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Research Article
Effect of Water Extract of Two Leaves (*Allium sativum* and *Sauropus androgynus*) on Egg Production and Yolk Cholesterol Levels in Egg Laying Hens

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Abstract
Objective: The present study was conducted to determine the effects of sweet leaf (*Sauropus androgynus*) and garlic (*Allium sativum*) leaves water extract on the egg production and yolk cholesterol level of 30-week-old hens. **Materials and Methods:** Ninety 30-week-old hens were colony caged in an environmentally controlled house to evaluate the effect of garlic and sweet leaf (Katuk, Indonesian) leaves water extract administration on hens. Sweet leaf and garlic extract were prepared by macerating sweet leaf and garlic leaves in distilled water (1:1, w/w). Hens were randomly divided into three equal groups: one served as a control and was administered with drinking water only. The other two groups were administered 5% water extract of sweet leaf or garlic leaves, respectively. **Results:** Garlic and sweet leaf water extract increased egg production ($p<0.05$), but not the efficiency of feed consumption ($p>0.05$). Garlic or sweet leaf water extract administration results in lower ($p<0.05$) serum and yolk cholesterol contents. **Conclusion:** Garlic or sweet leaf water extract increased egg production but decreased serum and yolk cholesterol contents of egg laying hens.

Key words: Garlic, sweet leaf (Katuk), cholesterol, egg laying hens

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

INTRODUCTION

Consumers desire low cholesterol animal product such as eggs because high cholesterol levels can cause atherosclerosis. Eggs are a delicate food product and lose quality rapidly during the period between collection and consumption. Thus, improving and extending egg shelf life is important to breeders and other poultry researchers. Numerous efforts have been made to lower the cholesterol content of eggs. Decreasing egg yolk cholesterol would be beneficial to the poultry industry and public health[1].

Studies on the efficacy of the use of plants to improve the quality of livestock production is very important because it can increase the diversity of plant resources and is the basis of economic botany. The plant leaves Katuk (*Sauropus androgynus*) and garlic (*Allium sativum*) are traditional medicinal plants and spices. They also have medical and antibacterial properties and contain beta-carotene as an active ingredient to color egg yolks. Katuk leaves contain phytochemical compounds with remarkable physiological functions. Preliminary observations indicate that the phytochemical compounds contained in Katuk leaves are flavonoids, saponins, sterols, quinone and tannins[2].

Garlic (*Allium sativum*) can prevent and treat many diseases. It has been broadly dispersed and consumed as a spice and herbal medicine for thousands of years. Recent studies have validated many of the medicinal properties attributed to garlic and its potential to lower the risk of diseases[1]. It been used for its antibiotic, antiviral, antibacterial and antifungal qualities. It is a potent inhibitor of food pathogens[3,4].

Garlic has a broad antibiotic spectrum against Gram-positive and Gram-negative bacteria. Garlic also lowers cholesterol levels and blood pressure, prevents cancer and strengthens the immune system. Previous study suggested that these functions were mainly attributed to the bioactive components of garlic including sulfur-containing compounds such as alliin, diallyl sulfides, allicin, flavonoids, saponins and fructans[3,5,6]. Therefore, the different garlic preparations used in various studies might be one of the reasons for the inconsistent results[7]. Allicin is mainly responsible for the pungent odor of garlic and is produced from an inert chemical in raw garlic called alliina-a derivative of cysteine via the alliinase enzyme in the presence of pyridoxal phosphate[3]. Garlic produces allicin to protect itself from bacteria and other diseases and is an antioxidant[4]. Studies on garlic as an alternative growth promoter in livestock production were conducted and were shown to have beneficial effects on growth, digestibility and carcass traits[8-10].

Katuk leaves contain 1411.06 mg L^{-1} antioxidants, which is much higher than Moringa leaves. They contain 8.73 mg/100 mL vitamin C[11]. Santoso *et al.*[2] reported that the 3% Katuk leaf meal in chicken feed might reduce the accumulation of fat, lower the fishy smell of meat and can reduce the number of *Salmonella* sp. and *E. coli* in meat. Santoso *et al.*[12] reported that administration of 5% Katuk leaf meal in could significantly lower the amount of fat and cholesterol in chicken meat. Syahruddin *et al.*[13] reported that administration of fermented katuk leaves up to 14% has no effect on weight gain and feed efficiency, but can lower cholesterol in broiler meat. Katuk leaves can lower cholesterol levels because of the content of beta-carotene[14]. This study was conducted to evaluate effect of water extract of two leaves (*Sauropus androgynus* and *Allium sativum*) on the egg production and yolk cholesterol contents of layers up to 30 weeks of age.

