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Regular Article

Serum biochemical properties and liver morphology of broiler chicken as affected by feeding Misai kucing (*Orthosiphon stamineus*) as supplement diet

Masnindah Malahubban[1]* and Zakry Fitri Ab. Aziz[2]

[1]Department of Animal Science and Fishery, [2]Department of Crop Science,
Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus, 97008 Bintulu, Sarawak, Malaysia
*Corresponding author email: masnindah@upm.edu.my

To evaluate the effects of ground leaf of Misai kucing (*Orthosiphon stamineus*) as a dietary supplement on serum biochemical parameters and liver morphology. One hundred and sixty one-day old male broiler chickens (n=160) were distributed into four treatment groups, with five replicates of eight birds in each group: the control group (basal diet without additive); the group dietary treatments, Diet OS2 (Basal diet + 2g/kg *O. stamineus*); Diet OS4 (Basal diet + 4g/kg *O. stamineus*) and Diet OS8 (Basal diet + 8g/kg *O. stamineus*). After 42 days, 40 birds were randomly selected for serum biochemical profile analysis involving pancreatic, renal, and hepatic functions (urea, sodium, potassium, chlorine, aspartate transaminase (AST), alkaline transaminase (ALT), alkaline phosphatase (ALP), glucose, cholesterol, triglycerides, total protein, albumin, and globulins). Present study found that serum levels of cholesterol, triglycerides, urea, AST, ALT and ALP were significantly lower suggesting that the *O. stamineus* ground leaf possibly do not cause kidney and liver impairment even at higher dosage (8g/kg). Present study concluded that the broiler chicken fed *O. stamineus* ground leaf at a rate 8 g/kg was the most promising dietary supplement to enhance health without deleterious effects on serum biochemical properties and morphological components of liver. In addition, it reduces abdominal fats and serum cholesterol. This study has provide evident that medicinal plant, *O. stamineus* can potentially substituted the use of additive synthetic.

Keywords: Broiler, liver, medicinal plant, *Orthosiphon stamineus*, serum.

Many researchers are experimenting with different feed additives mainly derived from natural compounds that have potential to alleviate problems resulting from the use of only chemical growth promoters. One such alternative is the addition of herbs in poultry fed. Unlike many synthetic antibiotics or inorganic compounds, these plant-derived products have low toxicity and reduced adverse effects.

Various commercial additives of plant origin, such as herbs, spices and various plant extracts have received increasing attention as possible substitutes for antibiotic-based growth promoters commonly used in the poultry to improve broiler performance (Hernandez *et al.,* 2004; Mahmood *et al.,* 2009). Few promising results have been demonstrated on the potential use of herbs incorporated in the broiler diets. However, report on the effect of *O. stamineus* on growth and health performance of broiler was unavailable. It was thought that *O. stamineus* may have contain immune enhancement properties and may improve performance in broilers.

Esonu *et al*. (2001) suggest that the haematological elements reflect the physiological responsiveness of the animals

and the influence of diet on haematological traits is very strong (Church *et al.,* 1984; Babatunde *et al.,* 1987). In addition, dietary ingredients cause changes in ontogeny and morphology of the liver. Keeping in view the herbs potential of *O. stamineus,* effect of this herbs was evaluated on the serum biochemical parameters and liver morphological of broiler chickens.

Materials and Methods

Plant Materials

Fresh samples of cultivated *Orthosiphon stamineus* (OS) was obtained from the Herbal Farm of Universiti Putra Malaysia. The plant sample was authenticated by Gene Bank Centre of Faculty of Agriculture, Universiti Putra Malaysia. Stem cutting is the most viable plant part used to cultivate the OS plant.

Stem cuttings were obtained from mature OS plant grown from field planting. Stem cuttings of OS were collected from a mature stems (semi-softwood) with 4-5 nodes. The stem cuttings were planted in black polythene bags containing a mixture of soil, sand, and peat moss (2:1:1) under nursery conditions with 2-3 nodes placed underground and the rest aboveground (2-3 nodes). The first harvest OS plant was at 10 weeks after planting. The shoots of OS were cut about 30 cm from the tip.

Preparation of Herbs for Animal Feed Additive

The fresh leaves from OS shoots were collected and oven-dried at 60 °C for 72 h. The dried leaves were ground to obtain powder using a Willey mill (Thomas® Willey cutting mill model 4) through a one mm screen and stored at 4 °C until further use.

