Effects of Supplementation of Alkaloid and Non Alkaloid from *Sauropus androgynus* Leaves on Egg Production and Lipid Profil in Layer Chicken

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Abstract. The present study was conducted to evaluate effects of supplementation of alkaloid or non alkaloid from *Sauropus androgynus* leaves on productive performance and the contents of lipid fractions in layer chickens. Forty two layer chickens aged 30 weeks were distributed to seven treatment groups. Each treatment group contained six layer chickens maintained in an individual cage, respectively. The present experiment used completely randomized experimental design. The seven treatment groups were as follows: 1) Control, layer chickens were fed diet without supplementation of alkaloid or non alkaloid extracted from *Sauropus androgynus* (P0); 2) Layer chickens were fed diet contained 30 mg non alkaloid/kg diet (P1); 3) Layer chickens were fed diet contained 60 mg non alkaloid/kg diet (P2); 4) Layer chickens were fed diet contained 90 mg non alkaloid/kg diet (P3); 5) Layer chickens were fed diet contained 30 mg alkaloid/kg diet (P4); 6) Layer chickens were fed diet contained 60 mg alkaloid/kg diet (P5); 7) Layer chickens were fed diet contained 90 mg alkaloid/kg diet (P6). Layer chickens were fed experimental diet with 2,750 kcal/kg Metabolizable Energy (ME) and 16.0% protein. Diet and drinking water were fed *ad libitum*. Experimental results showed that supplementation of alkaloid or non alkaloid from *Sauropus androgynus* leaves significantly affected productive performance in layer chickens. It appear that non alkaloid supplementation had no advantage in improving productive performance, whereas supplementation of 30 mg alkaloid/kg diet might have advantages in improving productive performance as indicated by better egg production and lower feed conversion ratio. Treatment had no effect on glucose and triglyceride concentration in serum, but it affected total cholesterol, HDL-cholesterol, LDL+VLDL-cholesterol and atherogenic index in serum. Cholesterol concentration in serum was significantly increased in P4 and P6, whereas HDL-cholesterol concentration was significantly increased in P1, P2, P3, P4 and P5 as compared with the control group. Atherogenic index was significantly lower in P1, P2, P3, P4 and P5 as compared with control group. Egg yolk content was significantly lower in P5 as compared with the control group. Fatty liver score was significantly lower in P1, P2, P4, P5 and P6 as compared with the control group. Abdominal fat was significantly lower in P1, P4 and P6 as compared with the control group. *Staphylococcus sp.* in eggshell was significantly reduced in layer chickens fed diet with 30 mg non alkaloid/kg diet or 90 mg alkaloid/kg diet, whereas *Escherichia coli* was not reduced by the treatments. In conclusion, both alkaloid and non alkaloid from *Sauropus androgynus* leaves were effective to reduce the risk of atherosclerosis occurrence. In addition, alkaloid was more effective in lowering egg cholesterol than non alkaloid. Both alkaloid and non alkaloid at a given level of supplementation reduced *Staphylococcus sp.* in eggshell.

Key Words: alkaloid, non alkaloid, *Sauropus androgynus*, atherogenix index, cholesterol

Introduction

Recently, egg industries were faced on the demand of eggs with low cholesterol. This demand was stimulated by experimental results that consuming cholesterol would increase serum cholesterol concentration (Santoso et al., 2001a; Santoso et al., 2005) and thus increasing the risk of atherosclerosis evident and coronary heart disease (Sparks, 2006). Many investigations have been conducted (e.g. Elkin and Yan, 1999; Elkin, 2007) to reduce cholesterol content in eggs. However, in many cases reduced in egg cholesterol was accompanied by reduced in egg production. Therefore, it is needed to find feed supplement which could reduce egg cholesterol without decreasing egg production. *Sauropus androgynus* leaves (Santoso and Sartini, 2001; Subekti, 2003) and its extract (Santoso et al.,

2005) were effective to reduce egg cholesterol without reducing egg production. This result was confirmed in broiler chickens (Santoso, 2001a,b).

