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The Effect of Aqueous Extract of two Leaves (*Sauropus Androgynus* and *Moringa Oleifera*) and Its Combination on Egg Production, Yolk Colour and Yolk Cholesterol Levels in Egg Laying Hens

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ABSTRACT

Objective: *The present study was conducted to determine the effects of Sauropus androgynus, Moringa oleifera leaves, and its combination administration in drinking water on the egg production and yolk cholesterol level of 40-week old hens.*

Material and Methods: *Two hundred and fourty 40-wk-old hens were colony caged in an environmentally controlled house to evaluate the effect of Sauropus androgynus (Katuk, Indonesian), Moringa oleifera leaf (Kelor, Indonesian) leaves and its combination via drinking water on laying hens. Sauropus androgynus leaf and Kelor extract was prepared by macerating Katuk leaf and Kelor leaves in distilled water (1:1, w/w). Hens were randomly divided into four equal groups: one served as a control and was administered with drinking water only. Theother three groups were administered 2% water extract of Katuk leaf or Kelor leaves, and its combination, respectively.*

Results: *An aqueous extract of Katuk and Kelor leaf and its combination in drinking water increased yolk colour and egg production ($P<0.05$), but not the efficiency of feed consumption ($P>0.05$). Katuk or Kelor leaf and its combination administration in drinking water results in lower ($P<0.05$) yolk cholesterol contents.*

Conclusion: *Katuk or Kelor leaf and its combinations administration in drinking water increased yolk colour and egg production, but decreased yolk cholesterol contents in egg laying hens.*

KEYWORDS: *Sauropus androgynus, Moringa oleifera,* yolk color, cholesterol, egg

INTRODUCTION

In many countries different types of plant extracts have been used in traditional medical systems to treat for microbial disease. Some researcher reported that phytochemicals present in those plants having antimicrobial and antioxidant properties are the reason for this ability to use them in disease treatments. Antimicrobial activities of some phytochemicals present in such plants have been investigated and the possibility of using them to develop new antimicrobial drugs has also been studied (Akinmoladun *et al.,* 2007; Dalukdeniya *et al., 2016;* Bukar *et al*., 2010; Adriani *et al*., 2015*).*

Kelor leaf (*Moringa oleifera*) is a traditional medicinal plant that has high nutrients, as an antibacterial, and contains beta carotene as an active ingredient for meat colouring (Ayssiwede *et al*., 2011), so it is very important role in improving colour egg yolks are very preferred by consumers, as well as with Katuk (*Sauropus androgynus*). Phytochemical compounds contained in it include saponins, flavonoids, and tannins (Santoso et al., 2005) and several other phenolic compounds that have antimicrobial activity (Bukar *et al*., 2010). Estrogen-like flavonoids can slow down the loss of bone mass (osteomalacia), lower blood cholesterol levels and increase HDL levels, while saponins are proven efficacious as anticancer, antimicrobial, and lower blood cholesterol levels. *Moringa oleifera* has been reported that different preparations of this plant having antibiotic, antitrypanosomal, hypotensive, antispasmodic, antiulcer, anti-inflammatory, hypo-cholesterolemic, and hypoglycemic activities (Dalukdeniya *et al., 2016)*. A few researchers (Ashok *et al*., 2014; Elangovan *et al.,* 2014) have investigated the antimicrobial activity of *Moringa oleifera* extracts against some pathogenic bacteria, as an effective feed supplement in poultry for its encouraging results in relation to total body weight gain and feed conversion efficiency in the broiler chicks (Akhouri *et al*., 2013; Godinez-Oviedo *et al*., 2016). Wardiny and Tuty (2012) explains that herbs leaves (Noni) contain other substances such as amino acids, phenolic compounds, ursulic acid, alkaloid compounds, phenols, and glycosides that are antimicrobial, antibacterial, and anti-inflammatory. It also functions as an endothelial vasodilatator and inhibits HMG Co-A (3-hydroxy-3-methyl-glutaryl Co-A) activity,thereby inhibiting lipoprotein lipase which is responsible for hydrolysis of plasma lipids and has beneficial health effects with regard to their ability in altering the lipid profile of broiler chicken (Adriani *et al*., 2015). Phytochemical compound in herbs (*Olea europaea)* leaf powder can be used for reducing egg yolk cholesterol content and egg yolk colouring agent in layer diets (Cayan and Erener, 2015).

Moringa pods are rich in bioactive compounds, especially carotenoids (β-carotene: 2.76 mg/100 g) (Ahmad *et al.,* 2017), flavonoids (quercetin), polyphenols, vitamins, and nutrients (Gopalakrishnanb *et al*., 2016). *Moringa* pod meal supplementation in diets affects egg mass, serum biochemistry, and bioactive compounds of the egg yolk positively (Ahmad *et al*., 2017). Katuk (*Sauropus androgynus*) leaves have large potential used for animal feed. Katuk leaves also contains a beta-carotene 173 ppm can lower cholesterol levels because of the content of beta-carotene (Syahruddin *et al*., 2013).

