Effect of Sauropus Androgynus (Katuk) Extract on Egg Production and Lipid Metabolism in Layers*

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ABSTRACT : The present study was conducted to evaluate effect of *Sauropus androgynus* extract (SAE) on egg production and lipid metabolism in layer chickens. Forty-eight layers aged 42 weeks (strain RIR) were distributed to 6 treatment groups as follows. One group was fed diet without SAE as the control (P_0), and other five groups were fed diet plus hot water-extracted SAE at level of 9 g/kg diet (W_9), diet plus ethanol extracted SAE at level of 0.9 g/kg diet ($E_{0.9}$), diet plus ethanol extracted SAE at level of 1.8 g/kg ($E_{1.8}$), diet plus methanol extracted SAE at level of 0.9 g/kg ($M_{0.9}$), and diet plus methanol extracted SAE at level of 1.8 g/kg ($M_{1.8}$). It was shown that SAE inclusion significantly increased egg production ($p<0.05$). Methanol-extracted SAE groups had lower egg production than ethanol-extracted SAE group ($p<0.05$). SAE supplemented groups had better feed conversion efficiency than the unsupplemented group ($p<0.05$). It was shown that ethanol extracted SAE resulted in the lowest feed conversion efficiency among the SAE supplemented groups ($p<0.05$). SAE supplementation significantly reduced abdominal fat, gizzard surrounded fat, liver fat ($p<0.05$), serum triglyceride, total cholesterol, VLDL+LDL-c ($p<0.01$), atherogenic index ($p<0.05$), egg cholesterol and triglyceride ($p<0.05$), but it had no effect on mesenteric fat, sartorial fat and fatty liver score. In conclusion, SAE supplementation could increase egg production but reduced egg cholesterol. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 3 : 364-369)***

Key Words : Egg Production, *Sauropus androgynus*, Cholesterol, Atherogenic Index

INTRODUCTION

There is evidence that poultry industries are faced on the problem of falling demand for eggs (Paik and Blair, 1996; USDA, 1997). This tendency is caused by the possibility of the increased risk of cardiovascular and associated diseases that may increase from the consumption of egg cholesterol (Sim, 1984; Voet and Voet, 1990). These diseases are the major mortality reason in all Western civilizations and other developed countries. Therefore, it is urgent to reduce cholesterol content of eggs. However, when some efforts have successfully reduced egg cholesterol it is followed by a significant reduction in egg production (Paik and Blair, 1996).

Traditional drug, synthetic chemists and medical herbs have been proven to be effective to decrease triglyceride, cholesterol, crude fat and lipogenic-related enzyme activities (Santoso et al., 2001a,b; Santoso and Sartini, 2001; Shim et al., 2004) and to increase body weight gain (Santoso et al., 2001b). However, synthetic chemists was believed to have greater side effect (e.g. impaired humoral immune) as compared with natural chemists (Cao et al., 2004).

Sauropus androgynus (Katuk) might be potential to modify fat metabolism in layers without reducing egg production. This hypothesis is supported by the fact that *Sauropus androgynus* is rich in benzoic acid (Agustal et al., 1997) that might be able to converted to benzoic estradiol. Benzoic estradiol is known to stimulate follicle growth and to improve reproduction (Siswandono and Soekardjo, 1995). In addition, Santoso and Sartini (2001) found that *Sauropus androgynus* leaf meal supplementation at level of 3% resulted in lower fat deposition in abdomen and carcass of broiler chickens and better feed conversion efficiency. Furthermore, Santoso et al. (2001) also found that in broiler chickens, *Sauropus androgynus* extract (SAE) inclusion at level of 4.5 g/drinking water also reduced fat accumulation in abdomen, neck and surrounded gizzard with better feed conversion efficiency. Similar results were found when SAE was supplemented to diet at level of 18 g/kg (Santoso, 2000a,b,c). In addition, feeding method (through feed vs. drinking water) of SAE also affected the responses of broiler chickens to SAE addition (Santoso et al., 2002b). In layer chicken, SAE supplementation significantly improved egg quality (Santoso et al., 2002a).

No study of effect of SAE on egg production and fat metabolism in layers has been published elsewhere. Therefore, the present study was conducted to evaluate effect of SAE on egg production and fat metabolism in layers.

