cm from the tip. Fresh OS leaves were harvested and oven-dried for 72 hours at 60°C.

Using a Willey mill (Thomas® Willey cutting mill model 4) and a one mm screen, the dried leaves were pulverised into powder and kept at 4 °C until needed.

### Birds and experimental design

A local hatchery provided 160 one-day-old male broiler chickens (Cobb 500). The broilers were fed a commercial broiler starting meal (0–20 days) and provided unlimited access to water. The broilers were weighed and reassigned to four different feeding regimens at the end of week three in order to attain comparable average weights for each treatment. Each treatment comprised five replications, each containing eight broilers. Broilers were randomly assigned to 20 cages, each measuring 122 cm (length) x 91 cm (width) x 50 cm (height) and equipped with round feeders and round drinkers, with the temperature and humidity set to ambient (28°C) (60 to 89%, respectively).

### Dietary treatments

The basal diet should be made without antimicrobials, anticoccidial medicines, or feed enzymes, according to the National Research Council's suggestion (NRC 1994; Applegate & Angel 2014). The feed was provided ad libitum and refilled at 08:30 and 17:30 every day, with the leftovers gathered in the raising cages. AOAC International techniques were used to analyze dietary and nutrition-related chemical composition (George & Latimer 2019).

The broilers were fed the following diets from the 21<sup>st</sup> to the 42<sup>nd</sup> day of the experiment: 1) Diet C (Control, basal diet); 2) Diet VE (Positive control) (Basal diet+200 mg/kg Vitamin E); 3) Diet T20 (Positive control) (Basal diet+20 mg/kg Tetracycline); and 4) Diet OS8 (Basal diet+8 g/kg OS). The rate of supplementation at 8 g/kg of OS was the best rate in promoting the growth and carcass characteristics of broiler chickens as reported in the earlier work (Malahubban et al. 2013b). The compositions of the above dietary treatments are as shown in Table 1.

Sunzen Corporation Sdn. Bhd. (Malaysia) supplied tetracycline. Vitamin E in the form of d-1- $\alpha$ -tocopherol acetate was provided by Lutavit E 50 (BASF, Germany). Tetracycline is used as a positive control for antibacterial activity, while Vitamin E served as a positive control for its antioxidant activity.

### Parameter measurement

Birds were weighed individually at weekly intervals. Daily observation was conducted for survival and mortality. Total feed intake was recorded per cage

at weekly intervals. Feed intake and feed conversion ratio were modified for mortality.

On day 42, ten broilers from each treatment group were selected at random and individually weighed to the closest gram unit before being slaughtered by severing the carotid artery and jugular veins (Department of Standard Malaysia 2004). Each bird was bathed in hot water for 20 seconds and manually de-feathered for 30 seconds after 5 minutes of bleeding. Manual removal of the feet, skull, and viscera followed.

The dressed bird was opened up, and the liver, kidney, heart, gizzard, proventriculus and intestine were removed and weighed. Carcass samples and liver were

stored immediately in a deep freezer at minus 80°C until required for further analysis. The alimentary tracts of five of the ten slaughtered birds in each treatment group were promptly dissected. The ceca contents and the ileal (about 30 cm long segment of the lower ileum measured from the Meckels diverticulum) were collected and put into plastic 50 mL Falcon tubes. Prior to the evaluation of the intestinal population of bacteria, the collected contents were maintained on ice. The collected ceca contents were processed within 60 to 90 min of collection. All samples were weighed after being diluted 1:10 with normal saline solution. Ten serial dilutions of ceca content were made in normal saline,

**Table 1.** Ingredients in the dietary treatments and nutritional analysis (adopted from earlier work of Masnindah Malahubban et al. 2013b)