Birds and Experimental Design

Experiments were conducted at the Poultry Research Unit, Department of Animal Science, Universiti Putra Malaysia (3°00′20 N, 101°42′18 E). One hundred and sixty one-day old male broiler chickens (Cobb 500) were obtained from a local hatchery. Broilers were randomly assigned into 20 cages with dimension of each cage at 122 cm (length) x 91 cm (width) x 50 cm (height) and heated with 24 hours lighting (for seven days) by two-incandescent light bulb (60 watts) located at each cage, and the temperature and humidity approximately recorded at 32 °C and, 62 to 90%, respectively. From 8-day-old to the end of experiment, no lighting was supplied and chicken were grown at ambient temperature (28 °C) and humidity (60 to 89%).

Broilers had *ad libitum* access to water and diet, and were fed on commercial broiler starter (0–20 days) diets. The chicks were vaccinated against Newcastle disease (Animal Health, Fort Dodge, Iowa, USA) on day 7 (Interocular) and on day 21 (Intranasal).

At 21 days of age, the chicks were weighed and reassigned to four different dietary treatments based on the average weight. Each treatment had five replicates containing eight broilers in each replicate. During 21 to 42 days of the experimental period, broilers were fed the following diets; 1) Diet C (Control, basal diet); 2) Diet OS2 (Basal diet + 2g/kg *O. stamineus*); 3) Diet OS4 (Basal diet + 4g/kg *O. stamineus*) and 4) Diet OS8 (Basal diet + 8g/kg *O. stamineus*).

The level of inclusion was designed according to quadratic increments. Dietary treatments were formulated according to the National Research Council (NRC, 1996). The compositions of the above dietary treatments are as shown in Table 1. No antimicrobial, anticoccidial drugs or feed enzymes were included in the basal diet. The feed was offered *ad libitum* and refilled at 08:30 and 17:30 hours daily, and the residual feed in the feeders was collected and weighed. Dietary and nutrition-related chemical composition analyses were performed using AOAC International procedures (AOAC International, 1995).

At the end of the trial, two birds (at day-42) per replicate were randomly selected, weighed to the nearest gram and slaughtered by severing the carotid artery and jugular veins (Halal method). The

procedures were according to the guidelines set up by Department of Standard Malaysia (Malaysian Standard, MS1500: 2004).

Table 1: Ingredients in the dietary treatments and nutritional analysis.

Ingredients	Dietary treatments[1]			
	C	OS2	OS4	OS8
Corn	61.00	60.80	60.60	60.20
Soy Bean Meal (SBM) (44%)	25.00	25.00	25.00	25.00
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[a]	0.25	0.25	0.25	0.25
Vitamin Mix[b]	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 cloride	0.10	0.10	0.10	0.10
Orthosiphon stamineus (g/kg)	-	2.00	4.00	8.00
Calculated Analysis (%)				
Metabolize Energy (ME) Kcal/kg	3201	3201	3201	3201
Crude Protein, %	20.00	20.00	20.00	20.00
Crude Fibre, %	4.35	4.38	4.38	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

[a]Premix provided per kg of diet: Mg = 56 mg; Fe = 20 mg; Cu = 10 mg; Zn = 50 mg; Co = 125 mg; I = 0.8 mg.

[b]Premix provided the following per kg of diet: Vitamin A = 50 MIU; Vitamin D_3 = 10 MIU; Vitamin E = 75 MIU; Vitamin K = 20 g; Vitamin B1 = 10 g; Vitamin B2 = 30 g; Vitamin B6 = 20 g; Vitamin B12 = 0.10 g; Calcium D-Panthothenate = 60 g; Nicotinic acid = 200g; Folic acid = 5 g; Biotin = 235 mg.

[1]Diet C = Control (0 g/kg herb *O. stamineus*); Diet OS2 = 2g/kg *O. stamineus* (34.64 mg Rosmarinic acid /kg); Diet OS4 = 4g/kg *O. stamineus* (69.28 mg Rosmarinic acid /kg); Diet OS8 = 8g/kg *O. stamineus* (138.56mg Rosmarinic acid /kg).

After 5 min of bleeding, each bird was dipped in hot water for 20 s and mechanically defeathered for 30 s. The feet, head and viscera were then removed manually. After slaughtered, ten birds from each treatment were selected for liver morphology examinations.

