

ISSN 1682-8356
ansinet.org/ijps



INTERNATIONAL JOURNAL OF
POULTRY SCIENCE

ANSI*net*

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com



Research Article

The Effect of Fermented *Sauropus androgynus* Plus Bay Leaf Inclusion on the Hematologic and Lipid Profiles of Female Broiler Chickens

Urip Santoso, Yosi Fenita and Kususiyah

Department of Animal Science, Faculty of Agriculture, Bengkulu University, Jalan Raya WR Supratman, Kandang Limun, Bengkulu, Indonesia

Abstract

Background and Objective: The use of antibiotics as a feed additive for poultry has been prohibited in many countries because the antibiotics accumulate in the meat, which may stimulate microbial pathogen resistance. The present study was conducted to evaluate the effect of fermented *Sauropus androgynus* plus bay leaf inclusion on the hematologic and lipid profiles of female broiler chickens.

Materials and Methods: Two hundred and eighty female broilers aged 14 days were divided into 7 treatment groups as follows: 1) the control broiler chickens were fed a diet with a commercial feed additive (contained zinc bacitracin) (P0), 2) broiler chickens were fed a diet with medicinal herb mixture formula 1 at 2.5% (P1), 3) broiler chickens were fed a diet with medicinal herb mixture formula 2 at 2.5% (P2), 4) broiler chickens were fed a diet with medicinal herb mixture formula 3 at 2.5% (P3), 5) broiler chickens were fed a diet with medicinal herb mixture formula 1 at 5% (P4), 6) broilers were fed a diet with medicinal herb mixture formula 2 at 5% (P5) and 7) broilers were fed a diet with medicinal herb mixture formula 3 at 5%. **Results:** Experimental results showed that the inclusion of fermented *Sauropus androgynus* plus bay leaves had no effect on thrombocyte levels, erythrocyte sedimentation rate (ESR), WBC, RBC, PCV, MCV, MCH, MCHC, liver and spleen weights, intestine length and toxicity but significantly affected the gizzard; the intestine ($p < 0.01$) and heart weights ($p < 0.05$); triglyceride, cholesterol, HDL ($p < 0.01$) LDL and VLDL ($p < 0.05$) levels; and the LDL/HDL ratio ($p < 0.01$). **Conclusion:** The inclusion of fermented *Sauropus androgynus* plus bay leaves improved the lipid profile without modifying the hematologic status in female broiler chickens.

Key words: Fermented *Sauropus androgynus*, bay leaves, hematologic status, lipid profiles, internal organ, broiler

Citation: Urip Santoso, Yosi Fenita and Kususiyah, 2018, The effect of fermented *Sauropus androgynus* plus bay leaf inclusion on the hematologic and lipid profiles of female broiler chickens, Int. J. Poult. Sci., 17: 410-417.

Corresponding Author: Urip Santoso, Department of Animal Science, Faculty of Agriculture, Bengkulu University Jalan Raya WR Supratman, Kandang Limun, Bengkulu, Indonesia

Copyright: © 2018 Urip Santoso *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Commercial feed additive contains antibiotics such as zinc bacitracin to promote the growth of poultry. Antibiotics destroy certain bacteria in the gastrointestinal tract and help poultry convert feed to muscle more quickly, thus causing more rapid growth. Moreover, antibiotics also improve the hematological profile and reduce blood lipid profiles¹. The hematological profile is an important indicator of the physiological or pathophysiological status of an individual. However, the use of antibiotics as a growth promoter has been prohibited in many countries because these antibiotics accumulate in poultry products, which may stimulate microbial pathogen resistance. Antibiotics are shown to have high side effects such as damage to the hormonal and immune systems². In addition, antibiotic residue in animal products may cause an allergy when the animals are consumed by people. Thus, an alternative feed additive is needed to substitute for antibiotics. An alternative feed additive should have the ability to stimulate growth, lower blood lipid concentrations and lead to normal hematological profiles as well as antibiotics.

Some antioxidant medicinal plants have been suggested as a substitute for antibiotics³ because they contain antibacterial and antilipid compounds. These medicinal plants have a low side effect and are able to maintain product quality and livestock performance^{4,5}. These natural antioxidant compounds include α -tocopherol, β -carotene, ascorbic acid, flavonoids, carotenoids, anthocyanins, phenol compounds, zinc and selenium^{6,7}.

Sauropus androgynus leaves have been shown to be potential substitutes for antibiotics⁸⁻¹² because this medicinal plant contains linolenic acid, palmitic acid, chlorophyll, benzoic acid and alkaloid¹³, flavonoids¹⁴ and phenols¹⁵. Bay leaves contain flavonoids and glycosides^{16,17}. Santoso *et al.*¹² showed that *Sauropus androgynus* or bay leaf inclusion modified the hematologic and blood lipid profiles of broiler chickens. Bay leaves reduce the blood lipid profile¹⁸. A change in the hematologic profile may result in a change in poultry performance, whereas a change in the blood lipid profile may indicate a change in fat deposition in poultry. However, the changes generated by the administration of those medicinal herbs were still not high. Thus, a method to improve the medicinal herb quality is needed.

Fermentation may improve the quality of medicinal herbs. Fermentation reduces anti-nutrients such as trypsin inhibitor¹⁹, oligosaccharide²⁰, tannin²¹, phytic acid²², phenol, phytin phosphorus and oxalate²², saponin²¹ and alkaloid²³.

In addition, fermentation improves nutritional values and feed utilization in poultry²⁴ and modifies lipid profiles²⁵⁻²⁷. Previous research²⁸ has shown that fermentation of the *Sauropus androgynus* leaf with *Saccharomyces cerevisiae* improved the quality of the *Sauropus androgynus* leaf. Bay leaf inclusion at 0.5% reduced serum triglyceride, total cholesterol, LDL and VLDL but increased serum HDL²⁹. Furthermore, bay leaf inclusion had no effect on hemoglobin, leukocytes, erythrocytes and lymphocytes.