Ingredients	Dietary Treatments <sup>1</sup>			
	C	VE	T20	OS8
Corn	61.0	61.0	61.0	60.2
Soy Bean Meal (SBM) (44%)	25.0	25.0	25.0	25.0
Fish Meal	6.41	6.41	6.41	6.41
Palm Oil	5.00	5.00	5.00	5.00
Limestone	1.26	1.26	1.26	1.26
Salt	0.28	0.28	0.28	0.28
Dicalcium Phosphate (DCP)	0.10	0.10	0.10	0.10
Mineral Mix <sup>a</sup>	0.25	0.25	0.25	0.25
Vitamin Mix <sup>b</sup>	0.25	0.25	0.25	0.25
L-Lysine	0.20	0.20	0.20	0.20
DL-Methionine	0.15	0.15	0.15	0.15
Choline chloride	0.10	0.10	0.10	0.10
<i>Orthosiphon stamineus</i>	-	-	-	0.80
Tetracycline	-	-	(20) <sup>c</sup>	-
Vitamin E	-	(200) <sup>d</sup>	-	-
<b>Calculated analysis (%)</b>				
Metabolize Energy (ME) Kcal/kg	3211	3211	3211	3211
Crude Protein, %	20.00	20.00	20.00	20.00
Crude Fibre, %	4.35	4.35	4.35	4.40
Crude Fat, %	3.21	3.21	3.21	3.21
Calcium, %	0.99	0.99	0.99	0.99
Available P, %	0.33	0.33	0.33	0.33

<sup>a</sup>Premix was given per kg of diet: Mg = 56 mg, Fe= 20 mg, C = 10 mg, Zn= 50 mg, C = 125 mg, I= 0.8 mg. <sup>b</sup>Premix was given the following per kg of diet: Vitamin A= 50.000 MIU; Vitamin D<sub>3</sub>= 10.000 MIU; Vitamin E = 75.000 MIU; Vitamin K= 20.000 g; Vitamin B1= 10.000 g; Vitamin B2= 30.000 g; Vitamin B6= 20.000 g; Vitamin B12= 0.100 g; Calcium D-Panthenate= 60.000 g; Nicotinic acid= 200.000 g; Folic acid= 5.000 g; Biotin= 235.000 mg. <sup>c</sup>Tetracycline (20 mg/kg dry matter intake). <sup>d</sup>d- 1-α tocopherol acetate (200 mg/kg dry matter intake). <sup>1</sup>Dietary treatments: C= Control; VE= 200 mg/kg d-1-α tocopherol acetate (positive control); T20= 20 mg/kg Tetracycline (positive control); OS8= 8g/kg *O. stamineus*

and a 100-µL portion of 10<sup>-7</sup> to 10<sup>-9</sup> diluted aliquot plated on fresh agar plates.

The total count of facultative anaerobic bacteria was determined on nutrient Agar (NA) (OXOID, U.K.) after incubation for 24 hour at 37°C (Kırkpınar et al. 2011). The nutrient agar medium could provide nutrients for most cultivable bacteria, including facultative anaerobic bacteria that can live without oxygen and not inhibited when oxygen is available. *Escherichia coli* count was determined from the green metallic surface sheen of colonies following incubation on Eosin–methylene blue (EMB) agar medium (OXOID, U.K.) under the same conditions. *Lactobacillus* count was determined from white colonies appearing on Rogosa Agar (RA)(OXOID, U.K.) after plates incubated in an anaerobic jar at 37°C for 48-72 hour. Results were expressed as log<sub>10</sub> of colony-forming units (CFU) per gram of ileal digesta. Blood samples (4.0 mL) from birds were collected from the wing vein using sterile gauge 23 needles and syringe. Blood samples were taken in a standard vacutainer and centrifuged at 3000 g for 10 minutes to separate them. For the analysis of serum glucose, cholesterol, triglycerides, albumin, total protein, sodium (Na), potassium (K), chlorine (Cl), urea, aspartate transaminase (AST), alkaline transaminase (ALT), and alkaline phosphatase, serum samples were kept at minus 20°C (ALP). Using an auto-analyzer, to analyse certain commercial kits (Roche Diagnostica, Basal, Switzerland) (HITACHI 902, Automatic Auto-analyser). By subtracting serum albumin from serum total protein levels, serum globulin was determined. Lipid oxidation was assessed based on malondialdehyde (MDA) for blood serum and liver.

Malondialdehyde, the end-product of lipid peroxidation by reactive oxygen species, was evaluated using the TBARS assay kit (Oxiselect™, CellBiolabs, U.S.A.).

### Statistical Analysis

SPSS software was used to conduct statistical data analysis (IBM SPSS version 21). Differences between means for all parameters were determined using a one-way analysis of variance (ANOVA). Multiple comparisons of means conducted using Duncan’s test to show differences among treatments. Differences were considered significant at the 0.05 level.

