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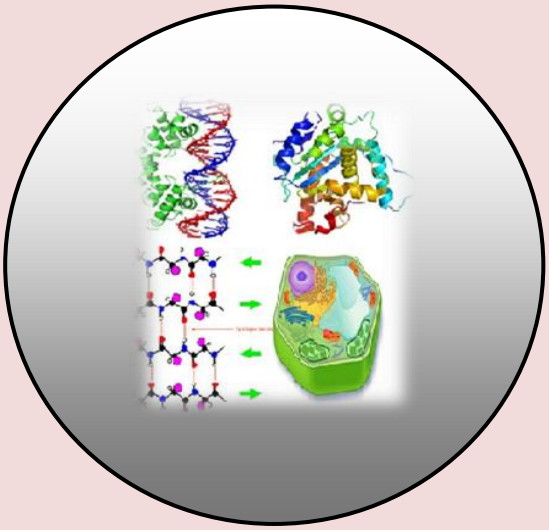

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The Effect of Fermented *Sauropus Androgynus* Leaf Extract in Drinking Water of Laying Hens on Egg Production and Egg Cholesterol

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ABSTRACT
This research aims to study the effect of fermented Sauropus androgynus leaf in drinking water of hens on egg production and egg cholesterol. The experimental design used was completely Randomized Design (CRD) with three treatments and six replications, where each replication used tens Lohmann Brown chickens 70 weeks old. The three treatments were Lohmann Brown chicken which was given drinking water without addition of katuk leaf water extract as control (A), Lohmann Brown hens given of 2% fermented Katuk leaf extract in drinking water, and Lohmann Brown hens given of 4% fermented Katuk leaf in drinking water (C). The results showed that 2% and 4% fermented leaves of katuk extract in drinking water of birds were increased significantly different effect (P<0.05) on feed efficiency, number of eggs, egg weight, protein content, yolk colour, and significantly effect (P <0.05) on decreased both cholesterol and fat levels in egg yolk of Lohmann Brown aged 70 weeks old. It was concluded that fermented of Sauropus androgynus leaf in drinking water of hens can improve egg production and egg yolk colour, but may decrease yolk cholesterol in laying hens.
Keyword: Sauropus Androgynus, Probiotic, Cholesterol and Eggs.

INTRODUCTION
High levels of cholesterol and fat in egg products from animals that are consumed are known to be a source of body obesity and coronary heart disease. The increase in the number of diseases and deaths from coronary heart disease is caused by lifestyle changes in the form of a diet with fat intake exceeding 30%, saturated fatty acids 10% higher than total energy, and cholesterol higher 300 mg per day (Sartika, 2008). According to Ezema and Eze (2015), the fat content of an egg is quite high, and contains cholesterol as much as 824.5 mg/dl. If we consume food products with excessive cholesterol can be one of the risk factors for the emergence of generative diseases. Efforts to reduce the cholesterol content of chicken eggs are very necessary, especially for consumers who are elderly.

Katuk leaf (*Sauropus androgynus*) is a traditional medicinal plant that has high nutritional properties, as an antibacterial, and contains beta carotene as a carcass color active substance (Ayssiwede *et al*., 2011), so its role is very important in increasing the preferred color of egg yolk by consumers. Phytochemical compounds contained in it are: saponins, flavonoids, and tannins (Santoso, 2000) and several other phenolic compounds that have antimicrobial activity (Bukar *et al*., 2010). Estrogen-like flavonoids turned out to be able to reduce reduced bone mass (osteomalacia), increase blood cholesterol levels and increase HDL levels, while saponins proved efficacious as anticancer, antimicrobial, and reduce blood cholesterol levels. Giving katuk leaf extract to laying hens increases egg production and decreases cholesterol, triglycerides, and LDL-cholesterol, but increases HDL-cholesterol in serum (Santoso *et al*., 2002). Based on the results of research conducted by several researchers (Bidura *et al*., 2017; Ekayuni *et al*., 2017; Santoso *et al*., 2015; Siti *et al*., 2018) showed that the administration of herbal leaves in chicken rations proved to reduce fat accumulation, reduce fishy odor, and be able to reduce the amount of *Salmonella sp*. and *E. choli*. Increased efficacy of katuk leaf herbs can be done through fermentation. Microbes used as fermentation inoculants should be inoculants that function as probiotics (Siti *et al*., 2018). Thus, these microbes will be able to function double in the digestive tract of chickens. *Saccharomyces sp*. can function as a probiotic and can improve the efficiency of ration use and reduce cholesterol in serum and eggs (Bidura *et al*., 2016a; Bidura *et al*., 2016b). Provision of unfermented Katuk leaves (Ekayuni *et al*., 2017) and fermented ones (Syahruddin *et al*., 2013) in rations can increase growth and reduce cholesterol content in chicken meat.