MATERIALS AND METHOD

Forty-eight layers aged 40 weeks (strain RIR) were distributed to 6 treatment groups as follows. One group was

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Table 1. Composition of experimental diet

Feedstuff	
Yellow corn (%)	50.0
Soybean meal (%)	14.0
Rice bran (%)	20.0
Fish meal (%)	7.0
Oil (%)	2.0
Calcium carbonate (%)	3.5
Mineral mix[1] (%)	3.0
Premix[2] (%)	0.5
Calculated analysis	
Metabolizable energy (kcal/kg)	2,850.0
Protein (%)	16.5

[1] Commercial mineral mixture contained 32.5% calcium and 10% phosphor.

[2] Supplied per kg diet 6.000 IU vitamin A, 1.000 IU vitamin D_3, 4 IU vitamin E, 1 mg vitamin B_1, 2.5 mg vitamin B_2, 0.25 vitamin B_6, 1 mg vitamin K, 1.25 mg vitamin C, 20 mg Niacin, 10 mg iron, 3 mg Ca-D Panto-thenate, 5 mg choline chloride, 15 mg methionine, 60 mg manganese, 0.1 mg iodine, 50 mg zinc, 0.1 mg cobalt, 2 mg cuprum, 5 mg santoquin, 10.5 mg zinc bacitracine.

fed diet without SAE as the control (P_0), and other five groups were fed diet plus hot water-extracted SAE at level of 9 g/kg diet (W_9), diet plus ethanol extracted SAE at level of 0.9 g/kg diet ($E_{0.9}$), diet plus ethanol extracted SAE at level of 1.8 g/kg ($E_{1.8}$), diet plus methanol extracted SAE at level of 0.9 g/kg ($M_{0.9}$), and diet plus methanol extracted SAE at level of 1.8 g/kg ($M_{1.8}$). The composition of basal diet is presented in Table 1. All layers were given drinking water *ad libitum*, and they were fed diets for 100 g daily. They were raised in stainless steel individual cages with continuous lighting, water was provided *ad libitum*, whereas diet was provided 100 g/head/day. Layers were provided SAE for 10 weeks.

Hot water extracted SAE was prepared as described by Santoso et al. (2001). *Sauropus androgynus* leaf (waste leaf which is not consumable) was collected from the field, dried under the sun, and mixed with water (one kilogram of leaf was mixed in 5 liters of water). The mixture was boiled at 90°C for 20 minutes, filtered and the supernatant obtained was the dried at 55°C for 36 h. The dried extract was then stored in a sealed plastic bag.

Ethanol extracted SAE was prepared as described by Darise and Wiryowidagdo (1997). Dried *Sauropus androgynus* leaf was percolated using a maceration technique with 96% alcohol as a solvent. The leaf was mixed with ethanol 96% (one kilogram of leaf was macerated in 10 liters of ethanol) and then stirred for about 6 h. After this, the mixture was stored for 18 h. The mixture was filtered and the residue was percolated in the same way until the solvent appeared clear. The filtrate was then evaporated using rotary-evaporator.

Methanol extracted SAE was prepared as described by Risfaheri et al. (1997). Dried *Sauropus androgynus* leaf was percolated using a maceration technique with methanol as a solvent. The leaf was mixed with methanol (one kilogram of leaf was macerated in 10 liters of ethanol) and then stirred for about one hour. After this, the mixture was stored for 5 days. The mixture was stirred every 24 h. The mixture was filtered and the residue was percolated in the same way until the solvent appeared clear. The filtrate was then evaporated using rotary-evaporator.

Egg production, feed intake and feed conversion efficiency were calculated. At 50 weeks of age, 4 layers of each group were selected by weight, and the blood was collected through wing vein. Blood was then centrifuged at 600×g for 10 minutes. The concentration of triglyceride, total cholesterol and HDL-cholesterol were measured by the procedure of manufacturer using commercial kits (Bio System SA, Spain). VLDL+LDL-cholesterol was calculated using the following equation: Total cholesterol-HDL-cholesterol. To estimate the risk of atherosclerosis, the atherogenic index was calculated as follows:

$$\frac{\text{Total Cholesterol - HDL-cholesterol}}{\text{HDL-cholesterol}}$$

After collecting blood, layers were then slaughtered and four areas of adipose tissue (abdomen, gizzard, mesenteric and sartorial), liver, gizzard, spleen, heart, intestine and caecum were removed and weighed. Liver fat was measured by the method of AOAC (1980). Four eggs from each treatment groups were collected and egg yolk was then separated. Egg yolk was diluted with distilled water (1:11, w/w), and the contents of yolk cholesterol and triglyceride were then measured by the procedure of manufacturer using commercial kits (Bio System SA, Spain).

All data were analyzed using ANOVA, and orthogonal contrast was used if there was significantly different at $p<0.05$.