## RESULTS AND DISCUSSION

### Result

#### Broiler Weight Performance

Table 2 shows the weight gain and live weight of broilers fed various diets. No improvement in live weight of broilers fed various experimental diets over the control basal diet was observed from day-21 to day-28. Significant live weight variation was observed only from day-35 to day-42 (final observation day). On day-35, even though broilers fed basal diet treated with OS08 had no significant difference with control diet (C) (P>0.05), the effect of OS08 treatment on live weight of broiler was similar to VE treatment and nearly identical with T20 treatment.

**Table 2.** Live weight and weight gain of broilers fed different diets for three weeks

Performance	Dietary Treatments			
	C	VE	T20	OS8
Live Weight (g)				
Initial (day 21)	767.3±15.3	784.4 ± 13.6	763.3 ± 22.4	756.6 ± 22.0
Week 4 (day 28)	1217.3 ±22.7	1256.5 ±38.6	1269.8 ±25.3	1230.1 ± 20.2
Week 5 (day 35)	1618.2 ±40.3 <sup>b</sup>	1714.9 ±33.9 <sup>ab</sup>	1776.2 ±35.6 <sup>a</sup>	1717.7 ±38.9 <sup>ab</sup>
Final (day 42)	2164.2 ±35.5 <sup>b</sup>	2305.8 ±55.2 <sup>a</sup>	2379.5 ±41.5 <sup>a</sup>	2296.8 ±47.5 <sup>a</sup>
Cumulative Weight gain (g)	1384.2 ±42.4 <sup>b</sup>	1477.1 ±61.4 <sup>ab</sup>	1569.7 ±57.5 <sup>a</sup>	1491.1 ±57.7 <sup>ab</sup>
Week 4	450.0 ±23.9	472.1 ±38.1	506.5 ±37.9	473.6 ±28.1
Week 5	400.9 ±32.1	454.4 ±39.0	507.1 ±39.2	489.0 ±34.4
Week 6	547.4 ±49.5	591.6 ±43.8	598.8 ±53.3	584.6 ±57.8

Treatment means ± standard errors are presented. Treatment values with the same superscript letters are not significantly different at P>0.05. C= Control; VE = 200 mg/kg d-1-α tocopherol acetate (positive control); T20= 20 mg/kg Tetracycline (positive control); OS8= 8g/kg *O. stamineus*

**Table 3.** Feed intake, FCR, and mortality of three-week-old broilers fed various diets

Performance	Dietary Treatments			
	C	VE	T20	OS8
Cumulative Feed intake (g)	3117.4±181.4	2998.2±67.7	3264.0±150.5	3112.0±134.5
IWeek 4	840.4±35.3	884.4±49.4	878.0±79.1	882.0±46.6
Week 5	894.6±56.1	907.4±41.9	1030.0±69.1	968.0±84.6
Week 6	1382.4±160.3	1206.4±71.4	1356.0±87.7	1262.0±95.4
Cumulative FCR (g)	2.21±0.12	1.97±0.04	2.01±0.09	1.99±0.08
Week 4	1.87±0.07	1.87±0.10	1.73±0.16	1.86±0.09
Week 5	2.23±0.14	2.00±0.09	2.03±0.14	1.98±0.17
Week 6	2.53±0.29	2.04±0.12	2.26±0.15	2.15±0.16
Mortality (%)	1.88	1.25	1.25	1.25

Treatment means ± standard errors are presented. No significant differences between treatments were found ( $P>0.05$ ). No significant difference on mortality rate ( $X^2 = 0.949$ ,  $P<0.05$ ). C= Control; VE = 200 mg/kg d-1- $\alpha$  tocopherol acetate (positive control); T20= 20 mg/kg Tetracycline (positive control); OS8= 8g/kg *O. stamineus*

On day-42, birds in OS8 diets showed a significant ( $P<0.05$ ) increase in the live weight (2296.8±47.5 g) as compared to control basal diet (2164.2±35.5 g), and similar to positive control diets, T20 at 2379.5±41.5 g and VE at 2305.8±55.2 g.