An improvement in the quality of medicinal herbs may support the improvement of hematologic status and blood lipid profiles in terms of a lower blood lipid profile and an optimum hematologic status of broiler chickens. Therefore, this study was designed to evaluate the effectiveness of fermented *Sauropus androgynus* plus bay leaves as a substitute for commercial feed additives on the hematologic and lipid profiles of female broiler chickens.

MATERIALS AND METHODS

Fermentation of medicinal herb mixture: The medicinal herb mixture was composed of fermented *Sauropus androgynus* and bay leaves. *Sauropus androgynus* and bay leaves obtained from the field or from traditional markets were air-dried for 5 days. They were subsequently dried in the sun for 1 h until the dryness was approximately 10-12% and then were milled and stored in a plastic bag. Fermentation of *Sauropus androgynus* and bay leaves was conducted according to the method of Santoso *et al.*²⁸.

Animals and diets: Seven hundred broiler chicks aged one day were placed in a litter house. New broiler chickens were given sugar water to reduce their stress due to travel. The brooder temperature was set in accordance with standard maintenance procedures. At the age of 4 and 21 days, broiler chickens were vaccinated against Newcastle disease. At the age of 1-13 days, broiler chickens were fed a commercial diet.

At the age of 14 days, female broiler chicks were selected and distributed into experimental units and were fed experimental diets up to age 34 days. The composition of experimental diets used is presented in Table 1. The medicinal herbs used were fermented *Sauropus androgynus* leaf and bay leaf. The level of medicinal herb mixture was 2.5 or 5%, as recommended by Santoso *et al.*²⁸.

Two hundred and eighty female broilers aged 14 days were distributed into the 7 treatment groups as follows: (P0) the control, broilers were fed a diet with a commercial feed additive (zinc bacitracin), (P1) broiler chickens were fed a diet

Table 1: The composition of experimental diets

Feed stuffs (%)	P0	P1	P2	P3	P4	P5	P6
Yellow corn	57.0	57.0	57.0	57.0	54.5	54.5	54.5
Rice bran	5.0	4.0	4.0	4.0	4.0	4.0	4.0
Broiler concentrate	34.2	33.2	33.2	33.2	33.2	33.2	33.2
Mineral mixture	1.7	1.7	1.7	1.7	1.7	1.7	1.7
Salt	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Commercial feed additive*	0.5	0	0	0	0	0	0
Medicinal herb formula 1		2.5			5.0		
Medicinal herb formula 2			2.5			5.0	
Medicinal herb formula 3				2.5			5.0
Palm oil	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Total	100	100	100	100	100	100	100
Calculated nutrient composition							
Crude protein (%)	19.86	19.82	19.79	19.75	20.09	20.02	19.95
ME (kcal kg ⁻¹)	3088.07	3097.63	3096.93	3096.22	3070.26	3068.85	3067.44
Ca (%)	0.947	0.975	0.961	0.964	1.031	1.017	1.003
P (%)	0.692	0.681	0.678	0.675	0.693	0.686	0.680

*Top mix (contained zinc bacitracin), FSAL: Fermented *Sauropus androgynus* leaves, FBL: Fermented bay leaves

with medicinal herb mixture formula 1 at 2.5%, (P2) broiler chickens were fed a diet with medicinal herb mixture formula 2 at 2.5%, (P3) broiler chickens were fed a diet with medicinal herb mixture formula 3 at 2.5%, (P4) broiler chickens were fed a diet with medicinal herb mixture formula 1 at 5%, (P5) broilers were fed a diet with medicinal herb mixture formula 2 at 5%, (P6) broilers were fed a diet with medicinal herb mixture formula 3 at 5%.

All treatment groups consisted of 4 replications and each replication consisted of 10 female broilers. The broilers were maintained in accordance with the standard procedure of broiler maintenance. Diet and drinking water were provided *ad libitum*.

Sampling and laboratory analysis: At the end of the study (34 days), the blood of 4 broilers for each treatment group was collected for an analysis of the hematologic and lipid profiles. The Packed Cell Volume (PCV) was determined by the microhematocrit method³⁰. Hemoglobin was determined using the cyanmethemoglobin method, while the Red Blood Count (RBC) was carried out using the hemocytometer method³¹. The thrombocyte count was determined using the Res-Ecker method³² and the White Blood Count (WBC) was determined using the hemocytometer method³¹. To obtain the plasma, blood samples were collected, then bottled with anticoagulant agent and centrifuged at 3000 rpm. The obtained blood plasma was then analyzed for triglyceride, cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL). The total cholesterol and LDL were determined by the method of Kulkarni³³ and triglyceride concentrations were determined by the method of Fossati and Prencipe³⁴.

Data analysis: All data were subjected to one-way analysis of variance and if significantly different, the results were tested further with Duncan's Multiple Range Test (using SPSS software). Statistical significance was set at $p < 0.05$ and $p < 0.01$.

RESULTS

Fermented medicinal herb composition: Fermented *Sauropus androgynus* leaf powder contained 12.39% moisture, 21.13% protein, 2.13% crude fat, 15.12% crude fiber, 2.53% calcium, 0.47% phosphorus, 4.97 ppm iron and 1.56 ppm potassium, whereas fermented bay leaf powder contained 14.56% moisture, 15.56% protein, 3.56% crude fat, 14.34% crude fiber, 1.45% calcium, 0.25% phosphorus, 1.68 ppm iron and 1.67 ppm potassium.

Hematologic profiles: Table 2 shows the effect of the herbal mixture on the hematologic profile of broiler chickens. Experimental results showed that the inclusion of the herbal mixture had no effect on thrombocytes, ESR, WBC, RBC, PCV, MCV, MCH and MCHC.