RESULTS

Table 2 shows effect of SAE on egg production and feed

Table 2. Effect of *Sauropus androgynus* extract (SAE) on egg production and feed conversion ratio in layers

Variable	P_0	W_9	$E_{0.9}$	$E_{1.8}$	$M_{0.9}$	$M_{1.8}$	SD	ANOVA
Henday egg, %	47.1	53.2	57.8	63.5	56.1	48.7	3.4	$p<0.05$
, g/bird	1,942	2,186	2,625	2,705	2,362	2,018	50.5	$p<0.05$
Feed conversion ratio	3.63	3.30	2.70	2.62	2.98	3.48	0.25	$p<0.05$

P_0=without SAE; W_9=hot-water SAE at 9 g/kg; $E_{0.9}$=ethanol-SAE at level 0.9 g/kg.

$E_{1.8}$=ethanol-SAE at level of 1.8 g/kg; P_4=methanol SAE at level of 0.9 g/kg; P_5=methanol SAE at level of 1.8 g/kg.

Table 3. Result of orthogonal contrast of egg production and feed conversion ratio

Variables	P_0 vs. W_9, $E_{0.9}$, $E_{1.8}$, $M_{0.9}$, $M_{1.8}$	W_9 vs. $E_{0.9}$, $E_{1.8}$, $M_{0.9}$, $M_{1.8}$	$E_{0.9}$, $E_{1.8}$ vs. $M_{0.9}$, $M_{1.8}$	$E_{0.9}$ vs. $E_{1.8}$	$M_{0.9}$ vs. $M_{1.8}$
Henday egg, %	*	NS	*	NS	NS
, g	*	*	*	NS	NS
Feed conversion ratio	*	*	*	NS	NS

P_0=without SAE; M_9=hot-water SAE at 9 g/kg; $E_{0.9}$=ethanol-SAE at level 0.9 g/kg.
$E_{1.8}$=ethanol-SAE at level of 1.8 g/kg; $M_{0.9}$=methanol SAE at level of 0.9 g/kg; $M_{1.8}$=methanol SAE at level of 1.8 g/kg.

Table 4. Effect of *Sauropus androgynus* extract on fat accumulation, serum lipid fractions and egg cholesterol and triglyceride in layer

Variable	P_0	W_9	$E_{0.9}$	$E_{1.8}$	$M_{0.9}$	$M_{1.8}$	SD	ANOVA
Abdominal fat (g)	24.0	9.7	18.3	11.5	7.4	11.6	3.8	p<0.05
Gizzard surrounded fat (g)	9.8	4.4	6.1	3.5	4.0	5.6	1.3	p<0.05
Mesenteric fat (g)	5.7	3.2	4.0	2.2	2.3	3.8	1.2	NS
Sartorial fat (g)	5.2	2.7	4.2	3.3	2.2	3.4	2.1	NS
Hepatic fat (%)	5.2	3.8	4.2	4.6	3.3	3.5	0.6	p<0.05
Fatty liver score	2.9	2.1	2.3	2.6	1.8	1.9	0.8	NS
Serum (mg/dl)								
- triglyceride	140	95.3	135.6	181.0	103.2	147.5	12.5	p<0.01
- total cholesterol	120.7	98.7	160.4	149.1	121.9	105.2	15.2	p<0.01
- HDL-c	55.6	59.5	66.1	66.8	60.6	63.2	4.0	p<0.01
- VLDL+LDL-c	65.0	39.0	94.1	82.1	60.9	41.8	5.3	p<0.01
Atherogenic index	1.17	0.66	1.43	1.23	1.01	0.67	0.03	p<0.05
Egg (mg/g wet yolk)								
- triglyceride	67.8	63.5	58.9	73.1	48.5	57.5	7.5	p<0.05
- cholesterol	21.6	12.9	11.7	16.4	19.6	22.1	4.3	p<0.01

P_0=without SAE; W_9=hot-water SAE at 9 g/kg; $E_{0.9}$=ethanol-SAE at level 0.9 g/kg.
$E_{1.8}$=ethanol-SAE at level of 1.8 g/kg; $M_{0.9}$=methanol SAE at level of 0.9 g/kg; $M_{1.8}$=methanol SAE at level of 1.8 g/kg.