Cumulative weight gain of broilers fed OS8 diet had no significant difference with control diet ( $P>0.05$ ). However, broilers on the OS8 diet had similar weight gain with broilers on VE diet at 1491.1±57.5 g and 1477.1±61.4 g. Moreover, broilers fed on the OS8 diet challenge cumulative weight gain of broilers on the T20 diet at 1569.7±57.5 g.

#### **Feed intake, feed conversion ratio and mortality**

No significant differences in feed intake and feed conversion ratio observed in broilers fed different diets in the present study, as shown in Table 3.

Mortality rate was also low, ranging from 1.25 to 1.88% and no significant difference was detected among treatments ( $X^2 = 0.949$ ,  $P<0.05$ ). On average, broilers fed OS8 recorded slightly lower cumulative feed intake than the control diet at 3112.0±134.5 g and 3117.4±181.4 g, respectively. However, broilers fed on T20 recorded higher cumulative feed intake than broilers fed OS8 and lower cumulative feed intake compared to broilers on the VE diet.

#### **Carcass and Organ Characteristics**

Table 4 shows the percentage of carcass yield and relative organ weight of broilers fed different dietary treatments for 42 days.

No significant differences ( $P>0.05$ ) found on relative percentage weight of carcass, kidney, heart, gizzard, proventriculus and small intestine of broilers fed in all different dietary treatments. However, significant differences found in the abdominal fat and liver weight of treated broilers. Broilers fed diet OS8 showed significantly reduced abdominal fat ( $P<0.05$ ) at 1.34±0.10% as compared to the control (1.96±0.22%) and diet T20 (2.51±0.26%).

Broilers fed diet T20 increased their abdominal fat significantly ( $P<0.05$ ) at 2.51±0.26% over control basal diet at 1.96±0.22%. The present study also showed that broilers fed diet OS8 increased their relative liver weight (2.92±0.18%) as compared to the control (2.34±0.09%), diet T20 (2.26±0.09%), and diet VE (2.25±0.11%).

#### **Blood Characteristics**

Serum biochemical parameters and experimental outcomes presented in Table 5. In general, serum cholesterol, glucose, triglycerides, potassium (K), aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) were found to vary significantly between the experimental treatments ( $P<0.05$ ). No significant differences found on serum total protein, albumin, globulin, sodium (Na), chlorine (Cl), and urea. In the present study, significantly lower cholesterol recorded in broilers fed diet OS8 (2.90±0.19 mmol/L) as compared to those fed the control diet (3.49±0.22 mmol/L), diet T20 (3.50±0.17 mmol/L), and diet VE (3.52±0.09 mmol/L).

Result of the present study also indicated that diet OS8 lowered serum glucose significantly at 4.18±0.36

**Table 4.** Effect of experimental diets on carcass yield and relative organ/tissue weights of broilers on day-42

Relative weight (%)	Dietary Treatments <sup>1</sup>			
	C	VE	T20	OS8
Carcass	70.89±2.07	72.41±1.82	73.51±1.65	72.15±1.39
Abdominal Fat	1.96 ±0.22 <sup>b</sup>	1.51±0.11 <sup>bc</sup>	2.51±0.26 <sup>a</sup>	1.34 ±0.10 <sup>c</sup>
Kidney	0.20±0.02	0.21±0.02	0.21±0.01	0.23±0.01
Liver	2.34±0.09 <sup>b</sup>	2.25±0.11 <sup>b</sup>	2.26±0.09 <sup>b</sup>	2.92±0.18 <sup>a</sup>
Heart	0.60±0.02	0.59±0.02	0.57±0.03	0.64±0.01
Gizzard	3.09±0.17	2.86±0.07	2.89±0.22	3.14±0.14
Proventriculus	0.96±0.05	0.88±0.04	0.92±0.03	0.87±0.04
Small intestine	3.99±0.22	4.46±0.21	4.37±0.23	4.40±0.30

Treatment means ± standard errors are presented. Treatment values with the same superscript letters are not significantly different at P>0.05.  
<sup>1</sup>Dietary treatments: C = Control; VE = 200 mg/kg d-1-α tocopherol acetate (positive control); T20 = 20 mg/kg Tetracycline (positive control); OS8 = 8g/kg *O. stamineus*.