Internal organ weight and toxicity score: The effect of the medicinal mixture on internal organ weight and the toxicity score is presented in Table 3. Experimental results showed that the inclusion of a medicinal mixture to the diet had no effect on the liver, spleen, intestine length and toxicity but significantly affected the gizzard, intestine ($p < 0.01$) and heart weights ($p < 0.05$). The control group had higher gizzard and intestine weights than the other treatment groups ($p < 0.01$). The P1 group had a lower heart weight than the other treatment groups.

Table 2: Effect of the medicinal herb mixture on the hematologic status of female broiler chickens

	P0	P1	P2	P3	P4	P5	P6	P
Thrombocyte ($\times 10^3 \text{ mm}^{-3}$)	5.25 \pm 2.22	4.00 \pm 2.45	4.75 \pm 2.50	5.50 \pm 1.00	3.50 \pm 2.38	3.25 \pm 2.06	4.75 \pm 1.89	0.684
ESR (mm h ⁻¹)	3.00 \pm 0.82	3.25 \pm 0.96	3.25 \pm 0.50	3.50 \pm 1.00	3.25 \pm 0.50	3.00 \pm 0.00	3.25 \pm 0.50	0.954
Hb (g dL ⁻¹)	9.40 \pm 1.57	9.58 \pm 0.94	9.30 \pm 1.38	9.30 \pm 1.19	10.08 \pm 0.83	9.83 \pm 0.75	9.75 \pm 0.64	0.935
WBC ($\times 10^3 \text{ mm}^{-3}$)	276.1 \pm 12.8	279.0 \pm 15.0	271.7 \pm 21.9	270.8 \pm 17.6	281.3 \pm 8.7	282.7 \pm 9.6	282.0 \pm 9.2	0.821
RBC ($\times 10^6 \text{ mm}^{-3}$)	2.33 \pm 0.34	2.40 \pm 0.24	2.13 \pm 0.67	2.35 \pm 0.39	2.65 \pm 0.24	2.50 \pm 0.16	2.48 \pm 0.22	0.554
PCV (%)	29.75 \pm 4.92	30.75 \pm 2.75	27.25 \pm 7.50	29.25 \pm 3.77	32.50 \pm 3.11	31.50 \pm 1.73	31.00 \pm 1.83	0.738
MCV (fL)	126.8 \pm 6.0	126.8 \pm 5.7	130.0 \pm 6.5	124.8 \pm 3.6	122.3 \pm 2.6	122.8 \pm 1.50	125.00 \pm 2.7	0.259
MCH (pg)	34.25 \pm 3.95	30.00 \pm 0.82	33.75 \pm 6.85	31.00 \pm 0.82	30.00 \pm 0.00	30.25 \pm 0.96	30.50 \pm 1.00	0.257
MCHC (g dL ⁻¹)	30.63 \pm 0.62	30.70 \pm 0.37	30.70 \pm 0.73	31.03 \pm 0.97	31.25 \pm 1.62	31.03 \pm 0.97	31.03 \pm 0.97	0.961

P0: Control, P1: Broiler chickens were fed a diet with formula 1 at 2.5%, P2: Broiler chickens were fed a diet with formula 2 at 2.5%, P3: Broiler chickens were fed a diet with formula 3 at 2.5%, P4: Broiler chickens were fed a diet with formula 1 at 5%, P5: Broilers were fed a diet with formula 2 at 5%, P6: Broilers were fed a diet with formula 3 at 5%

Table 3: Effect of the medicinal herb mixture on the internal organ weight of female broiler chickens

Internal organ	P0	P1	P2	P3	P4	P5	P6	P
Liver (%)	2.28 \pm 0.20	2.22 \pm 0.14	2.12 \pm 0.09	2.37 \pm 0.28	2.21 \pm 0.11	2.42 \pm 0.16	2.32 \pm 0.21	0.102
Spleen (%)	0.16 \pm 0.07	0.11 \pm 0.03	0.12 \pm 0.02	0.09 \pm 0.03	0.11 \pm 0.02	0.15 \pm 0.04	0.12 \pm 0.03	0.057
Gizzard (%)	2.01 \pm 0.12 ^c	1.82 \pm 0.11 ^b	1.85 \pm 0.05 ^b	1.58 \pm 0.11 ^a	1.75 \pm 0.12 ^b	1.77 \pm 0.06 ^b	1.82 \pm 0.08 ^b	0.000**
Heart (%)	0.26 \pm 0.03 ^b	0.21 \pm 0.02 ^a	0.26 \pm 0.02 ^b	0.26 \pm 0.02 ^b	0.26 \pm 0.02 ^b	0.27 \pm 0.04 ^b	0.25 \pm 0.03 ^b	0.019*
Intestine (%)	4.92 \pm 0.33 ^c	3.83 \pm 0.20 ^a	3.96 \pm 0.28 ^a	4.38 \pm 0.33 ^b	4.52 \pm 0.26 ^b	4.58 \pm 0.25 ^b	3.72 \pm 0.21 ^a	0.000**
Intestine length (cm 100 ⁻¹ g BW)	14.4 \pm 0.98	14.16 \pm 0.95	13.53 \pm 1.16	14.26 \pm 1.43	14.18 \pm 1.20	14.89 \pm 1.19	13.21 \pm 0.35	0.181
Toxicity (%)	2.433 \pm 0.25	2.327 \pm 0.15	2.34 \pm 0.10	2.469 \pm 0.29	2.31 \pm 0.11	2.57 \pm 0.17	2.44 \pm 0.23	0.103

BW: Body weight, P0: Control, P1: Broiler chickens were fed a diet with formula 1 at 2.5%, P2: Broiler chickens were fed a diet with formula 2 at 2.5%, P3: Broiler chickens were fed a diet with formula 3 at 2.5%, P4: Broiler chickens were fed a diet with formula 1 at 5%, P5: Broilers were fed a diet with formula 2 at 5%, P6: broilers were fed a diet with formula 3 at 5%