Producing low cholesterol egg products with high ration efficiency using the extract of Kelor leaf and Katuk leaf extract will provide knowledge to small-scale farmers to achieve established food security, as well as enhance the competitiveness of their businesses to increase their income and welfare. Based on this study, researchers are interested in studying the effect of leaf water supply of Katuk (*Sauropus androgynus*) and Kelor leaf (*Moringa oleifera*), and its combination tin drinking drinking water to increase the egg production and colour of egg yolks, and lower cholesterol in chicken egg yolks.

MATERIALS AND METHODS

Animals, treatments, and experimental design: This study used 240 Lohmann Brown hens, 40 weeks of age, with a homogeneous body weight of 1762.48 ± 28.51 grams obtained from a commercial poultry farm. All chickens were given commercial feed specific for laying hens containing 2.750 kcal/kg of metabolizable energy (ME); 17% of CP; 3.5% of Ca; and

available phosphor of 0.45%. For the treatments, hens were placed into four groups each containing 10 hens: (A) hens were only given water as a drink, (B) hens were given 2 cc of *Sauropus androgynus* leaf extract in 100 cc of drinking water, (C) hens were given 2 cc of Kelor leaf extract in 100 cc of drinking water; and (D) hens were given 1 cc of Katuk leaf extract in 100 cc + 1 cc of Kelor leaf extract in 100 cc of drinking water. Each treatment was repeated 6 times for a total of 240 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 Katuk (*Sauropus androgynus)* and Kelor leaf (*Moringa oleifera*) Extract: Fresh leaves of the Katuk leaf and Kelor leaf 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) (Parwata *et al*., 2016). 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 08.00 h and 14.00 h throughout the experimental period. The percent egg production was calculated. Once every two weeks, the eggs from three consecutive days were used to measure egg weight and quality. Yolk color was determined by using a Roche colour fan (1 to 15). Yolk cholesterol content was analyzed for two consecutive weeks. The blood samples were randomly collected from two (2) birds per replicate at the end of the study at 10^{th} week and analyzed for the estimation of blood to determine the total serum cholesterol content. Cholesterol levels were analyzed following the Liberman-Burchard methods (Lieberman and Burchard, 1980).
Statistical analysis: All data were analyzed with 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 showed that leaf extract of Katuk (*Sauropus androgynus*) and Kelor leaf (*Moringa oleifera*) and its combination in drinking water significantly ($P<0.05$) increased total egg weight, egg count, and feed efficiency (feed consumption : total egg weight). However, there was no significant difference ($P>0.05$) in feed consumption, water consumption, and egg weight per hens (g/head).

The average value of the Feed conversion ratio (feed consumption: total egg weight) for ten weeks of observation in the control chicken group was 2.51/head (Table 1). The mean value of FCR in treated chickens B, C, and D showed significant differences ($P<0.05$) higher than controls: 9.56%; 7.57%; and 10.36%, respectively higher than the control.

The number of eggs and daily production of eggs in each treatment is presented in Table 1.

The results showed that the average number of eggs in chicken treated B (*Sauropus androgynus*); Treatment C (*Moringa oleifera*), and treatment D (combination of *Sauropus androgynus* and *Moringa oleifera*) had significantly higher ($p<0.05$) egg/egg production than controls. The average number of eggs in chickens treated B, C, and D was 8.08%; 8.25%; and 8.64% higher than control (A), respectively. The number of eggs in the treatment group B, C, and D did not show any significant difference ($P>0.05$).

The results of observation on the yolk colour values (1-15) showed that additional leaf extract of Katuk (*Sauropus androgynus*) and Kelor leaf (*Moringa oleifera*) and its combination through drinking water gave a significant increase (P<0.05) in egg yolk Table 1). The highest yolk colour was obtained in chickens of the C group of 11.06 (1-15) followed by group D with an egg yolk value of 10.93 (1-15); and group B that is 10.37 (1-15) increased significantly (P<0.05) than group A.

In addition, the results showed that the addition of leaf extract of Katuk (*Sauropus Androgynus*) and Kelor leaf (*Moringa oleifera*) and its combination in drinking water resulted in a significant decrease (P<0.05) in cholesterol levels in chicken egg yolks (Table 1). *Sauropus androgynus* leaf extract (B), Kelor leaf (C), and its combination (D) in drinking water resulted in a significant decrease (p<0.05) in yolk cholesterol content compared to control (group A). The lowest cholesterol content in yolk was found in treatment D (470.62 mg/dl) or (7.67% lower than control), followed by group B with cholesterol level of 475.28 mg/dl or (6.75% lower than control), and group B recorded cholesterol in yellow at 483.93 mg/dl (5.06% lower than control). The cholesterol content in the egg yolk of the treatment groups B, C, and D showed no significant difference (p<0.05).