**Table 5.** Serum biochemical parameters and experimental outcomes of broilers on day-42

Serum Parameters	Dietary Treatments <sup>1</sup>			
	C	VE	T20	OS8
Cholesterol (mmol/L)	3.49±0.22 <sup>a</sup>	3.52±0.09 <sup>a</sup>	3.50±0.17 <sup>a</sup>	2.90±0.19 <sup>b</sup>
Glucose (mmol/L)	8.18± 0.24 <sup>a</sup>	6.94± 0.51 <sup>a</sup>	4.81± 0.60 <sup>b</sup>	4.18± 0.36 <sup>b</sup>
Triglycerides (mmol/L)	1.03± 0.19 <sup>a</sup>	0.60± 0.02 <sup>b</sup>	0.98± 0.07 <sup>a</sup>	0.76± 0.04 <sup>ab</sup>
Total protein (g/L)	28.78±0.88	29.48±1.79	31.46±1.82	29.12±1.75
Albumin (g/L)	18.73±1.05	21.82±1.77	19.85±2.14	19.31±1.30
Globulin(g/L)	10.05±1.25	7.66±1.54	11.61±1.53	9.81±1.37
Na (mmol/L)	114.20± 6.90	127.30± 8.40	126.00± 8.90	110.90± 6.60
K (mmol/L)	15.20± 1.30 <sup>b</sup>	22.90± 1.40 <sup>a</sup>	17.00± 2.20 <sup>b</sup>	11.20± 1.10 <sup>b</sup>
Cl (mmol/L)	79.80± 2.40	75.60± 4.40	74.60± 3.70	69.30± 3.00
Urea (mmol/L)	0.33± 0.04	0.24± 0.02	0.24± 0.03	0.28± 0.04
AST (U/L)	254.13± 8.43 <sup>b</sup>	248.49± 8.52 <sup>b</sup>	287.98± 6.71 <sup>a</sup>	213.16± 3.23 <sup>c</sup>
ALT (U/L)	5.52±1.01 <sup>a</sup>	3.97±0.96 <sup>ab</sup>	4.25±0.50 <sup>ab</sup>	2.13±0.12 <sup>b</sup>
ALP (U/L)	990.90± 252.68 <sup>b</sup>	1081.70±225.97 <sup>b</sup>	3682.20±38.84 <sup>a</sup>	874.30±187.28 <sup>b</sup>

Treatment means ± standard errors are presented. Treatment values with the same superscript letters are not significantly different at P>0.05.  
<sup>1</sup>Dietary treatments: C = Control; VE = 200 mg/kg d-1-α tocopherol acetate (positive control); T20 = 20 mg/kg Tetracycline (positive control); OS8 = 8g/kg *O. stamineus*.

mmol/L than the control, and diet VE at 8.18±0.24 mmol/L 6.94±0.51 mmol/L, respectively. The results indicated that diet OS8 was similar to diet T20 4.81±0.60 mmol/L of serum glucose. Broilers fed had significantly lower ALP at 874.30±187.28 U/L than birds fed on diet T20 at 3682.20±38.84 U/L. diet OS8 had equivalent serum triglycerides levels (0.76±0.04 mmol/L) with birds on diet VE (0.60±0.02 mmol/L). However, birds fed on diet VE had significantly lower than control, and T20 diets. Broilers fed diet OS8

showed lowered serum potassium significantly at 11.20±1.10 mmol/L, similar to broilers on control and T20 diets, compared to broilers on diet VE at 22.90±1.40 mmol/L.

Broilers fed diet OS8 had significantly lowered AST levels at 213.16±3.23 U/L as compared to those on diet C (254.13±8.43 U/L), diet VE (248.49±8.52 U/L), and diet T20 (287.98±6.71 U/L). As in serum AST enzyme activity, broilers fed diet OS8 showed significantly lower ALT at 2.13±0.12 U/L than the control at

5.52±1.01 U/L. All in all, broilers fed diet OS8 exhibited a serum enzyme-lowering effect. From the present study also found that birds provided diet OS8 had significantly lower ALP at 874.30 ± 187.28 U/L than birds fed on diet T20 at 3682.20 ± 38.84 U/L.