Blood Collection

On day-21 and 42, five birds from each treatments group were randomly selected and blood samples (4.0 mL) were collected from the wing vein using a 23-ga needle, serum was separated by centrifugation at 3000 g for 10 min. The serum samples were stored at minus 20 °C for the analyses of serum glucose, cholesterol, triglycerides, albumin, total protein, sodium (Na), potassium (K), chlorine (Cl), urea, aspartate transaminase (AST), alkaline transaminase (ALT), and alkaline phosphatase (ALP) and were measured by specific commercial kits (Roche Diagnostica, Basal, Switzerland) using an auto-analyser (HITACHI 902,

Automatic Auto-analyser). The serum globulin was calculated by subtracting serum albumin from serum total protein levels.

Histological Procedures

After slaughtering, two birds were sampled per replicate group for liver and morphology. The liver of birds was removed, weighed and preserved.

Tissue Preservation and Processing

The liver was preserved in 10% buffered formalin for at least 48 hours and then undergone a series of dehydration and clearing process using a histokinette (Leica® ASP 300). A small fraction of the liver was transferred into tissue cassette. The sample was rinsed in graded series of alcohol baths beginning with 70% alcohol and increasing the concentration up to 100% alcohol in order to remove water (dehydration) and fat. The tissue was subsequently treated with chloroform to remove excessive alcohol from the previous processing procedures. The whole procedures of tissue processing in the histokinette took 16 hours (pre-set time) to complete. To stabilize tissue structure, the liver was transferred into warmed wax (pre-set temperature at 58 °C) and immediately frozen. Subsequently, processed liver was embedded in paraffin (wax) to form paraffin blocks and then serially sectioned using a microtome (Leica® Model RM2155) with the thickness size at 5 μm.

Staining Procedures

Paraffin was first removed from the tissue sections using xylene. Subsequently, the tissue sections were soaked in a descending series of 100%, 95% and 70% alcohol baths for removal of xylene and formalin fixative. These sections were later washed in running water before staining the nucleus with hematoxylin. The sections were washed again in running water before dipping them into alcohol.

Subsequently, the sections were soaked in running water for 5 min before stained the cytoplasm with eosin. Excessive eosin was then removed in a series of alcohol baths. Xylene was used again to remove the wax. Stained tissue sections were then wiped dry and mounted.

Liver Examination

Liver tissues were visually examined using light microscope (Leica®) and observation was made from magnifications 10X, 20X and 40X and any histological changes and/or abnormalities were recorded.

Statistical Analysis

Statistical data analysis was carried out using the SPSS software (IBM SPSS version 21). Differences between means for serum biochemistry among treatments were analyzed for ANOVA followed by Duncan's test at 0.01 level of significance.

Results

Serum Biochemical Parameters

The results of serum analysis in the present study are shown in Table 2, Table 3 and Table 4, which presented the levels of biochemical components in serum of broilers fed on different dosages of *O. stamineus* leaf ground which was recorded at day 21 and day 42. At day-21, data were recorded as a baseline as no dietary treatments inclusion except commercial feed, therefore, no significant differences of serum samples were detected among broiler chickens. At day-42, serum cholesterol level showed that most of the herbal diets exhibited cholesterol-lowering activity. Birds fed on Diet OS2 (2.66 mmol/L), Diet OS4 (2.61 mmol/L) and Diet OS8 (2.59 mmol/L) showed significantly lowered serum cholesterol level as compared to Diet C (3.78 mmol/L). These results showed that cholesterol levels were in the normal range as 2.59 to 3.78 mmol/L (Table 2). Serum triglycerides level of broilers decreased significantly in broilers fed Diet OS4 (0.64 mmol/L) and Diet OS8 (0.48 mmol/L) as compared to those fed Diet C (0.99 mmol/L). The albumin, total protein and globulin in serum of broilers fed on different dosages of OS ground leaf were unchanged.

Table 2: Serum biochemical parameters of broilers at initial (day-21) and at the end (day-42) of dietary treatments (Mean±SE).