Sauropus androgynus leaves were known to have antibacterial properties (Santoso, 2001c; Santoso et al., 2001b). This compound might be benefit to substitute antibiotics. The substitution of antibiotic in poultry diet might be a crucial item because antibiotics could cause bacteria resistancy to antibiotics (Barton and Hart, 2001). Antibiotics or its substitution such as antibacterial natural substance is an important feed additive in broiler or layer diet, because broiler meat and egg are one of the sources of *Salmonella* contamination of human (Van-Immerseel et al., 2009). However, which compounds in these leaves which have anticholesterol and anti-bacterial properties without reducing egg production was unknown.

Agustal et al. (1997) found that *Sauropus androgynus* leaves contained five main secondary compounds, namely monomethyl succinate, 2-phenylmalonic acid, cyclopentanol, 2 methyl-acetate, cis, benzoic acid, 2-pyrrolidinone and methylpyroglutamate. Bender and Ismail (1975) found papaverine in *Sauropus androgynus* leaves at value of 5.8 g/kg fresh leaves. Suprayogi (2000) found that *Sauropus androgynus* contained steroid (adrostan-17 one, 3-ethyl-3-hydroxy 5 alpha), 3,4-dimethyl-2-oxocyclopent-3-enylacetic acid, and polyunsaturated fatty acid. In addition, *Sauropus androgynus* also contained tannin, flavonoid, saponin and others. Such compounds could be classified to alkaloid and non-alkaloid. Therefore, the present study was conducted to evaluate effect of alkaloid and non alkaloid extracted from *Sauropus androgynus* leaves on egg production and lipid profile in layer chickens.

Materials and Methods

The present study was conducted in four steps, namely: 1) extraction of alkaloid and non alkaloid from *Sauropus androgynus* leaves, 2) layer maintenance, 3) laboratory and data analysis. Alkaloid and non alkaloid from *Sauropus androgynus* were extracted by the method as decribed by Sutedja et al. (1997).

Forty two layer chickens aged 30 weeks were distributed to seven treatment groups. Each treatment group contained six chickens, respectively, and maintained in individual cages for 5 weeks. Completely randomized experimental design was used in the present study. The seven treatment groups were as follows 1) Control, layer chickens were fed diet without alkaloid or non alkaloid extracted from *Sauropus androgynus* (P0); 2) Layer chickens were fed diet contained 30 mg non alkaloid/kg diet (P1); 3) Layer chickens were fed diet contained 60 mg non alkaloid/kg diet (P2); 4) Layer chickens were fed diet contained 90 mg non alkaloid/kg diet (P3); 5) Layer chickens were fed diet contained 30 mg alkaloid/kg diet (P4); 6) Layer chickens were fed diet contained 60 mg alkaloid/kg diet (P5); 7) Layer chickens were fed diet contained 90 mg alkaloid/kg diet (P6).

Layer chickens were fed experimental diet with 2,750 kcal/kg Metabolizable Energy (ME) and 16.0% protein. Drinking water was given *ad libitum*, whereas feed was given at level of 120 g/bird/day. Eggs were collected and weighed daily. At the end of experiment, the blood of four layer chickens in each treatment group was collected. The blood was then centrifuged at 3,000 rpm for 10 minutes to obtain serum. Serum was then analyzed for the concentration of triglyceride, glucose, total cholesterol and HDL-cholesterol. VLDL+LDL cholesterol was determined by the following equation: VLDL+LDL-c = total cholesterol – HDL-c. After blood collecting, all layer chickens were slaughtered, and abdominal fats were removed and weighed. Fatty liver score was also determined.

Four eggs in each treatment group were also collected. Cholesterol and triglyceride contents

of eggs were then determined (AOAC, 1980), whereas *Salmonella sp*., *Escherichia coli* and *Staphylococcus sp.* were determined by the method of Collins (1989). All data were analyzed by analysis of variance and if there were significantly different they were then determined by DMRT.