Table 1. Effect of Katuk leaf extract (*Sauropus androgynus*) and Kelor leaf (*Moringa oleifera*) and its combination in drinking water on egg production, egg yolk colour, and cholesterol content in egg yolk in Lohmann Brown laying hen

Variables	Groups[1)]				SEM[2)]
	A	B	C	D	
Feed consumption (g/head/days)	128.03a	125.81a	128.94a	124.78a	2.074
Water consumption (ml/head/days)	314.82a	278.37a	305.49a	269.83	24.97
Total egg weight (g/head/70 days)	3570,62b[3)]	3879.59a	3890.41a	3882.04a	70.601
Egg weight (g/head)	62.70a	63.03a	63.10a	62.75a	1.873
The numer of eggs (egg/70 days)	56.95b	61.55a	61.65a	61.87a	1.296
Hen-day production (%)	81.35a	87.93b	88.07b	88.39b	1.275
Feed conversion ratio (feed consumption: total egg weight)	2.51a	2.27b	2.32b	2.25b	0.074
Yolk colour (1 to 15)	8.63a	10.37b	11.06b	10.93b	0.584
Yolk cholesterol (mg/dl)	509.71a	483.93 b	475.28 b	470.62 b	6.095

Notes:

1. (A) hens were only given water as a drink, (B) hens were given 2 cc of Katuk leaf extract in 100 cc of drinking water, (C) hens were given 2 cc of Kelor leaf extract in 100 cc of drinking water; and (D) hens were given 1 cc of Katuk leaf extract in 100 cc + 1 cc of Kelor leaf extract in 100 cc of drinking water, respectively.
2. SEM: Standard Error of Treatment Means

3. Means with different superscripts within raw values are significantly different (P<0.05)

The results of observation of the effect of extract from leaf Katuk (*Sauropus androgynus*) and Kelor leaf (*Moringa oleifera*) and its combination in drinking water on egg production, feed efficiency, egg yolk, and cholesterol in egg yolk are presented in Table 1. Herbs extract was can significantly increase total egg weight and egg production, but there is no significant change in feed consumption and drinking water. The yolk cholesterol content showed a significant decrease in the chicken given leaf katuk (*Sauropus androgynus*) and Kelor leaf extract (*Moringa oleifera*) and its combination through drinking water. Herbs extract is used as an oral additive to improve animal performance especially under intensive management systems (William and Losa, 2001). The same thing was reported by Gill *et al*. (2002) and Hernandez *et al.* (2014) that the health status and performance of livestock can be supported by plant extracts.

In addition, the results showed that additional leaf extract of Katuk (*Sauropus androgynus)*, Kelor leaf (*Moringa oleifera*) and its combination in drinking water resulted in a significant increase in egg production, ie a significant increase in total egg weight and number of eggs. Similarly reported by Bidura *et al*. (2017) that laying hens given herbal extract (*Allium sativum* and *Sauropus androgynus*) showed higher egg production intensity and increased egg mass. However, the average egg weight did not change significantly when compared to the control group. Additional *Morinda citrifolia* leaf water extract was able to increase egg and egg yolk (Suarjana *et al*., 2018). *Moringa oleifera* leaf extract in drinking water resulted in increased egg production and feed efficiency in laying hens (Siti *et al.,* 2017).

We found that extracts from Katuk leaf (*Sauropus androgynus*), Kelor leaf (*Moringa oleifera*), and its combination in drinking water significantly improved feed efficiency (feed consumption : total egg weight). This is caused by the content of phytochemical compounds in Katuk leaf extract (*Sauropus androgynus*) and Kelor leaf (*Moringa oleifera*), and its combination in drinking water. Adibmoradi (2006) reported that extracts of herbs leaves (Garlic) can increase the villous height and depth of the small intestine crypt. Nusairat (2007) reported that the thickness of the epithelium and the number of goblet cells in the duodenum, jejunum, and poultry ileum decreased and this feature could increase nutrient uptake. It was also reported that morphological changes in the poultry intestine can increase digestion capacity. The same is reported by Santoso *et al*. (2015) that supplementation of herbs plant extracts can improve nutrient digestibility in the gastrointestinal tract. Ramakrishna *et al.* (2003) reported studies in rats that phytochemical compounds might increase pancreatic enzyme activity and provide micro-environments for better utilization of nutrients. All types of *Moringa oleifera* extract showed antimicrobial properties against both gram-negative bacteria (*Salmonella enteritica, Vibrio parahaemolyticus, Escherichia coli*) and gram-positive bacteria (Listeria *monocytogenes*) (Dalukdeniya *et al*., 2016). Furthermore, the extract was shown to be evident with higher antibacterial activity against gram-negative bacteria compared with gram-positive bacteria.