Producing low-cholesterol eggs with high efficiency of feed use, namely through the use of Katuk leaf extract will provide knowledge to small-scale farmers to establish an established food security, and improve business competitiveness to increase income and welfare. This study aims to examine the effect of giving Katuk leaves (*Sauropus androgynus*) fermented with probiotics in drinking water laying hens on the egg production and egg cholesterol levels.

MATERIALS AND METHODS

Animals, treatments, and experimental design: This study used 180 Lohmann Brown hens, 70 weeks of age, with a homogeneous body weight of 1932.70 ± 30.65 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%. Each cage equipped with food and drinking holder placed outside the cage. Sanitation of the equipment is done everyday by cleaning places for feeding and drinking. For the treatments, hens were placed into three groups each containing 10 hens: (A) hens were only given water as a drink, (B) hens were given 2 cc of fermented Katuk leaf (FKL) extract in 100 cc of drinking water; and (C) hens were given 4 cc of FKL extract in 100 cc of drinking water, respectively. Each treatment was repeated 6 times for a total of 180 hens. Food and drinking water were given *ad libitum*. The individual hens were weighted weekly, food consumption and egg production was recorded daily.

Process of making flour *Sauropus andogynus* leaves and fermentation: Fresh leaves of the Katuk leaf were obtained from the local fresh food market. Before being fermented, Katuk leaf that have dark green, thinly sliced, and dried in room temperature for 1-2 days, then dried in oven at temperature 50°C for 24 hours. Furthermore Katuk leaf was ground to fine powder form. Powdered Katuk leaves were then analyzed and prepared for fermentation using khamir *Saccharomyces spp*. Gb-7 isolated from colon of native chickens (Bidura *et al*., 2016), with inoculum dose of 5% incubated for 4 days with the thickness of 2 cm substrate at pH 5.5 and temperature 30°C and than macerated overnight in distilled water (1:1, w/w) (Parwata *et al*., 2016). The blended extract was then filtered using a cheese cloth. This (FKL) 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 colour was determined by using a Roche yolk colour fan (1 to 15). Yolk cholesterol content was analyzed for two consecutive weeks. The yolk 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 yolk to determine the total yolk cholesterol content.

The percent egg production was calculated, feed conversion ratio (FCR, feed DM intake/total egg weight). 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. 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 Total egg weight, feed consumption, feed conversion ratio (feed consumption: total egg weight), and the number of eggs in groups are shown in Table 1. The results study shows that 2-6% fermented Katuk leaf (FKL) extract in drinking water significantly increased ($P<0.05$) the average total egg weight and the average number of eggs produced as well as the average hen-day production were noted among the treatments (Table 1). However, no significant differences ($P>0.05$) in the feed consumption and water consumption. The average value of FCR (feed consumption: total egg weight) over eight weeks of observation in the control group was 2.442/head (Table 1). This was significantly different ($P<0.05$) from hens in treatment groups B and C, an average of 6.96% and 5.81% more higher than control (Group A), respectively.

Table 1. The effect of FKL aqueous extract added in drinking water and administered to 70-78 weeks aged of egg laying hens to the egg production and feed efficiencies.

Variables	Treatments[1)]			SEM[2)]
	A	B	C	
Feed consumption (g/head/56 days)	6691.16[a]	6710.83[a]	6742.83[a]	36.075
Water consumption (l/head/56 days)	16.910[a]	17.241[a]	17.463[a]	0.286
Total egg weight (g/head/56 days)	2741.00[a]	2965.84[b]	2930.44[b]	21.274
Feed conversion ratio (feed consumption: total egg weight)	2.442[a]	2.272[b]	2.300[b]	0.019
The number of eggs (egg/56 days)	41.065[a]	42.523[b]	42.308[b]	0.287

Notes:

1. A: drinking water without FKL extract as control; (B): drinking water with 2 cc/100 cc FKL extract; and (C): drinking water with 4 cc/100 cc FKL extract, respectively.
2. SEM: Standard Error of Treatment Means
3. Means with different superscripts within raw values are significantly different ($P<0.05$)

In addition, the results show that an additional FKL extract (2-4 cc/100 cc) in drinking water resulted in a significant ($P<0.05$) increase in crude protein (CP) of yolk and yolk colour of eggs (Table 2). Eggs yolk colour of the group C was 9.83 followed by group B was 9.602(B); and 8.122 (Group A), respectively. The cholesterol content in egg yolk in chickens decreased significantly ($P<0.05$) in the presence of FKL extract in drinking water (Table 2). Increased level of FKL in drinking water was significantly ($P<0.05$) lowered yolk cholesterol levels. Yolk cholesterol content of the group A was 173.348 mg/dl followed by group B was 9.31% and 9.06 % (C) were lowered significantly different ($P<0.05$) than control (A), respectively.

Table 2. The effect of FKL aqueous extract added in drinking water and administered to 70-78 weeks aged of egg laying hens to the egg yolk quality.