Table 4: Effect of the medicinal herb mixture on the serum lipid profiles of female broiler chickens

Lipid (mg dL ⁻¹)	P0	P1	P2	P3	P4	P5	P6	P
Triglyceride	115.8 \pm 3.3 ^d	105.6 \pm 8.1 ^c	104.8 \pm 4.8 ^c	103.5 \pm 3.7 ^{bc}	93.9 \pm 5.3 ^a	96.8 \pm 1.8 ^{ab}	91.8 \pm 4.3 ^a	0.000
Cholesterol	127.8 \pm 3.4 ^e	118.0 \pm 4.6 ^{cd}	122.5 \pm 4.1 ^{de}	119.3 \pm 3.3 ^d	114.5 \pm 6.9 ^{bc}	106.5 \pm 1.9 ^a	109.0 \pm 5.3 ^{ab}	0.000
HDL	45.0 \pm 1.4 ^a	48.25 \pm 1.7 ^b	57.75 \pm 1.3 ^d	50.0 \pm 2.2 ^{bc}	51.0 \pm 1.2 ^c	48.50 \pm 1.7 ^{bc}	51.0 \pm 1.8 ^c	0.000
LDL	51.25 \pm 3.6 ^c	45.0 \pm 2.6 ^{ab}	49.0 \pm 3.7 ^{bc}	48.0 \pm 0.8 ^{abc}	44.75 \pm 4.0 ^{ab}	42.75 \pm 5.3 ^a	43.5 \pm 2.7 ^{ab}	0.020
VLDL	31.5 \pm 5.1 ^b	24.75 \pm 6.4 ^{ab}	15.75 \pm 4.6 ^a	21.75 \pm 3.2 ^a	18.75 \pm 7.6 ^a	15.25 \pm 5.8 ^a	14.50 \pm 9.3 ^a	0.010
LDL/HDL	1.138 \pm 0.06 ^c	0.935 \pm 0.06 ^{ab}	0.845 \pm 0.06 ^a	0.96 \pm 0.03 ^b	0.878 \pm 0.09 ^{ab}	0.880 \pm 0.08 ^{ab}	0.853 \pm 0.03 ^a	0.000

P0: Control, P1: Broiler chickens were fed a diet with formula 1 at 2.5%, P2: Broiler chickens were fed a diet with formula 2 at 2.5%, P3: Broiler chickens were fed a diet with formula 3 at 2.5%, P4: Broiler chickens were fed a diet with formula 1 at 5%, P5: Broilers were fed a diet with formula 2 at 5%, P6: Broilers were fed a diet with formula 3 at 5%

Blood lipid profiles: Table 4 shows the effect of the herbal mixture on blood lipid profiles. Experimental results showed that the inclusion of the herbal mixture significantly affected the levels of triglycerides, cholesterol, HDL ($p < 0.01$), LDL and VLDL ($p < 0.05$) and the LDL/HDL ratio ($p < 0.01$). P4 and P6 had lower triglyceride levels than P0, P1, P2 and P3 but were statistically similar to P5. P0 (control) had the highest triglyceride level. P5 had a lower cholesterol level than P0, P1, P2, P3, P4 but was statistically similar to the P6. P0 had a higher cholesterol level than that of the other treatment groups, except for P2. P2 had the highest HDL level, whereas P0 had the lowest HDL level. P0 had a higher LDL level than P1, P4, P5 and P6 but was statistically similar to other treatment groups. P0 had a higher VLDL level than P2, P3, P4, P5 and P6 but was statistically similar to P1. P0 had a higher LDL/HDL ratio than the other treatment groups.

DISCUSSION

Fermented *Sauropus androgynus* leaf had higher protein, calcium, phosphorus and iron, whereas fermented bay leaf had higher crude fat and potassium. It appears that *Sauropus androgynus* leaf is rich in protein, calcium, phosphorus and iron. There was no change in the hematological status by fermented *Sauropus androgynus*. This result agrees with the observation of Santoso *et al.*¹², who reported that cassava yeast-fermented *Sauropus androgynus* leaf inclusion did not change the hematological status of broiler chickens. The results of this study are in contrast with those of Brata *et al.*³⁵, who found that *Sauropus androgynus* leaf extract supplementation decreased the number of erythrocytes with no effect on the Hb, PCV, MCH, MCV and MCHC. Furthermore, *Sauropus androgynus* leaf powder

increased Hb, PCV and RBC in anemia-induced rats³⁶. This difference in result may be caused by the difference in *Sauropus androgynus* leaf processing.

The RBC ranged from $1.45-4.64 \times 10^6/\text{mm}^3$ ^{4,37-40}; the Hb ranged from 6.88-13 g dL⁻¹³⁷⁻⁴¹; PCV ranged from 22-35%^{37,38,40,41}; the WBC ($\times 10^6 \mu\text{L}^{-1}$) ranged from 4.10-5.12⁴⁰; the MCV ranged from 90 to 140 fL³⁹; the MCH ranged from 33-69.6 pg^{37,39}; and the MCHC ranged from 26-35%^{37,39}. Santoso *et al.*¹² found that in broilers aged 35 days, the RBC was 2.40-2.85, thrombocyte level was 2-6.25, PCV was 32.25-37.75, Hb was 9.88-11.95, MCH was 38.5-41.75, MCV was 128.75-135.5 and MCHC was 29.75-31.0. Santoso *et al.*¹² reported that the ESR ranged from 3.25 to 8.25 mm h⁻¹. Thus, the present study showed a normal range of hematologic status in broiler chickens.