Parameters	Day	Diet[1]			
		C	OS2	OS4	OS8
Cholesterol (mmol/L)	D_{21}	2.69±0.27	2.86±0.21	2.66±0.20	2.63±0.23
	D_{42}	3.78±0.20^{a}	2.66±0.24^{b}	2.61±0.27^{b}	2.59±0.15^{b}
Glucose (mmol/L)	D_{21}	5.40±0.40	5.50±0.29	6.11±0.19	6.29±0.28
	D_{42}	5.52±0.46^{a}	5.40±0.70^{a}	5.58±0.36^{a}	5.42±0.25^{a}
Triglycerides (mmol/L)	D_{21}	0.96±0.11	0.86±0.04^{ab}	0.64±0.15^{bc}	0.90±0.12
	D_{42}	0.99±0.12^{a}	12.48±1.41	12.38±1.28	0.48±0.02^{c}
Albumin (g/L)	D_{21}	11.50±0.87	16.76±1.41	14.33±2.23	10.86±0.97
	D_{42}	16.27±1.17	18.43±1.50	23.34±2.75	12.45±1.19
Total protein (g/L)	D_{21}	24.30±1.83	1.06±0.17	0.82±0.13	19.71±1.76
	D_{42}	26.63±2.62	23.95±1.93	26.72±1.37	22.08±1.79
Globulin (g/L)	D_{21}	12.90±5.15	5.96±1.99	10.96±2.18	8.85±1.42
	D_{42}	10.37±2.20	7.19±1.56	12.39±1.85	9.63±2.34

Different letters within the same column differ significantly (P<0.01).
[1]Diet C = Control (0 g/kg medicinal herb; *O. stamineus*); Diet OS2 = 2 g/kg *O. stamineus* (34.64 mg Rosmarinic acid /kg); Diet OS4 = 4 g/kg *O. stamineus* (69.28 mg Rosmarinic acid /kg); Diet OS8 = 8 g/kg *O. stamineus* (138.56mg Rosmarinic acid /kg).

Table 3: Sodium, potassium, chlorine and urea levels in serum of broiler fed on diets supplemented with different rates of *A. paniculata* and *O. stamineus* ground leaf

Parameters	Day	Diet[1]			
		C	OS2	OS4	OS8
Na (mmol/L)	D_{21}	114.2 ± 6.9	121.3 ± 6.2	113.8 ± 6.1	128.6 ± 8.9
	D_{42}	124.3 ± 9.6^{b}	141.6 ± 4.0^{ab}	134.8 ± 1.2^{ab}	147.5 ±4.0^{a}
K (mmol/L)	D_{21}	15.2 ± 1.3	15.1 ± 1.6	15.2 ± 1.4	14.2 ± 1.6
	D_{42}	19.3 ± 0.4^{b}	19.4 ± 1.2^{b}	21.9 ± 1.3^{ab}	24.0 ± 0.3^{a}
Cl (mmol/L)	D_{21}	79.8 ± 2.4	73.8 ± 3.7	80.4 ± 2.2	76.3 ± 2.2
	D_{42}	77.1 ± 6.8^{b}	93.6 ± 3.1^{a}	91.6 ± 0.7^{a}	96.7 ± 3.9^{a}
Urea (mmol/L)	D_{21}	0.33 ± 0.04	0.29 ± 0.02	0.25 ± 0.03	0.23 ± 0.03
	D_{42}	0.77 ± 0.04^{a}	0.60 ± 0.03^{b}	0.37 ± 0.03^{c}	0.48 ± 0.03^{c}

Different letters within the same column differ significantly (P<0.01).
[1]Diet C = Control (0 g/kg medicinal herb; *O. stamineus*); Diet OS2 = 2 g/kg *O. stamineus* (34.64 mg Rosmarinic acid /kg); Diet OS4 = 4 g/kg *O. stamineus* (69.28 mg Rosmarinic acid /kg); Diet OS8 = 8 g/kg *O. stamineus* (138.56mg Rosmarinic acid /kg).

Table 4: Serum enzymes in broiler fed different dietary treatments (Mean ± SE).

Parameters	Days	Diet[1]			
		C	OS2	OS4	OS8
AST (U/L)	D_{21}	199.4±10.6	186.4 ± 17.2	167.8 ±16.1	174.4±12.8
	D_{42}	246.0±17.3^{a}	216.6±0.9^{ab}	211.5±9.9^{ab}	187.9 ±7.1^{b}
ALT (U/L)	D_{21}	4.72 ±0.71	3.62±0.64	4.23±0.54	5.01 ±0.53
	D_{42}	5.07 ±0.51^{a}	2.81±0.31^{b}	3.30±0.46^{b}	2.87 ±0.28^{b}
ALP (U/L)	D_{21}	1876.0 ±209.6	1253.9 ±137.1	1606.9 ±156.1	1798.8 ±209.1
	D_{42}	1745.8 ±211.9^{a}	680.7 ±123.2^{b}	807.2 ±55.7^{b}	805.6 ±18.8^{b}