Variables	Treatments[1)]			SEM[2)]
	A	B	C	
Ether extract of yolk (% dry matter)	27,912[a]	26,092[b]	26,158[b]	0,221
Crude protein of yolk (% dry matter)	16,466[a]	17,870[b]	17,940[b]	0,249
Yolk cholesterol (mg/dl)	173,348[a]	157,193[b]	157,627[b]	1,556
Yolk colour (1 to 15)	8,122[a]	9,602[b]	9,838[b]	0,237

DISSCUSSION

The giving of Katuk leaf water extract fermented with probiotics in drinking water will help absorb nutrients due to the active compounds contained in the water extract of Katuk leaves. The most important plant bioactive compounds are alkaloids, flavonoids, tannins, and phenolics (Edeoga *et al*., 2005). This is due to the active compounds in FKL extract, such as flavonoids, saponins, tannins, and beta-corotene. The main workings of these active ingredients are by inhibition of pathogenic microbes and endotoxins in the intestines, increasing pancreatic activity, increasing metabolism, and absorption of nutrients in the gastrointestinal tract of chicken (Windisch *et al.,* 2008; Grashorn, 2010). These active compounds in the digestive tract of poultry will be able to help absorb food substances. Reported by Adibmoradi *et al.* (2006) that the active compounds of herbal plants such as Garlic can increase the villus height and crypto depth, and reduce epithelial thickness and the number of villi cells in the duodenum, jejunum, and poultry ileum. The villus height increases, and the thickness of the epithelium and goblet in the duodenum, jejunum, and ileum will increase absorption of nutrients (Nusairat, 2007). The increase in total egg weight is due to the phytochemical efficacy of Katuk leaves which can increase protein digestibility and ration dry matter, as reported by Issa and Omar (2012) that dry matter digestibility (DM), Protein (CP), and Ether Extract (EE) increases with the administration of phytochemical compounds contained in garlic. Hernandes *et al.* (2004) reported that administration of plant extracts can increase digestibility of nutrients in the digestive tract of poultry. According to Adibmoradi *et al.* (2006) that administration of herbal extracts (garlic) can increase villus height and crypt depth, as well as decrease epithelial thickness and the number of goblet cells in the duodenum, jejunum, and poultry ileum (Nusairat, 2007), so that the absorption of food substances can increase. Reported by Bidura *et al.* (2017) that the administration of phytochemical compounds (Katuk or Garlic leaf water extract) in real drinking water can increase production and the efficiency of feed use in laying hens. The results of the study showed that the colour of the egg yolk turned out to be influenced by the food substances contained in katuk leaves, namely the carotenoid content in katuk leaves. According to Hulshof *et al.* (1997), Katuk leaves have a fairly high carotene compared to other vegetables such as spinach, carrots, and kale. The color of the yolk is determined by nutrient consumption, in other words the higher the addition of FKL in drinking water. The colour of the yolk is influenced by the use of different FKL in drinking water. The role of xanthophyll and carotene in Katuk leaves besides being a precursor of vitamin A, also acts as a source of pigment in eggs. The higher the addition of FKL in drinking water, the better the color of the yolk produced. This is due to the presence of carotenoid pigments contained in FKL. The more addition of FKL in drinking water, the better color can be produced. High yolk colour intensity is supported by the results of a study by Piliang (2003) who reported that the addition of 9% Katuk leaves in the feed, giving the yolk colour score was 11.5 roche color fan scale (1-15). According to VanKoetsveld and Gimbergen (1981) that in Leghorn chicken yolk contains as much as 2-10 µg β-carotene. The results of the observations on the color of the yolk showed a significant difference compared to the control treatment. There was a decrease in fat and cholesterol levels in egg yolk with the giving of Katuk leaves fermented by the probiotics *Saccharomyces sp* in chicken drinking water. This is due to the role of the probiotic *Saccharomyces sp*. which was used as an inoculant for FKL before extracting. According to Mallo *et al*. (2010) and Mountzouris *et al.* (2010), probiotics can improve egg weight, feed efficiency, yolk colour, egg shell quality, pathogen inhibition, and have the ability to lower serum cholesterol levels (Kusumawati *et al*., 2003). The lowest cholesterol level was obtained by feeding the chickens with diets containing 14% fermented Katuk leaf (Syahruddin *et al.,* 2013). Likewise, the results of the Ekayuni *et al.* (2017) study reported that 50 ml/liter *Moringa oleifera* leaf water extract in drinking water significantly can reduce abdominal fat and cholesterol levels in meat in broiler chickens. Santoso *et al.* (2015) reported that *Saccharomyces cerevisiae* fermented *Sauropus androgynus* leaves inclusion resulted in the best broiler meat quality as indicated by lower fat and cholesterol.

CONCLUSION

It was concluded that an additional 2-4 cc/100 cc of fermented *Sauropus androgynus* leaf in drinking water of hens can improve egg production and egg yolk colour, but may decrease yolk cholesterol in laying hens.

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