**Lipid peroxidation in serum and liver**

Occurrence of lipid peroxidation in serum and liver broilers fed different dietary treatments after 42 days presented in Table 6. Broilers fed diet OS8 (0.20±0.033 nmol/mL) showed significant lower serum lipid peroxidation rates as compared to broilers on the control basal diet (0.31±0.043 nmol/mL), and had significantly similar with broilers on diet VE (0.15±0.027 nmol/mL) and diet T20 (0.29±0.032 nmol/mL). In the liver, lipid peroxidation was also lowered significantly in broilers fed diet OS8 (1.64±0.09 nmol/g) as compared to those on the control (2.55±0.15 nmol/g), and diet T20 (2.29±0.16 nmol/g) but was similar with broilers fed diet VE (1.47±0.13 nmol/g).

**Intestinal Microbial Population**

The intestinal microbial population of broilers fed different dietary treatments over 42 days shown in Table 7. No significant differences (P>0.05) found in an intestinal population of *Lactobacillus* and *Escherichia coli* in broilers subjected to different dietary treatments in the present study. However, broilers fed diet OS8 had significantly lower (P<0.05) facultative anaerobe populations at 6.19±0.15 log CFU/g as compared to broilers fed the control diet (6.70±0.07 log CFU/g). It was also found that population of facultative anaerobe in broilers provided OS8 was similar to broilers on T20 (6.14±0.17 log CFU/g).

**Discussion**

OS8 supplemented in broiler chicken diet showed an increase in the live weight compared to the control basal diet. The improvement in the live weight of broiler chicken after fed on OS8 could be due to

antioxidant and antimicrobial properties constituted in the *O. stamineus* ground leaf, as Malahubban et al. (2013a) reported. Herbs’ chemical composition regulates the intestinal microflora then stimulating the digestion process and eventually increasing weight gain and feed utilization (Elkatcha et al. 2016). However, the effect of phytochemical composition may not stand alone because it could vary significantly due to variety, location, and climate (Xiao et al. 2012). It may also affect broad aspects of physiology, being positive interaction with the biochemistry of the body of broiler chicken (Mbikay 2012). Therefore, the following data could provide helpful growth-promoting indication and broiler chicken status after fed *O. stamineus* ground leaf supplemented in the diet.

No significant differences in feed intake and feed conversion ratio were observed in broilers fed different diets in the present study (Table 2). They reflected similar results where broilers were fed oregano and garlic essential oils (Kırkpınar et al. 2011). The mortality rate was considerably low in the present study being in the range of 1.25 to 1.88%, comparable to rates experienced in commercially produced broilers where 2 to 7% mortality is common (Idan et al. 2020; Yerpes et al. 2020).

The observed reduction of abdominal fat deposition and the increment of liver weight was consistent with reports from Malahubban et al. (2013b) studies. Similar findings have also been reported in broilers fed green tea extract (Mohammadpour et al. 2021) or turmeric extract (Utami et al. 2020). Other herbal supplements have, nevertheless, been less successful in this respect. For example, Taufik & Maruddin (2019) reported that broilers fed garlic supplement did not alter abdominal fat deposition. The excessive gain in liver weight of broilers fed diet OS8 demonstrated vigorous hepatic use. A similar incidence was found in rats fed rosemary extract with higher hepatic metabolism and better liver mass (Wang et al. 2019).

In terms of serum biochemical parameters, dietary treatments in the present study affected mainly serum cholesterol, glucose, triglycerides, potassium, and the enzymes aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP). Broilers fed the OS supplement confirmed reduced AST and ALT rates, suggesting that a normal liver

**Table 6.** Lipid peroxidation in serum and liver of broilers on day-42

Parameters	Dietary Treatments			
	C	VE	T20	OS8
Serum (nmol/mL)	0.31±0.043 <sup>a</sup>	0.15±0.027 <sup>c</sup>	0.29±0.032 <sup>ab</sup>	0.20±0.033 <sup>bc</sup>
Liver (nmol/g)	2.55±0.15 <sup>a</sup>	1.47±0.13 <sup>b</sup>	2.29±0.16 <sup>a</sup>	1.64±0.09 <sup>b</sup>

Treatment means ± standard errors are presented. Treatment values with the same superscript letters are not significantly different at P>0.05. C= Control; VE= 200 mg/kg d-1-α tocopherol acetate (positive control); T20= 20 mg/kg Tetracycline (positive control); OS8= 8g/kg *O. stamineus*

**Table 7.** Intestinal bacteria population (log CFU/g of intestinal contents) of broiler chickens fed different diets (Readings on Day-42)