Table 1. Composition of basal diet (g/kg diet)

Feedstuff	Amount (kg)
Yellow corn	50.0
Rice bran	24.0
Mungbean meal	4.0
Soybean meal	4.0
Peanut meal	6.0
Fish meal	7.0
Palm oil	1.0
Bone meal	2.5
Calcium carbonate	1.0
Premix	0.5
Chemical composition	
Protein, %	16.5
Metabolizable energy (kcal/kg)	2,752.0

Results and Discussion

Effect of alkaloid or non alkaloid from *Sauropus androgynus* on productive performance in layer chickens are shown in Table 2. Experimental results showed that supplementation of alkaloid or non alkaloid from *Sauropus androgynus* leaves significantly affected productive performance in layer chickens. It appeared that non alkaloid supplementation had no advantage in improving productive performance, whereas supplementation of 30 mg alkaloid/kg diet might have advantages in improving productive performance as indicated by better egg production and lower feed conversion ratio. Agustal et al. (1997) alkaloid from *Sauropus androgynus* leaves is methylpyroglutamate, whereas Bender and Ismail (1975) show that this medical herb contained papaverin. Methylpyroglutamate might be hydrolyzed to produce the glutamate, glutamine or glutamic acid. These substances are very important in the metabolism of amino acid and ammonia in muscle and kidney cells (Munro, 1979). Besides, glutamate plays an important role in the inter conversions between amino acids (histidine, proline, glutamine and arginine) and the citric acid cycle through transamination in the gluconeogenesis process (Ganong, 1993). Glutamic acid is also involved in the biosynthesis of leukotrienes from arachidonic acid. Thus, it appears that the improvement of egg production might result from this mechanism.

Effect of alkaloid or non alkaloid from *Sauropus androgynus* on lipid fraction contents, abdominal fat and Fatty Liver Score in layer chickens are shown in Table 3. Abdominal fat was lower in P1, P4, and P6 as compared with the control group ($P<0.05$). P1, P4 and P6 had lower abdominal fat as compare with the control group at level of 51.3, 56.3 and 56.3%, respectively. Fatty Liver Score (FLS) was lower in P1, P2, P4, P5 and P6 as compared with the control group ($P<0.05$). P1, P2, P4, P5 and P6 had lower FLS at level of 19, 24, 19, 52.5 and 43%, respectively. Treatment had no effect on glucose and triglyceride concentration in serum, but it affected total cholesterol, HDL-cholesterol, LDL+VLDL-cholesterol and atherogenic index in serum ($P<0.05$). Cholesterol concentration in serum was significantly increased in P4 and P6, whereas HDL-cholesterol concentration was significantly increased in P1, P2, P3, P4 and P5 as compared with the control group. Atherogenic index was significantly lower in P1, P2, P3, P4 and P5 as compared with control group. Egg yolk content was significantly lower in P5 as compared with the control group. Fatty liver score was significantly lower in P1, P2, P4, P5 and P6 as compared with the control group.

Table 2. Effect of supplementation of alkaloid or non alkaloid from *Sauropus androgynus* leaves on productive performance in layer chicken

Variable	P_0	P_1	P_2	P_3	P_4	P_5	P_6	ANOVA
Week 1								
Egg production,%/bird	91.4^{c}	80.0^{b}	82.8^{b}	94.3^{c}	91.4^{c}	71.4^{a}	82.8^{b}	P<0.05
Egg production, g/bird	1830.1^{c}	1645.8^{b}	1672.2^{b}	1951.2^{c}	1778.8^{bc}	1392.7^{a}	1702.5^{b}	P<0.05
Feed consumption, g/bird	3.500	3.500	3.500	3.500	3.500	3.500	3.500	
Feed conversion	1.91	2.13	2.09	1.79	1.97	2.51	2.06	NS
Week 2								
Egg production,%/bird	87.1^{b}	78.6^{a}	85.7^{c}	88.6^{b}	90.0^{b}	77.1^{a}	81.4^{a}	P<0.05
Egg production, g/bird	3465.8^{b}	3273.3^{a}	3492.0^{b}	3616.9^{bc}	3519.6^{b}	3014.3^{a}	3345.3^{ab}	P<0.05
Feed consumption, g/bird	7.000	7.000	7.000	7.000	7.000	7.000	7.000	
Feed conversion	2.02	2.14	2.00	1.93	1.99	2.32	2.09	NS
Week 3								
Egg production, g/bird	4954.1	4523	5067	4982	4999	4118	4575	P<0.05
Feed consumption, g/bird	10.500	10.500	10.500	10.500	10.500	10.500	10.500	
Feed conversion	2.12	2.32	2.07	2.11	2.10	2.55	2.30	P<0.05
Week 4								
Egg production, g/bird	6.281.9^{bc}	5.551.0^{a}	6.225.3^{bc}	5.922.2^{b}	6.533.9^{c}	5.389.9^{a}	5.865.5^{b}	P<0.05
Feed consumption, g/bird	14.000	14.000	14.000	14.000	14.000	14.000	14.000	
Feed conversion	2.23^{a}	2.52^{b}	2.25^{a}	2.36^{ab}	2.14^{a}	2.60^{b}	2.39^{ab}	P<0.05
Week 5								
Egg production, g/bird	7.506.9^{c}	6.407.6^{a}	7.149.4^{b}	7.180.2^{b}	7.904.^{d}	6.416.1^{a}	6.911.3^{b}	P<0.05
Feed consumption, g/bird	17.500	17.500	17.500	17.500	17.500	17.500	17.500	
Feed conversion	2.33^{a}	2.73^{b}	2.45^{ab}	2.44^{ab}	2.21^{a}	2.73^{b}	2.53^{ab}	P<0.05