Table 5. Result of orthogonal contrast of egg production and feed conversion ratio

Variables	P_0 vs. W_9, $E_{0.9}$, $E_{1.8}$, $M_{0.9}$, $M_{1.8}$	W_9 vs. $E_{0.9}$, $E_{1.8}$, $M_{0.9}$, $M_{1.8}$	$E_{0.9}$, $E_{1.8}$ vs. $M_{0.9}$, $M_{1.8}$	$E_{0.9}$ vs. $E_{1.8}$	$M_{0.9}$ vs. $M_{1.8}$
Abdominal fat (g)	*	NS	NS	NS	NS
Gizzard surrounded fat (g)	*	NS	NS	NS	NS
Hepatic fat (%)	*	NS	NS	NS	*
Serum (mg/dl)					
- triglyceride	NS	*	***	*	**
- total cholesterol	NS	*	***	NS	*
- HDL-c	*	*	****	NS	NS
- VLDL+LDL-c	NS	*	***	NS	*
Atherogenic index	*	*	*	*	*
Egg (mg/g)					
- triglyceride	NS	NS	*	*	NS
- cholesterol	*	*	*	*	NS

P_0=without SAE; W_9=hot-water SAE at 9 g/kg; $E_{0.9}$=ethanol-SAE at level 0.9 g/kg.
$E_{1.8}$=ethanol-SAE at level of 1.8 g/kg; $M_{0.9}$=methanol SAE at level of 0.9 g/kg; $M_{1.8}$=methanol SAE at level of 1.8 g/kg.

conversion efficiency. It was shown that SAE inclusion significantly affected egg production ($p<0.05$). SAE inclusion significantly increased egg production ($p<0.05$) (Table 3). Methanol-extracted SAE groups had lower egg production than ethanol-extracted SAE group ($p<0.05$). It was shown that SAE supplementation significantly affected feed conversion efficiency ($p<0.05$). SAE supplemented groups had lower feed conversion ratio than the unsupplemented group ($p<0.05$). It was shown that ethanol extracted SAE resulted in the lowest feed conversion efficiency among the SAE supplemented groups ($p<0.05$).

Table 4 shows effect of SAE on fat profiles in layers. SAE supplementation significantly affected abdominal fat, gizzard surrounded fat, liver fat ($p<0.05$), serum triglyceride, total cholesterol, VLDL+LDL-cholesterol ($p<0.01$), atherogenic index ($p<0.05$), egg cholesterol and triglyceride ($p<0.05$), but it had no effect on mesenteric fat, sartorial fat and fatty liver score. SAE supplementation significantly reduced abdominal fat, gizzard surrounded fat, hepatic fat as compared with unsupplemented group (Table 5), whereas it increased HDL-cholesterol ($p<0.01$).

DISCUSSION

The main secondary metabolic compounds in katuk extract were monomethyl succinate, cis-2-methyl-

cyclopenthanol acetate, benzoic acid, phenyl malonic acid, methylpyroglutamate and 2-pyrolidinone (Agustal et al., 1997). In addition, Suprayogi (2000) found that katuk leaf contained androstan-17-one, 3-ethyl-3-hydroxy-5 alpha (steroid), 3,4-dimethyl-2-oxocyclopent-3-enylacetic acid and polyunsaturated fatty acids such as octadecanoic acids, 9-eicosyne, 5,8,11-heptadecatrienoic acid ethyl ester, 11,14,17-eicosatrienoic acid methyl ester. The increase in egg production might be stimulated by an increase in follicle growth and improved reproduction. Benzoic acid might be converted to benzoic-estradiol (Siswandono and Soekardjo, 1995). Furthermore, they stated that benzoic estradiol improved the function of reproduction and stimulated the growth of follicle and FSH. In addition, androstan-17-one, 3-ethyl-3-hydroxy-5 alpha will most probably be converted to the estradiol, estrone and progresteron in females or might also be changed to the mineralocorticoid (corticosterone, aldosteron) or glucocorticoid (corsisone, cortisol) (Suprayogi, 2000). These hormones regulate repdoduction, growth, lactation and other physiological process.

It was also assumed that continuous supplementation of SAE might improve the balance of microorganism in digestive tract by lowering pathogenic microorganisms such as *Escherichia coli* and *Salmonella sp.* (Santoso et al., 2001; Santoso et al., 2002a). *Salmonella typhosa* and *Staphylococcus aureus* (Darise and Sulaeman, 1997) and increasing effective microorganism such as *Lactobacillus sp.* and *Bacillus subtilis* (Santoso et al., 2001). It was known that increasing number of *Bacillus subtilis* (Santoso et al., 1995) and/or *Lactobacillus sp.* improved feed conversion efficiency.