MATERIALS AND METHODS

Animals, treatments and experimental design: This study used 90 Lohmann Brown hens, 30 weeks of age, with a homogeneous body weight of 1654.36±14.85 g obtained from a commercial poultry farm. All chickens were given commercial feed specific for laying hens containing 2.750 kcal kg^{-1} of Metabolizable Energy (ME), 18% of CP, 3.5% of Ca and available phosphor of 0.45%. For the treatments, hens were placed into three groups each containing 5 hens: (1) Hens were only given water as a drink, (2) Hens were given 5cc of sweet leaf extract in 100cc of drinking water and (3) Hens were given 5cc of garlic leaf extract in 100cc of drinking water. Each treatment was repeated 6 times for a total of 90 hens. Food and drinking liquid were given *ad libitum*. The individual hens were weighted weekly and food consumption and egg production was recorded daily.

Preparation of garlic (*Allium sativum*) and sweet leaf (*Sauropus androgynus*) extract: Fresh leaves of the sweet leaf and garlic were obtained from the local fresh food market. The leaves of both plants were blended and macerated overnight in distilled water (1:1, w/w)[15]. The blended extract was then filtered using a cheese cloth. This extract was used for the treatment.

Performance, egg quality metrics and laboratory analysis: Eggs were collected and labeled on a daily basis at 800 and 1400 h throughout the experimental period. The percentage of egg production was calculated. Once every two weeks, the eggs from three consecutive days were used to measure egg weight and quality. Yolk cholesterol content was analyzed for

two consecutive weeks. Blood samples from 36 hens were allowed to clot and the serum were harvested to determine the total serum cholesterol content. Cholesterol levels were analyzed following the Liebermann-Burchard method[16].

Statistical analysis: All data were analyzed with one-way ANOVA to determine the differences among treatments. If differences were found, then further analysis was performed with Duncan's multiple range test.

RESULTS

The results of the present study showed that 5.0% Katuk leaf extract and 5.0% garlic leaf extract in drinking water significantly increased ($p<0.05$) the average number of eggs produced and the average total egg weight as well as the average hen-day production. However, no significant differences ($p>0.05$) in the efficiency of feed consumption, water consumption and egg weight per head (g head^{-1}) were noted among the treatments (Table 1). The average value of Feed Conversion Ratio (FCR) (feed consumption:total egg weight) over 10 weeks of observation in the control group was 3.37/head (Table 1). This was significantly different ($p<0.05$) from hens in treatment groups B and C an average of 10.39 and 8.61%, respectively. In addition, the results showed that an additional 5.0% (5cc/100cc) of Katuk and garlic leaves extract in drinking water resulted in a significant ($p<0.05$) decrease in levels of cholesterol in the serum and yolk of birds.

It was measured the effects of oral administration of Katuk or garlic leaf extract on egg production and yolk cholesterol levels in layer hens. The extract increased egg weight and mass, but there was no significant change in egg production. Serum and egg-yolk cholesterol content decreased significantly in the egg-laying hens administered Katuk or garlic leaves. Chowdhury *et al.*[17] found that no effect on laying hens by mixing diets with 2-10% sun-dried garlic paste. It has been suggested the levels of garlic juice used in the present study were insufficient to influence egg yolk cholesterol[1]. Syahruddin *et al.*[13] reported that feed consumption and FCR were not affected by the levels of fermented Katuk leaf in the diet. However, the cholesterol content in broiler carcass was significantly affected by the dietary treatments. Another study found that garlic powder had no significant effects on broilers weight gain, Feed Intake (FI) and feed conversion[18]. Ao *et al.*[7] reported that administration of 1-3% of fermented garlic powder in the diet had no effect on egg production and egg weight.

It was found that Katuk or garlic significantly increased the efficiency of feed (feed consumption:total egg weight). This could be the a result of phytochemical contained in Katuk leaves and garlic. Administration of garlic can markedly increase villus height and crypt depth[1]. Thickness of the epithelium and the number of goblet cells in the duodenum, jejunum and ileum poultry decreased[8]. These features can increase nutrient absorption. They concluded that the morphological changes in the intestines of birds increases digestive capacity.