Values bearing different superscript at the same row differ significantly (P<0.05). NS = non significant.
P_0 = Diet without alkaloid or non alkaloid; P_1 = Diet with non-alkaloid of 30 mg/kg diet; P_2 = Diet with non-alkaloid of 60 mg/kg diet; P_3 = Diet with non-alkaloid of 90 mg/kg diet; P_4 = Diet with alkaloid of 30 mg/kg diet; P_5 = Diet with alkaloid of 60 mg/kg diet; P_6 = Diet with alkaloid of 90 mg/kg diet. [1] represents 6 birds.

It appeared that the effect of alkaloid and non alkaloid from *Sauropus androgynus* leaves was doses dependent. Both alkaloid and non alkaloid supplementation might reduce the risk of atherosclerosis occurrence as indicated by lower atherogenic index except for P6. It appeared that supplementation of alkaloid above 60 mg/kg diet might increase the risk of atherosclerosis occurrence. The lower risk of atherosclerosis occurrence could be explained by higher HDL-cholesterol concentration in serum rather than serum total cholesterol concentration. As shown in Table 3 alkaloid or non alkaloid extracted from *Sauropus androgynus* leaves had no antidiabetic properties.

Alkaloid was more effective in lowering egg cholesterol than non alkaloid from *Sauropus androgynus*. It appeared that lower cholesterol of egg in P5 was not caused by lower serum cholesterol, because serum cholesterol was not reduced by the treatments. Another possible mechanism of lower egg cholesterol was higher excretion of cholesterol through bile acid. However, it appeared that both alkaloid and non alkaloid was not effective in lowering egg cholesterol as compared with *Sauropus androgynus* leaves extract (Santoso et al., 2005). These investigators found that the extract reduced egg cholesterol at level of 40%, whereas supplementation of 60 mg alkaloid reduced egg cholesterol at level of 22%. Therefore, *Sauropus androgynus* leaves extract might contain several active compounds which had antilipid properties. Food and Drug Administration of the United States (1997) stated that changing in nutrient content could be claimed to have significant value in animal industries if that nutrition content decreased or increased above 25%. Therefore, supplementation of alkaloid or non alkaloid extracted from *Sauropus androgynus* leaves

Table 3. Effect of supplementation of alkaloid or non alkaloid from *Sauropus androgynus* leaves on the contents of fat, glucose, abdominal fat in layer chickens (week 5)