Fenita *et al.*⁴² reported that the inclusion of unfermented *Sauropus androgynus* leaf or bay leaf at 5% had no effect on the internal organ weight. The present study showed that although the intestine length was not reduced, the intestine weight of the treatment groups was lighter than that of the control. It is assumed that the thickness of the intestine was thinner in the treatment groups. Fernandes *et al.*⁴³ showed that the inclusion of probiotics tended to lower the thickness of the small intestine. Hu *et al.*⁴⁴ reported that fermentation increased the villus height and the villus height-to-crypt depth ratio and decreased the crypt depth and intestinal wall thickness in the duodenum, jejunum-ileum and cecum in broiler chickens. Furthermore, they assumed that the change in intestinal morphology may be due to lower antinutrition and an increase in the degradation of protein to peptides after fermentation. In addition, that improvement may result from the increased numbers of beneficial bacteria (*Saccharomyces cerevisiae*) in the intestine. This may explain the tendency for higher body weight gain in broiler chickens fed diet containing a fermented medicinal herb mixture.

The function of the gizzard includes particle size reduction, nutrient degradation, feed flow regulation and reduction in the coarseness of the diet. Fermentation causes a softer physical feed and a decrease in the crude fiber so that gizzard activity decreases to produce a gentle feed. This may be one of the reasons for the lower weight of gizzards in broilers fed diet containing the fermented medicinal herb mixture.

Santoso *et al.*⁹ reported that the supplementation of unfermented *Sauropus androgynus* leaf extract reduced total cholesterol, triglyceride and LDL levels but increased the HDL concentration in the serum of layer chickens. Kamalia *et al.*⁴⁵ reported that the inclusion of *Sauropus androgynus* leaf at 3% increased serum HDL levels but decreased serum LDL

levels. Santoso *et al.*¹² found that *Sauropus androgynus* or bay leaf powder at a 5% level decreased blood triglyceride and LDL levels but increased blood HDL levels. Bay leaves reduced total cholesterol, LDL, triglyceride and glucose levels and increased HDL levels in people with type 2 diabetes¹⁸. The bay leaf and its isolated flavonoids and glycosides reduced TC, TG, LDL-C and VLDL-C levels; therefore, bay leaf is a useful agent for reducing hyperlipidemia⁴⁶. Belawa *et al.*⁴⁷ found that the inclusion of bay leaves reduced blood total cholesterol, LDL and triglyceride levels but increased blood HDL levels in local chickens. Khan *et al.*⁴⁸ reported that giving capsules containing 1 or 2 g of ground bay leaves per day for 30 days reduced serum triglyceride, total cholesterol and LDL levels increased HDL levels in people with type 2 diabetes. Aljamal⁴⁹ reported that bay leaves reduced the total cholesterol, LDL and triglyceride levels and increased HDL levels in people with type 1 diabetes. Sutrisna *et al.*⁵⁰ found that bay leaf extract reduced serum cholesterol, triglyceride and LDL levels and increased HDL levels in hypercholesterolemia male rats of the Wistar strain.

The phenolic content of bay leaf ranges from 69.76-103.91 depending on the method of extraction⁵¹. Bay leaf contains flavonoids and glycosides, such as kaempferol, quercetin, apigenin, luteolin, quercetin 3-O- α -L-rhamnopyranoside, kaempferol-3-O- β -glucopyranoside, quercetin-3'-O- β -glucopyranoside, quercetin-3-O- β -galactoside, isorhamnetin-3-O- β -glucopyranoside, isorhamnetin-3-O- β -galactopyranoside, quercetin-3-O-rutinoside, kaempferol-3-O-rutinoside, isorhamnetin-3-O-rutinoside and isorhamnetin^{16,17}. Bay leaf has also been reported to contain flavonoids, saponin and steroid. *Sauropus androgynus* contains linolenic acid (31.75%), palmitic acid (12.14%), chlorophyll (9.33%), benzoic acid (8.58%) and alkaloid (7.2%)¹³, flavonoids, tannins, saponins, triterpenoids¹⁴ and phenols¹⁵.

Phenolic substances^{52,53} and flavonoids⁵⁴ inhibit LDL oxidation and decrease cholesterol and LDL levels by inhibiting HMG CoA reductase activity and oxidation of LDL⁵⁵. Tannin reduces cholesterol levels by inhibiting cholesterol absorption in the intestine⁵⁶. Saponin decreases the total cholesterol, triglyceride and LDL cholesterol levels and increases HDL cholesterol levels⁵⁷⁻⁵⁹ as a result of lipase inhibition^{60,61}, thus stimulating the activity of superoxide dismutase and improved lipid peroxidation⁶².

An increase in the level of HDL may be beneficial because Nofer *et al.*⁶³ documented its role in reverse cholesterol transport. HDL has recently been recognized to have several other important cardioprotective properties, including the ability to protect LDL from oxidative modification.

Santoso *et al.*¹¹ reported the following levels in broilers aged 35 days: cholesterol 113.8-126.3, TG 18.0-39.8, HDL 66.0-74.3, LDL 38.0-48.8 and VLDL 4-8.2 mg dL⁻¹. TG ranges from 104-224.28 mg dL⁻¹^{36,37,39}; cholesterol ranges from 184.25-221.65 mg dL⁻¹^{36,37,39}; HDL ranges from 80.0-151.6 mg dL⁻¹^{37,39} and LDL ranges from 45.8-72.4 mg dL⁻¹³⁷.

CONCLUSION

The inclusion of a medicinal herb mixture improves the lipid profile without modifying the hematologic status and lowers the gizzard and intestine weights in female broiler chickens.

SIGNIFICANCE STATEMENT

This study discovers the possible uses of a medicinal herb mixture as beneficial for reducing triglyceride, LDL and VLDL levels and LDL/HDL ratios; these findings indicate a lower risk of atherosclerosis occurrence in broiler chickens. This study helps to reveal the critical area of atherosclerosis problems and free antibiotic livestock practices that many researchers were unable to previously explore. Thus, a new theory may be created based on the usefulness of medicinal herb mixtures in lowering the risk of atherosclerosis occurrence and developing organic farming for broiler chickens. In addition, the possible natural medicines for hyperlipidemia in poultry and human beings might be developed.