Different letters within the same column differ significantly (P<0.01).
[1]Diet C = Control (0 g/kg medicinal herb; *O. stamineus*); Diet OS2 = 2 g/kg *O. stamineus* (34.64 mg Rosmarinic acid /kg); Diet OS4 = 4 g/kg *O. stamineus* (69.28 mg Rosmarinic acid /kg); Diet OS8 = 8 g/kg *O. stamineus* (138.56mg Rosmarinic acid /kg).

The serum sodium level in broilers was significantly higher in birds fed Diet OS8 (147.5 mmol/L) compared to those on Diet C at 124.3 mmol/L (Table 3). Serum potassium also significantly higher in broiler fed on Diet OS8 (24 mmol/L) diet as compared to Diet C (19.3 mmol/L).

Chlorine serum was also significantly higher in all herbal supplemented diets over control basal diet. Serum urea was significantly reduced ($P<0.01$) in all herbal dietary treatments at varying dosages as compared to the control diet (0.77 mmol/L).

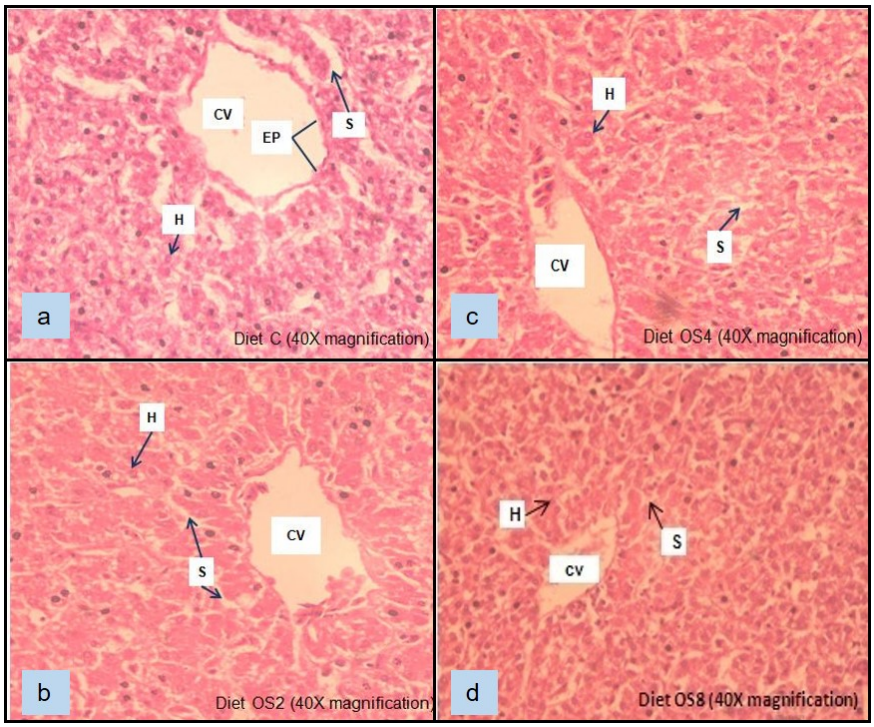

Figure 1: Histological examination on liver of 42-day old broilers fed control diet, Diet OS2, Diet OS4, and Diet OS8. All figures show normal liver histology where hepatocytes (H), sinusoid (S), central vein (CV) and portal area conditions were at normal (40X magnification). Note for images a-d: (a) Micrograph of liver (Diet C) the lumen of central vein (CV) is lined by a simple squamous epithelium (EP),and observed also hepatocytes (H), and sinusoid (S) at normal architectures, (40X magnification). (b) Micrograph of liver (Diet OS2) central vein (CV) of the liver lobule, collects blood from the sinusoid (S), and the liver plate are composed of hepatocytes (H) appear to radiate as spokes of a wheel from the central vein, (40X magnification). (c) Micrograph of liver (Diet OS4), central vein (CV), which is continuous with the endothelial lining of the hepatic sinusoid (S), and the liver plate are composed of hepatocytes (H), (40X magnification). (d) Micrograph of liver (Diet OS8), central vein (CV), which is continuous with the endothelial lining of the hepatic sinusoid (S). Observed also that the liver plates are composed of hepatocytes (H), (40X magnification).