Hernandez *et al.*[19] reported that plant extract supplementation improved apparent whole tract digestibility of the nutrients. Adibmoradi *et al.*[20] reported that garlic administration enhanced villus height and crypt depth and decreased epithelial thickness and goblet cell numbers in duodenum, jejunum and ileum of birds and similar results were also have been shown by Nusairat[8]. Rao *et al.*[21] reported that garlic supplementation probably enhanced the activities of the pancreatic enzymes and provided micro-environment for better nutrient utilization in rats.

In more recent studies, Yalcin *et al.*[22] found that supplementing garlic powder at 5 or 10 g kg^{-1} increased layer production. Khan *et al.*[23] reported that laying hens fed on

Table 1: Effect of garlic (*Allium sativum*) and sweet leaf or Katuk (*Sauropus androgynus*) leaf water extract added in drinking water and administered to 30-40 weeks aged of egg laying hens to the egg production and yolk cholesterol level

	*Treatments			
Variables	Group A	Group B	Group C	SEM
Feed consumption (g head^{-1} days^{-1})	165.60^{a}	156.90^{a}	160.46^{a}	2.671
Water consumption (mL head^{-1} days^{-1})	354.73^{a}	348.75^{a}	361.07^{a}	5.384
Total egg weight (g head^{-1}/70 days)	3439.74^{b}	3636.69^{a}	3646.89^{a}	70.592
Egg weight (g head^{-1})	61.38^{a}	60.29^{a}	60.63^{a}	2.274
Number of eggs (egg/70 days)	56.04^{b}	60.32^{a}	60.15^{a}	1.078
Hen-day production (%)	80.05^{a}	86.17^{b}	85.93^{b}	1.106
Feed conversion ratio (feed consumption: total egg weight)	3.37^{a}	3.02^{b}	3.08^{b}	0.062
Serum cholesterol (mg dL^{-1})	177.39^{a}	145.07^{b}	144.85^{b}	8.571
Yolk cholesterol (mg dL^{-1})	562.84^{a}	527.95^{b}	530.37^{b}	9.173

*A: Drinking water without garlic or Katuk leaves extract as control, B: Drinking water with 5cc/100cc garlic water extract and C: Drinking water with 5cc/100cc Katuk water extract, respectively, SEM: Standard error of treatment means, Means with different superscripts within raw values are significantly different ($p<0.05$)

dried garlic (2-8%) showed higher egg-production intensity. This result contrasts with previous studies showing supplementation of 1% garlic powder caused a higher thigh yield the poorest thigh yield occurred with 3% garlic powder[24]. Groups with 1% garlic powder had significantly higher breast yield than others[24].

Ao *et al.*[7] reported that administration of 1-3% of fermented garlic powder in the diet increased egg yolk color compared with controls. Beta-carotene and vitamin E are natural antioxidants and antioxidants have an important role in inhibiting and scavenging free radicals. Therefore, they protect humans against infections and degenerative diseases. In addition, *Sauropus androgynus* leaves were also rich in flavonoids, vitamin C[25] and other phenolic compounds[26], these are natural antioxidants.

Andarwulan *et al.*[22] reported that supplementing garlic powder at 5 or 10 g kg^{-1} increased in hen egg production with a significant increase in egg weight similar to our results. Khan *et al.*[23] also reported that laying hens fed on dried garlic (2-8%) showed higher egg production intensity and increases in egg mass, however, the egg weight did not change significantly when compared to the control group. The diversity of garlic preparation and administration methods makes it harder to contrast our results with the literature.

Cholesterol levels in serum and egg yolks declined markedly by administration of Katuk leaves and leaves of garlic extract in drinking water. The decrease in plasma cholesterol via garlic powder supplementation might be due to the reduction of synthetic enzymes[18]. Significant decreases in hepatic 3-hydroxy-3-methylglutaryl-CoA reductase, cholesterol 7α-hydroxylase, fatty acid synthetase and pentose phosphate pathway activities were seen in various fractions of garlic (petroleum ether, methanol and water-soluble fractions). Results of the present study are consistent with the findings of Yalcin *et al.*[27] who reported that garlic powder supplementation significantly reduced the plasma cholesterol concentration when laying hens were fed 0.5 and 1.0% garlic powder. This decreased total cholesterol and triglycerides in diabetic rats[28].

Chowdhury *et al.*[17] and Ao *et al.*[7] reported a reduction in yolk cholesterol contents due to the use of garlic. Birrenkott *et al.*[29] reported that diet supplementation with garlic oil at 0.02% had no effect on yolk cholesterol or other lipid components even when fed for up to 8 months.