ACKNOWLEDGMENTS

The authors would like to thank the Director General of Higher Education, the Ministry of Research, Technology and Higher Education, Indonesia and the Institute for Research and Community Service of the University of Bengkulu for administrative services during the study. The authors are also grateful to Anindita, Popi Purwanto, Syatri Utami and Yusti Ari Daniel Nababan for supporting this research work.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

REFERENCES

1. Jenkins, D.J.A., C.W.C. Kendall, M. Hamidi, E. Vidgen and D. Faulkner *et al.*, 2005. Effect of antibiotics as cholesterol-lowering agents. *Metabolism*, 54: 103-112.

2. Cao, J., K. Li, X. Lu and Y. Zhao, 2004. Effects of florfenicol and chromium (III) on humoral immune response in chicks. *Asian-Aust. J. Anim. Sci.*, 17: 366-370.

3. Liu, B., W. Li, Y. Chang, W. Dong and L. Ni, 2006. Extraction of berberine from rhizome of *Coptis chinensis* Franch using supercritical fluid extraction. *J. Pharm. Biomed. Anal.*, 41: 1056-1060.

4. Simitzis, P.E., S.G. Deligeorgis, J.A. Bizelis, A. Dardamani, I. Theodosiou and K. Feggeros, 2008. Effect of dietary oregano oil supplementation on lamb meat characteristics. *Meat Sci.*, 79: 217-223.

5. Santoso, U., M. Ishikawa and K. Tanaka, 2000. Effects of fermented chub mackerel extract on lipid metabolism of rats fed a high-cholesterol diet. *Asian-Aust. J. Anim. Sci.*, 13: 516-520.

6. Atawodi, S.E., J.C. Atawodi, G.A. Idakwo, B. Pfundstein and R. Haubner *et al.*, 2010. Evaluation of the polyphenol content and antioxidant properties of methanol extracts of the leaves, stem and root barks of *Moringa oleifera* Lam. *J. Med. Food*, 13: 710-716.

7. Moyo, B., S. Oyedemi, P.J. Masika and V. Muchenje, 2012. Polyphenolic content and antioxidant properties of *Moringa oleifera* leaf extracts and enzymatic activity of liver from goats supplemented with *Moringa oleifera* leaves/sunflower seed cake. *Meat Sci.*, 91: 441-447.

8. Santoso, U. and Sartini, 2001. Reduction of fat accumulation in broiler chickens by *Sauropus androgynus* (Katuk) leaf meal supplementation. *Asian-Aust. J. Anim. Sci.*, 14: 346-350.

9. Santoso, U., J. Setianto and T. Suteky, 2005. Effect of *Sauropus androgynus* (Katuk) extract on egg production and lipid metabolism in layers. *Asian-Aust. J. Anim. Sci.*, 18: 364-369.

10. Santoso, U. and Y. Fenita, 2016. The effect of *Sauropus androgynus* leaf extract on performance, egg quality and chemical composition of eggs. *J. Indones. Trop. Anim. Agric.*, 41: 125-134.

11. Santoso, U., Y. Fenita and E. Sulistyowati, 2017. Effect of *Sauropus androgynus* leaf extract, fish oil and vitamin E on performance, egg quality and composition in laying hens. *J. Indones. Trop. Anim. Agric.*, 42: 88-98.

12. Santoso, U., Y. Fenita and Kususiayah, 2015. Effect of fermented *Sauropus androgynus* leaves on blood lipid fraction and haematological profile in broiler chickens. *J. Indonesian Trop. Anim. Agric.*, 40: 199-207.

13. Samad, A.P.A., U. Santoso, M.C. Lee and F.H. Nan, 2014. Effects of dietary katuk (*Sauropus androgynus* L. Merr.) on growth, non-specific immune and diseases resistance against *Vibrio alginolyticus* infection in grouper *Epinephelus coioides*. *Fish Shellfish Immunol.*, 36: 582-589.

14. Santoso, U., Y. Fenita and Kususiayah, 2017. The effect of medicinal herb inclusion on hematologic status and blood lipid profiles in broiler chickens. *Int. J. Poult. Sci.*, 16: 415-423.

15. Andarwulan, N., R. Batari, D.A. Sandrasari, B. Bolling and H. Wijaya, 2010. Flavonoid content and antioxidant activity of vegetables from Indonesia. *Food Chem.*, 121: 1231-1235.