In general, present study indicated that herbal diets lowering serum enzymes (Table 4). The Diet OS8 lowering aspartate transaminase (AST) significantly ($P<0.01$) as compared to Diet C at 187.9 U/L and 246.0 U/L, respectively. Almost all herbal diets had Alanine transaminase (ALT), alkaline phosphatase (ALP)-lowering effects.

Morphological Analysis of Liver

Histological study on the liver using light microscopic showed a normal structure and no alteration in the lives of the treated broiler chicken fed on diets treated with OS (Figure 1).

Discussion

In the present study, serum cholesterol level showed that most of the herbal diets exhibited cholesterol-lowering activity. Birds fed diets supplemented with 2 g/kg OS, 4 g/kg OS and 8 g/kg OS significantly lowered serum cholesterol level as compared to control diet. Cholesterol levels were in the normal range as 2.59 to 3.78 mmol/L. This in agreement with Sturkie *et al.* (2000) who suggested that the normal value of cholesterol in chicken varies from 2.59 - 3.88 mmol/L. However, breed of chicken, nutritional pattern, type of feed, environmental factors and the test ingredient used could be contributed to the variation in cholesterol status. Matawalli *et al.* (2004) reported that the methanolic leaf extract of *Adansonia digitata* lowered the lipid levels in rat and suggested that the possible hypolipiaemic effect could be attributed to the presence of saponins and fibre in the extract which has been shown to bind to serum lipids especially cholesterol, thereby easing their excretion from circulation. The present study found that *O. stamineus* contributes to reduction in cholesterol level in the serum. This might be the first report of feeding of *O. stamineus* ground leaf on lowering serum cholesterol level in broiler chicken. This beneficial activity could be related to the presence of triterpenoid in *O. stamineus* which were widely reported as the cholesterol-lowering components (Chen *et al.*, 2011; Choi *et al.*, 2012).

Serum triglycerides level of broilers was lowered significantly when fed diets supplemented with OS at 4 g/kg and 8 g/kg as compared to control diet. Present study indicated that the serum triglycerides level may be positively correlated with dosage of *O. stamineus* ground leaf may have led to triglyceride-lowering effect. As similar phenomenon with level of serum cholesterol, triglyceride-lowering activity of broiler fed on *O. stamineus* ground leaf could be related to its bioactive components, especially the presence of triterpenoid (rosmarinic acid) which were successfully extracted from *O. stamineus* (Unpublished data). Thus, this herb might be useful in preventing cardiovascular diseases, however, the role of triglycerides on heart diseases still unclear and questionable (Goldberg *et al.,* 2011). In the present study, albumin, total protein and globulin in serum of broilers fed on different dosages of *O. stamineus* ground leaves were unchanged. However, Elagib *et al*. (2012) reported that cinnamon (*Cinnamomum verum*), cumin (*Cuminum cyminum*), fenugreek (*Trigonella foenum-graecum*) and ginger (*Zingiber officinale*) inclusion in feed diets of broiler chicks exhibited some changes by increasing the serum total proteins and globulins, but albumin remains unchanged.

Present study observed a significant increase in the liver weight of broilers fed Diet OS4 and Diet OS8, and a significant decrease in serum enzymes. Thus the increase in liver weight was not associated with general liver damage action by hepatotoxin (Klaunig and Kolaja, 1998). In addition, the increase in liver weight could be due to the increase of macromolecules protein synthesis within hepatocytes and the proliferation of smooth endoplasmic reticulum (Han *et al.,* 2008). In the present study, histopathological study on the liver using light microscopic showed that a normal structure and no alteration in the livers of the treated broiler chickens with *O. stamineus* of varying dosages.

Conclusion

Present study found that broiler chicken fed *O. stamineus* ground leaf at a rate 8 g/kg was the most promising dietary supplement to enhance health without deleterious effects on serum biochemical properties and morphological components of liver. In addition, it reduces abdominal fats and serum cholesterol. It also maintain the integrity of liver indicating that no toxic

effect from OS supplementation at a rate up to 8 g/kg. Therefore, *O. stamineus* ground leaf dietary supplement at a rate 8 g/kg could be used as an alternative and potential growth promoter in the diet formulation for safe and sustainable broiler chicken production.

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