Variable	P_0	P_1	P_2	P_3	P_4	P_5	P_6	ANOVA
Serum, mg/dl								
Glucose	91.0	82.5	87.0	90.0	100.5	91.5	80.5	NS
Cholesterol	77.0^{a}	90.5^{ab}	77.5^{a}	72.5^{a}	100.5^{b}	88.5^{ab}	100.0^{b}	P<0.05
Triglyceride	204	209.5	197.0	193.0	203.0	211.0	206.5	NS
HDL-cholesterol	13.5^{a}	21.0^{b}	18.5^{b}	22.5^{b}	19.0^{b}	26.0^{c}	14.5^{a}	P<0.05
LDL+VLDL-Cholesterol	66.5^{b}	69.5^{b}	59.0^{ab}	50.0^{a}	81.5^{c}	62.5^{ab}	86.5^{c}	P<0.05
Atherogenic index	4.70^{c}	3.31^{b}	3.19^{b}	2.22^{a}	4.29^{bc}	2.40^{a}	5.96^{d}	P<0.05
Egg, mg/g yolk								
Cholesterol	16.80	16.87	15.97	18.75	17.47	13.20	18.0	NS
Triglyceride	52.2	60.75	51.9	57.52	54.45	50.77	57.45	NS
Egg, mg/yolk								
Cholesterol	262.1^{b}	258.1^{b}	231.6^{ab}	261.6^{b}	225.4^{ab}	203.3^{a}	275.4^{b}	P<0.05
Triglyceride	814.3	929.5	752.6	802.4	702.4	781.9	879.0	NS
Fatty liver score	2.63^{b}	2.13^{a}	2.0^{a}	3.0^{b}	2.13^{a}	1.25^{a}	1.5^{a}	P<0.05
Abdominal fat, % BW	2.38^{b}	1.16^{a}	2.39^{b}	2.43^{b}	1.04^{a}	2.4^{b}	1.04^{a}	P<0.05

Values bearing different superscript at the same row differ significantly (P<0.05). NS = non significant.
P_0 = Diet without alkaloid or non alkaloid; P_1 = Diet with non-alkaloid of 30 mg/kg diet; P_2 = Diet with non-alkaloid of 60 mg/kg diet; P_3 = Diet with non-alkaloid of 90 mg/kg diet; P_4 = Diet with alkaloid of 30 mg/kg diet; P_5 = Diet with alkaloid of 60 mg/kg diet; P_6 = Diet with alkaloid of 90 mg/kg diet. [1] represents 6 birds.

Table 4. Effect of supplementation of alkaloid or non alkaloid from *Sauropus androgynus* leaves on amount of microorganism in eggshell (week 2) (cell/egg)

Variable	P_0	P_1	P_2	P_3	P_4	P_5	P_6	ANOVA
Salmonella sp	0	0	0	0	0	0	0	
Escehrichia coli	7000^{a}	7200^{a}	27100^{bc}	34000^{c}	6800^{a}	4500^{a}	18250^{b}	P<0.001
Staphylococcus sp	1700^{b}	600^{a}	9650^{e}	8500^{d}	6700^{d}	2300^{c}	400^{a}	P<0.05

Values bearing different superscript at the same row differ significantly (P<0.05).
P_0 = Diet without alkaloid or non alkaloid; P_1 = Diet with non-alkaloid of 30 mg/kg diet; P_2 = Diet with non-alkaloid of 60 mg/kg diet; P_3 = Diet with non-alkaloid of 90 mg/kg diet; P_4 = Diet with alkaloid of 30 mg/kg diet; P_5 = Diet with alkaloid of 60 mg/kg diet; P_6 = Diet with alkaloid of 90 mg/kg diet. [1] represents 4 birds.

had no significant value for reducing egg cholesterol in egg industries.

The present study showed that alkaloid or non alkaloid from *Sauropus androgynus* was inconsistence in decreasing pathogenic microorganisms in eggshell (Table 4). *Escherichia coli* was significantly higher in treatment groups except in P1, P4 and P5, whereas *Staphylococcus sp.* was significantly lower in P1 and P6 as compared with the control. The present study did not agree with the previous study (Santoso, 2001c; Santoso et al., 2001b) who found that *Sauropus androgynus* leaves extract had antibacterial properties. Further study is needed to elucidate an active compound in *Sauropus androgynus* which have antibacterial properties. No *Salmonella sp.* was found in eggshell. Eggshell contaminated with *Salmonella sp.* might result from either oviduct or fecal contamination (Reu et al., 2008).

Conclusions

In conclusion, both alkaloid and non alkaloid from *Sauropus androgynus* leaves were effective to reduce the risk of atherosclerosis occurrence. In addition, alkaloid was more effective in lowering egg cholesterol than non alkaloid. Both alkaloid and non alkaloid at a given level of supplementation reduced *Staphylococcus sp.* in eggshell.

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