16. Ayoub, N.A., A.N. Hashim, S.A. Hussein, N.M. Hegazi, H.M. Hassanein and M.A. Nawwar, 2013. Hepatoprotective effect of bay leaves crude extract on primary cultured rat hepatocytes. *Eur. Scient. J.*, 2013: 647-655.
17. Abu-Dahab, R., V. Kasabri and F.U. Afifi, 2014. Evaluation of the volatile oil composition and antiproliferative activity of *Laurus nobilis* L. (Lauraceae) on breast cancer cell line models. *Records Nat. Prod.*, 8: 136-147.
18. Aljamal, A., 2011. Effects of bay leaves on the patients with diabetes mellitus. *Res. J. Med. Plant*, 5: 471-476.
19. Ari, M.M., B.A. Ayanwale, T.Z. Adama and E.A. Olatunji, 2012. Effects of different fermentation methods on the proximate composition, amino acids profile and some antinutritional factors in soybean (*Glycine max*). *Ferment. Technol. Bioeng.*, 2: 6-13.
20. Ibrahim, S.S., R.A. Habiba, A.A. Shatta and H.E. Embaby, 2002. Effect of soaking, germination, cooking and fermentation on antinutritional factors in cowpeas. *Food/Nahrung*, 46: 92-95.
21. Olaniyi, L.O. and S. Mehhizadeh, 2013. Effect of traditional fermentation as a pretreatment to decrease the antinutritional properties of rambutan seed (*Nephelium lappaceum* L.). *Proceedings of the International Conference on Food and Agricultural Sciences*, October 5-6, 2013, Melaka, Malaysia.
22. Olagunju, A.I. and B.O.T. Ifesan, 2013. Changes in nutrient and antinutritional contents of sesame seeds during fermentation. *J. Microbiol. Biotechnol. Food Sci.*, 2: 2407-2410.
23. Su, J.S., B.N. Liu, P.F. Tian, Q. Lin, Y.X. Zhao and X.Z. Ge, 2010. Effect of microbial fermentation on the extraction of alkaloids from radix aconiti and aconite. *J. Beijing Univ. Chem. Technol. (Nat. Sci. Edn.)*, 37: 97-101.
24. Sukrayana, Y., U. Atmomarsono, V.D. Yunianto and E. Supriyatna, 2011. Improvement of crude protein and crude fiber digestibility of fermented product of palm kernel cake and rice bran mixture for broiler. *J. Ilmu Teknologi Peternakan*, 1: 167-172.
25. Santoso, U., S. Ishikawa and K. Tanaka, 2001. Effect of fermented chub mackerel extract on lipid metabolism fed diets without cholesterol. *Asian-Austr. J. Anim. Sci.*, 14: 535-539.
26. Santoso, U., K. Tanaka, S. Ohtani and M. Sakaida, 2001. Effect of fermented product from *Bacillus subtilis* on feed conversion efficiency, lipid accumulation and ammonia production in broiler chicks. *Asian-Aust. J. Anim. Sci.*, 14: 333-337.
27. Santoso, U., S. Ishikawa and K. Tanaka, 2010. Effect of fermented chub mackerel extract on lipid metabolism of diabetic rats. *J. Indones. Trop. Anim. Agric.*, 35: 158-164.
28. Santoso, U., Y. Fenita, Kususiayah and I.G.N.G. Bidura, 2015. Effect of fermented *Sauropus androgynus* leaves on meat composition, amino acid and fatty acid compositions in broiler chickens. *Pak. J. Nutr.*, 14: 799-807.
29. Narahari, D., P. Michealraja, A. Kirubakaran and T. Sujatha, 2005. Antioxidant, cholesterol reducing, immunomodulating and other health promoting properties of herbal enriched designer eggs. *Proceedings of the 11th European Symposium on the Quality of Eggs and Egg Products*, May 23-26, 2005, Doorwerth, The Netherlands.
30. Coles, E.H., 1986. *Veterinary Clinical Pathology*. 4th Edn., W.B. Saunders Co., Philadelphia, London, ISBN: 978-0721618289, Pages: 486.
31. Schalm, O.W., N.C. Jain and E.J. Carroll, 1975. *Veterinary Haematology*. 3rd Edn., Lea and Febiger, Philadelphia.
32. Brown, B.A., 1976. *Direct Methods for Platelet Counts*, Rees and Ecker Method. In: *Hematology: Principles and Procedures*, Brown, B.A. (Ed.). 2nd Edn., Lea and Febiger, Philadelphia, USA., ISBN-13: 978-0812105339, pp: 101-103.
33. Kulkarni, K.R., 2006. Cholesterol profile measurement by vertical auto profile method. *Clin. Lab. Med.*, 26: 787-802.
34. Fossati, P. and L. Prencipe, 1982. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clin. Chem.*, 28: 2077-2080.
35. Brata, B., Y. Fenita, Nurmeiliasari and T. Yogaswara, 2015. Blood profile of layers in response to *Sauropus androgynus* leaves extract supplementation in fermented palm oil sludge based diet. *Proceedings of the International Seminar on Promoting Local Resources for Food and Health*, October 12-13, 2015, Bengkulu, Indonesia.
36. Adnyana, I.K., A. Rosmadi, J.I. Sigit and S.F. Rahmawati, 2012. The effect of *Sauropus androgynus* leaf juice, sweet potato leaf juice and kefir on hematology profile of aluminum sulfate-induced anemia mice. *Acta Pharm. Indones.*, 37: 54-58.
37. Attia, Y.A., A.E. Abd Al-Hamid, M.S. Ibrahim, M.A. Al-Harhi, F. Bovera and A. El-Naggar, 2014. Productive performance, biochemical and hematological traits of broiler chickens supplemented with propolis, bee pollen and mannan oligosaccharides continuously or intermittently. *Livest. Sci.*, 164: 87-95.
38. Beski, S.S.M. and S.Y.T. Al-Sardary, 2015. Effects of dietary supplementation of probiotic and synbiotic on broiler chickens hematology and intestinal integrity. *Int. J. Poult. Sci.*, 14: 31-36.
39. Bounous, D.J. and N.L. Stedman, 2000. *Normal Avian Hematology: Chicken and Turkey*. In: *Schalm's Veterinary Hematology*, Feldman, B.F. and J.C. Zinkl (Eds.). 5th Edn. Lea and Febiger, Philadelphia, USA., pp: 1147-1154.
40. Elsayed, M., A. Elkomy, M. Aboubakr and M. Morad, 2014. Tissue residues, hematological and biochemical effects of tilmicosin in broiler chicken. *Vet. Med. Int.*, Vol. 2014. 10.1155/2014/502872
41. Mushtaq, M., F.R. Durrani, N. Imtiaz, U. Sadique, A. Hafeez, S. Akhtar and S. Ahmad, 2012. Effect of administration of *Withania somnifera* on some hematological and immunological profile of broiler chicks. *Pak Vet. J.*, 32: 70-72.