Monomethyl succinate, cis-2-methyl-cyclopenthanol acetate, 3,4-dimethyl-2-oxocyclopent-3-enylacetic acid, phenylmalonic acid and methylpyroglutamate might be hydrolyzed to the succinate, acetate, malonic acid and glutamate, respectively. Succinate, malonic acid and acetate are involved in the sequence of reaction in the citric acid cycle. (Rodwell, 1988; Voet and Voet, 1990). Glutamate plays important role in the interconversion between amino acids (histidine, proline, glutamine and arginin) and the citric acid cycle through transamination in the gluconeogenesis (Ganong, 1993). Therefore, it is logical if SAE supplementation improved feed efficiency. SAE extracted by ethanol resulted in the best performance. Quantifying the main compounds is needed to elucidate this phenomenon. It was found that method of extraction affected egg production. Ethanol-extracted SAE had the highest value. It was hypothesized that ethanol-extracted SAE might contain the highest value of benzoic acid and androstan-17-one, 3-ethyl-3-hydroxy-5 alpha (steroid).

Considerable efforts have been made to reduce cholesterol content in chicken egg with little success (Noble et al., 1990; Michella and Slaugh, 2000). Some investigators succeeded to reduce egg cholesterol but it was followed by the reduction of egg production. However, the present study showed that the supplementation of hot water extracted SAE or ethanol-extracted SAE at level 0.9 g/kg drastically reduced egg cholesterol with better egg production. This occurrence might result from the synergism of active compounds in SAE. It means that certain compounds in SAE improved egg production, whereas others reduced egg cholesterol without any negative effects on egg production. It is needed further study to elucidate active compounds that influence cholesterol metabolism, and the mechanisms. Shim et al. (2004) found that the reduction of serum cholesterol by medical herb (e.g. *Codonopsis lanceolata*) was partly caused by the elevation of biliary excretion of cholesterol.

The reduction in cholesterol content of eggs found in layers fed diet supplemented hot-water SAE or ethanol-SAE at level of 0.9 g/kg, up to 40%, is probably significant in practical terms to the poultry industry, because the Food and Drug Administration in the United States (1997) has stated that for a product to legally claim "less" or "reduced amount of a nutrient", it has to have 25% less than the normal amount of that nutrient. However, normal content of egg cholesterol in control group was higher as compared with the observation of Hall et al. (1992) who found that egg cholesterol of RIR ranged from 15.15 to 18.50 mg/g wet yolk.

It is interest to note that lowering effect of SAE on cholesterol was influenced by the method of extraction. SAE extracted by hot water reduced both serum and egg cholesterol, whereas SAE extracted by ethanol (0.9 g/kg) resulted in lower egg cholesterol with higher serum cholesterol. These suggested that the concentration of active compounds extracted was affected by the method of extraction. It was also found that level of SAE affected both serum and egg cholesterol.

Another interesting result was SAE extracted by hot water or by methanol (1.8 g/kg) reduced serum VLDL+LDL-cholesterol with higher HDL-cholesterol, whereas ethanol extracted SAE increased HDL-cholesterol. From these results, SAE might be able to be used to reduce the risk of atherosclerosis. This was confirmed by lower atherogenic index in the present study. Further study is needed to elucidate the effect of SAE on atherosclerosis. It was unknown why ethanol extracted SAE caused higher serum cholesterol and VLDL+LDL-cholesterol concentrations with lower egg cholesterol. The latest fact suggested that cholesterol distribution from serum to egg was inhibited.

The reduction of abdominal fat, gizzard surrounded fat and liver fat by SAE confirmed previous investigations (Santoso and Sartini, 2001; Santoso, 2001b; Santoso et al.,

2002b). These authors found that feeding SAE or *Sauropus androgynus* leaf meal reduced fat accumulation in the body, abdomen and liver. However, the concentration of serum tryglyceride was influenced by the method of extraction. Hot-water extracted SAE reduced serum triglyceride, ethanol extracted SAE at level of 1.8 g/kg increased serum triglyceride, whereas methanol extracted SAE had no effect. This difference might relate to the different active compounds concentration in each extract. The main active compound affected fat accumulation might be methyl-pyroglutamate which is converted to glutamate. The stimulation of protein synthesis by glutamate may reduce substrate supply for fatty acid synthesis. Furthermore, glutamate could further be converted to L-arginine and then to nitric oxide. Nitric oxide could block LDL oxidation and reduced atherosclerosis (Cooke, 1998; Drexler, 1999; Napoli and Ignarro, 2001).

The remainder of methyl groups resulted from degradation of methylpyroglutamate, monomethyl succinate and cis-2-methyl-cyclopenthanol acetate might also have an important role in biochemical reaction as exogenous methyl sources in the body.

CONCLUSION

The present study concluded that supplementation of hot water extracted SAE or ethanol extracted SAE improved egg production and feed conversion efficiency, and reduced egg cholesterol.

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