Chowdhury *et al.*[17] reported that plasma cholesterol concentrations decreased on average by 15, 28, 33 and 43% with increasing levels of dietary garlic paste of 2, 4, 6 and 8%, respectively. Prasad *et al.*[30] reported similar findings for total cholesterol and triglycerides. These were significantly decreased by garlic supplementation in chickens up to 8 weeks of age in comparison to a control group. Khan *et al.*[23] reported that dried garlic powder in the diets of commercial laying hens reduced serum and yolk cholesterol concentrations and skewed the layer performance upwards significantly. However, Birrenkott *et al.*[29] also reported that supplementing the diet with 3% garlic powder is not effective in lowering cholesterol yolk (mg g^{-1}) or other lipid components of serum of laying hens even when given a diet for 8 months. Yalcin *et al.*[22] reported that total cholesterol was not affected by the yellow garlic supplements.

Katuk leaf can lower cholesterol levels because of the content of beta-carotene[14]. Beta-carotene can decrease the cholesterol associated with hydroxy methyl glutaril enzyme-CoA (HMG)[31]. This enzyme plays a role in the formation of mevalonic in the biosynthesis of cholesterol. Cholesterol synthesis and synthesis of beta-carotene are derived from acetyl CoA along with mevalonic. If the consumption of beta-carotene is greater than that of saturated fatty acid, then it makes the biosynthesis process by enzyme HMGCoA directed at beta-carotene. Thus, the saturated fatty acids are not converted into cholesterol[32]. The lowest cholesterol level was obtained by feeding the chickens with diets containing 14% fermented Katuk leaf[13].

More beta-carotene in hen diets lowers cholesterol in the carcass because beta-carotene can inhibit enzyme HMG-CoA reductase (hydroxy methyl glutaryl-CoA). This plays a role in the formation of mevalonic acid. Mevalonic is required for cholesterol synthesis by inhibition of the enzyme that blocks formation of cholesterol[13,33]. Oka *et al.*[11] reported that the antioxidant content in Katuk leaf extract is 1411.06 mg L^{-1}, which is much higher than the antioxidant content of Moringa leaves. However, its vitamin C content is 8.73 mg/100 mL, which is still lower than the Moringa leaf extract (19.13 mg/100 mL). Fermented *Sauropus androgynus* leaves offered the best broiler meat quality as indicated by lower fat and cholesterol with higher vitamin A, beta-carotene, protein, iron contents with better amino acid and fatty acid content[12]. *Sauropus androgynus* leaves also reduced cholesterol in broiler meat[34] and eggs[2].

Issa *et al.*[18] reported that garlic powder significantly decreased the levels of triglycerides. Garlic powder decreased triglyceride levels compared to the control group. This effect can be explained by the possible inhibition of the acetyl CoA synthetase enzyme, which is necessary for the biosynthesis of fatty acids. This might explain the hypocholesterolemic and hypolipidemic action of garlic products that depresses the hepatic activities of lipogenic and cholesterogenic enzymes, such as malic enzyme, fatty acid synthase and glucose-6-phosphatase dehydrogenase. Compounds that lower cholesterol in serum and egg yolk include drinking

water with 5cc/100cc garlic water extract and drinking water with 5cc/100cc Katuk extract, respectively. These might contain alkaloids and non-alkaloids[35], saponins[36], flavonoid[37] and polyphenol[38]. The reduction of cholesterol by alkaloids is due to reduction of lipogenic enzyme activities and increased bile acid excretion in the feces[39]. Garlic powder supplementation significantly reduced the plasma cholesterol concentration when laying hens were fed 0.5 and 1.0% garlic powder[27].

These results are consistent with the beneficial effects of garlic on cholesterol metabolism in human health. Birrenkott *et al.*[29] reported that diet supplementation with 3% powdered garlic was not effective in lowering yolk cholesterol (mg g^{-1}) or other lipid components in the serum of laying hen seven when fed for up to 8 months and on broiler chickens[10]. More recently, Yalcin *et al.*[22] reported that total yolk cholesterol was not affected by garlic supplementation. Similar findings were reported in rats-garlic powder failed to influence the lipid profiles in rats[40]. Raeesi *et al.*[24] reported that 1 and 3% garlic had no significant effects on relative weights of carcass, fat pad or digestive organs among different treatments except for the small intestine. The different commercial garlic products might explain these contradictory results[17].

CONCLUSION

It is concluded that 5.0% Katuk (*Sauropus androgynus*) or garlic (*Allium sativum*) water extract in drinking water improved egg production and may decrease both serum and yolk cholesterol in laying hens up to 30 weeks of age.

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