42. Fenita, Y., U. Santoso, Kusuyah, J. Supriyadi, N. Sari and N. Adiyana, 2017. The effect of medicinal herb inclusion on performance, carcass quality and organoleptic profiles in broiler chickens. Proceedings of the National Seminar on the Management, Development and Utilization of Agricultural and Livestock Genetic Resources to Support Sustainable Food Availability, June 2017, Mulawarman University, Samarinda, Indonesia, (In Indonesian).
43. Fernandes, B.C.S., M.R.F.B. Martins, A.A. Mendes, E.L. Milbradt and C. Sanfelice *et al.*, 2014. Intestinal integrity and performance of broiler chickens fed a probiotic, a prebiotic, or an organic acid. *Braz. J. Poult. Sci.*, 16: 417-424.
44. Hu, Y., Y. Wang, A. Li, Z. Wang and X. Zhang *et al.*, 2016. Effects of fermented rapeseed meal on antioxidant functions, serum biochemical parameters and intestinal morphology in broilers. *Food Agric. Immunol.*, 27: 182-193.
45. Kamalia, A. Mujenisa and A. Natsir, 2014. [The Effect of addition different levels of katuk leaf meal in the ration on cholesterol, triglyceride, LDL and HDL of broiler blood]. *Buletin Nutrisi Makanan Ternak*, 10: 12-18.
46. Al-Samarrai, O.R., N.A. Naji and R.R. Hameed, 2017. Effect of bay leaf (*Laurus nobilis* L.) and its isolated (flavonoids and glycosides) on the lipids profile in the local Iraqi female rabbits. *Tikrit J. Pure Sci.*, 22: 72-75.
47. Belawa, T.G., Yadhya and I.W. Wirawan, 2014. The effect of corn meal substituted with sengauk in diets contain starpig supplemented by *Syzygium polyanthum* on feed efficiency and blood lipid profile of Kampoeng chicken. *Majalah Ilmiah Peternakan*, 17: 95-99.
48. Khan, A., G. Zaman and R.A. Anderson, 2009. Bay leaves improve glucose and lipid profile of people with type 2 diabetes. *J. Clin. Biochem. Nutr.*, 44: 52-56.
49. Aljamal, A., 2010. Effects of bay leaves on blood glucose and lipid profiles on the patients with type 1 diabetes. *World Acad. Sci. Eng. Technol.*, 69: 211-214.
50. Sutrisna, E., Y. Nuswantoro and R.F. Said, 2018. Hypolipidemic of ethanolic extract of Salam bark (*Syzygium polyanthum* (Wight) Walp.) from Indonesia (Preclinical study). *Drug Invent. Today*, 10: 55-58.
51. Verawati, D. Nofiandi and Petmawati, 2017. Effect of extraction method on total phenolic content and antioxidant activity of bay leaf (*Syzygium polyanthum* (Wight) Walp.). *J. Katalisator*, 2: 53-60.
52. Frankel, E.N., J.B. German, J.E. Kinsella, E. Parks and J. Kanner, 1993. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *Lancet*, 341: 454-457.
53. Kerry, N.L. and M. Abbey, 1997. Red wine and fractionated phenolic compounds prepared from red wine inhibit low density lipoprotein oxidation *in vitro*. *Atherosclerosis*, 135: 93-102.
54. Reed, J., 2002. Cranberry flavonoids, atherosclerosis and cardiovascular health. *Crit. Rev. Food Sci. Nutr.*, 42: 301-316.
55. Bentz, A.B., 2009. A review of quercetin: chemistry, antioxidant properties and bioavailability. *J. Young Investigators*, 9: 120-128.
56. Tebib, K., P. Besancon and J.M. Rouanet, 1994. Dietary grape seed tannins affect lipoproteins, lipoprotein lipases and tissue lipids in rats fed hypercholesterolemic diets. *J. Nutr.*, 124: 2451-2457.
57. Afrose, S., M.S. Hossain, T. Maki and H. Tsujii, 2010. Hypocholesterolemic response to Karaya saponin and *Rhodobacter capsulatus* in broiler chickens. *Asian-Aust. J. Anim. Sci.*, 23: 733-741.
58. Elekofehinti, O.O., J.P. Kamdem, I.J. Kade, J.B.T. Rocha and I.G. Adanlawo, 2013. Hypoglycemic, antiperoxidative and antihyperlipidemic effects of saponins from *Solanum anguivi* Lam. fruits in alloxan-induced diabetic rats. *South Afr. J. Bot.*, 88: 56-61.
59. Khan, N., M.S. Akhtar, B.A. Khan, V.D.A. Braga and A. Reich, 2015. Antiobesity, hypolipidemic, antioxidant and hepatoprotective effects of *Achyranthes aspera* seed saponins in high cholesterol fed albino rats. *Arch. Med. Sci.*, 11: 1261-1271.
60. Xu, B.J., L.L. Han, Y.N. Zheng, J.H. Lee and C.K. Sung, 2002. *In vitro* inhibitory effect of triterpenoidal saponins from platycodi radix on pancreatic lipase. *Arch. Pharm. Res.*, 28: 180-185.
61. Kimura, H., S. Ogawa, M. Jisaka, Y. Kimura, T. Katsube and K. Yokota, 2006. Identification of novel saponins from edible seeds of Japanese horse chestnut (*Aesculus turbinata* blume) after treatment with wooden ashes and their nutraceutical activity. *J. Pharm. Biomed. Anal.*, 41: 1657-1665.
62. Wang, T., R.C. Choi, J. Li, C.W. Bi and W. Ran *et al.*, 2012. Trillin, a steroidal saponin isolated from the rhizomes of *Dioscorea nipponica*, exerts protective effects against hyperlipidemia and oxidative stress. *J. Ethnopharmacol.*, 139: 214-220.
63. Nofer, J.R., B. Kehrel, M. Fobker, B. Levkau, G. Assmann and A. von Eckardstein, 2002. HDL and arteriosclerosis: Beyond reverse cholesterol transport. *Atherosclerosis*, 161: 1-16.