Bacteria	Dietary Treatments			
	C	VE	T20	OS8
Facultative Anaerobic	6.70±0.07 <sup>a</sup>	6.56±0.17 <sup>ab</sup>	6.14±0.17 <sup>b</sup>	6.19±0.15 <sup>b</sup>
<i>Lactobacillus</i>	4.96±0.52	5.92±0.36	5.46±0.04	5.38±0.07
<i>Escherichia coli</i>	4.11±0.26	3.79±0.33	3.55±0.19	3.75±0.21

Treatment means ± standard errors are presented. Treatment means with different superscript letters within the same column differ significantly ( $P < 0.05$ ). C= Control; VE= 200 mg/kg d-1- $\alpha$  tocopherol acetate (positive control); T20= 20 mg/kg Tetracycline (positive control); OS8= 8g/kg *O. stamineus*

characteristic, this was similar to that reported by Utami et al. (2020) on the effect of turmeric extract in the diet of broiler chickens. In contrast, the increase in AST and ALP activities could indicate injury or damage to the liver as presently indicated in broiler chicken following tetracycline supplement.

Gut microbial population plays an essential role in broiler weight gain. OS supplementation might also affect the population of gut microbes in broilers due to its antimicrobial and antioxidant properties, as mentioned earlier, and as similarly reported in previous studies (Giannenas et al. 2018; Guo et al. 2019). Microflora in gut contributes significantly to overall health performance of the host (Diether & Willing 2019) by influencing the development of the gut system. When infections attach to the mucosa, for example, gut integrity and function are severely harmed, and the immune system is put to the test (Aguzey et al. 2020). Chickens raised in a pathogen-free environment grow 15% quicker than those raised in typical conditions where germs and viruses are present (Akpan et al. 2019). Therefore, the microbial population is one of the crucial indicators of the broiler's health status.

Furthermore, it is widely known that gut microbiota represents a nutritional "burden" in fast-growing broiler chicken (Ravindran & Abdollahi 2021), because activemicroflora components may have a higher energy demand for maintenance and worse nutrient utilization efficiency (Yadav & Jha 2019). With its antimicrobial properties (Malahubban et al. 2013a), OS could improve digestibility of the feed offered to the animals and regulate and limit growth and colonization of a variety of pathogenic and non-pathogenic species in the gastrointestinal tract (Teng & Kim 2018). Increasing the presence of pathogenic bacteria in broiler guts may result in poor growth and a high feed conversion ratio. While the dietary treatment with OS in the present study did not affect the population of *Lactobacillus* and *Escherichia coli*, the inclusion, however, suppressed the population of the facultative anaerobes that might include pathogenic species such as *Salmonella enterica* (Pham et al. 2022), and this was comparable with tetracycline. The

significant effect of OS on total facultative anaerobes might be collective and not selective by reducing the number of intestinal microbial communities rather than the number of specific individuals and characteristics of bacteria. The antibacterial properties of *O. stamineus* could be associated with phytochemical compounds, as demonstrated in the previous phytochemical screening (Malahubban et al. 2013a), which revealed alkaloids, tannins, etc. saponins and steroids in the methanol extract. Antibacterial action of phytochemicals is mediated by a variety of mechanisms. Tannins, for example, work by depriving essential proteins like enzymes of iron, hydrogen bonding, or non-specific interactions (Loo et al. 2020). *Bacteroides fragilis*, *Clostridium perfringens*, and *Enterobacter cloacae* are among the bacteria that are inhibited by tannic acid (Loo et al. 2020). Therefore, the inclusion of 8 g/kg OS in the diet can replace synthetic antibiotic tetracycline or its equivalents.

## CONCLUSION

Dietary supplementation of *Orthosiphon stamineus* leaf powder at a rate of 8 g/kg in the feed was comparable to tetracycline and Vitamin E supplementation to promote the growth of broilers. *O. stamineus* leaf powder also reduced abdominal fat deposition and cholesterol levels in the blood serum of the birds. From the present study it is also found that 8 g/kg *O. stamineus* supplementation in diet promoted lowering blood serum enzymes, suggesting metabolic stability. Therefore, *O. stamineus* leaf powder can replace conventional antibacterial and antioxidant compounds as broiler diet supplements in organic and sustainable poultry production.

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