Moringa oleifera leaf extract may be useful as an effective feed supplement in poultry for encouraging results in relation to weight and feed efficiency in broiler (Akhouri *et al*., 2013). It can also be used potentially before mass vaccination in chicks, because of its immunomodulatory properties, such as levamisole compounds. Herbs extract supplements show the potential for increasing the amount of breast meat and can improve the morphology of the small intestine by different mechanisms (Karukarach *et al.,* 2016). The primary mode

of action of these active ingredients is inhibition of pathogenic microbes and endotoxins in the gut and enhanced pancreatic activity, resulting in better nutrient metabolism and utilization (Windisch *et al*., 2008; Grashorn, 2010).

Colour is an important food quality trait, as it affects the perception of quality and intensity of consumer flavors and tastes, as well as purchasing decisions (Loetscher *et al.,* 2013). Most consumers associate egg yolk with age and animal health status and egg quality, as well as egg products, but objectively this is not the case. The colour of egg yolks given leaf extract Katuk and Kelor leaf extract in drinking water also has an egg yolk colour significantly higher than the control. The increase in egg yolk colour is strongly associated with the ingredients used in the ration. Carotenoid compounds play an important role in the development of various colours in the yolk. In particular, the lutein compound is an active yolk dye. The same is observed by Cayan and Erener (2015) that the increase in the amount of olive leaf powder in the diet results in a linear increase in the yolk colour. The increase in the yolk colour can be attributed to the carotenoid content of olive leaf powder. Beta-carotene and vitamin E are natural antioxidants, and antioxidants have an important role in inhibiting and scavenging free radicals. Some researchers report that supplements of herbs leaf extract show an increased potential for yolk colour. Several studies on the effects of mulberry leaf, ginkgo leaf markedly increase the colour of egg yolks (Lokaewmanee *et al*., 2009; Zhao *et al*., 2013), *Allium sativum* and *Sauropus androgynus* (Bidura *et al.,* 2017). Ahmad *et al.* (2017) reported that suplemented of *Moringa oleifera* pod meal (5-15 g/kg) in layers diet were increased β-carotene in egg yolk, whereas cholesterol levels in egg yolk and serum were decreased significantly, that is, 201.87 mg/100g and 8.47 mg/dl, respectively. The same reported by Mabusela *et al.* (2018), *Moringa oleifera* whole seed meal inclusion improved yolk colour, maintained external egg quality, and improved the fatty acid profile.

Adriani *et al.* (2015) reported that the use of *Morinda citrifolia* juice supplementation in drinking water significantly decreased total cholesterol, triglyceride, and low density lipoprotein (LDL) levels. According to Boger *et al.* (1997) that *Morinda citrifolia* may serve as an endothelial vasodilatator and may inhibit the activity of HMG Co-A (3-hydroxy-3-methyl-glutary Co-A), thereby inhibiting lipoprotein lipases responsible for the hydrolysis of plasma lipids. Kusnandar and Rahmawati (2003) reported that medical herbs contain active antioxidants, such as Selenium, which has an important function to activate glutathione peroxidase and neutralize free radicals that attack the fat molecules in the body. Sally (2003) reported that *Morinda citrifolia* has the ability to lower blood cholesterol, triglycerides and LDL in mice.

Patil *et al*. (2010) reported that cholesterol and triglyceride reduction by alkaloids was partly due to reduced lipogenic enzyme activity and increased excretion of bile acids in feces. In addition, according to Nuraini *et al*. (2008), the presence of beta-carotene in herbal extracts can lower cholesterol levels in the blood because it inhibits the action of HMG-CoA reductase enzyme that plays a role in the formation of mevalonate in cholesterol biosynthesis in the liver.

Thurber and Fahey (2009) reported that *Moringa oleifera* leaves have beneficial effects, including anti-inflammatory action, inhibition of platelet aggregation, antioxidants, antimicrobials, and antitumor activity. In addition, according to Iqbal and Bhanger (2006) reported that *M.oleifera* leaf contains polyphenol compounds (eg, kaempferol, rhamnetin, isoquercitrin, and kaempferitrin). These flavonoids have an important role in the binding of

free radicals. *M.oleifera* contains phytochemical compounds that have important biological activity. Several previous studies have confirmed that extracts or compounds isolated from *M.oleifera* have antioxidant, anti-carcinogenic, anti-diabetic, anti-imflamasi and anti-hypertensive properties, as well as the ability to protect against liver damage (Godinez-Oviedo *et al*., 2016).

CONCLUSION

We conclude that Katuk (*Sauropus androgynus*) or Kelor (*Moringa oleifera*) leaf extract and its combinations administration in drinking water improved yolk colour and egg production, but may decrease yolk cholesterol contents in laying hens up fourty weeks of age

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