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The Effect of Dietary Teak Leaf Extract (*Tectona grandis* Linn. *f*) on Egg Quality of Laying Hens

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Abstract: The purpose of this study was to determine the effect of teak leaf extract (*Tectona grandis* Linn. *f*) as feed additive on egg quality of laying hens. The materials used were 240 Isa Brown laying hens aged at 30 weeks old with average egg mass was 53.13±3.26 g. The method used was experiment in a completely randomized design. The birds were divided into six dietary treatments with four replications (10 birds of each replication). The treatments used were basal diet without teak leaf extract as negative control (R0), basal diet with 0.015% of virginiamycin as positive control (R1), and basal diet with teak leaf extract at 0.4% (R2), 0.8% (R3), 1.2% (R4), and 1.6% (R5). Variables measured in this study were egg weight, albumen weight, yolk weight, eggshell weight, albumen percentage, yolk percentage, eggshell percentage, Haugh unit, shell thickness, and yolk color score. Data were analyzed by using analysis of variance, while the significance of differences among treatment means were evaluated by using Duncan Multiple Range Test. The results showed that the addition of teak leaf extract in the laying hens diets significantly affect ($P<0.01$) egg weight, albumen weight, eggshell weight, eggshell percentage, Haugh unit, shell thickness, and yolk color score. The inclusion of 1.2% teak leaf extract in the diet gives the best effect on the egg quality of laying hens.
Keywords: antibiotics, *Tectona grandis* Linn. *f*, egg quality, laying hens, phytobiotics, virginiamycin.

INTRODUCTION

Maintaining egg quality is very important in the livestock industry because it affects the profits of farmers. Sharma *et al*. [1] stated that from 6 to 10% of egg damage during production, transport, and until to the consumers, is caused by the thin shell. Odabasi *et al*. [2] stated that the color of shell was an indication of egg quality and could affect the marketing, the consumers prefer brown color than white. Świątkiewicz *et al*. [3] Explained that egg quality was very important in terms of egg safety from pathogenic bacterial contamination for human consumption. In addition, in Indonesian Law No. 18, 2012, Concerning about Food, Article 67 Paragraph 2 [4] mandated that food security was intended to prevent the possibility of biological (microbiological) contamination, chemicals and other objects that can interfere, harm and endanger human health.

Improving egg quality can be done by increasing the feed quality and one of them can be done by adding phytobiotics [5]. Phytobiotics are herbal plant that has bioactive compound which usually used in traditional medicine and can be used as an alternative to antibiotics [6]. Teak leaves (*Tectona grandis* Linn. *f*) is one of the phytobiotics that have not been widely used for feed additives in laying hens. Teak leaves contains flavonoids, anthocyanins, and natural antioxidants. According to Khera and Bhargava [7], bioactive compounds in teak leaves have antibacterial and antifungal activities. By looking at bioactive compound of teak leaves, it is expected to improve the egg quality of laying hens.

MATERIALS AND METHODS

Birds

A total of 240 Isa Brown laying hens aged at 30 weeks old with 53.13±3.26 g of average egg mass were used in this study. The birds were randomly assigned into six dietary treatments with four replications (10 birds of each replication).

Diets and experimental design

Basal diet was formulated according to the Guideline of Hy-Line Brown Laying Hens Management [8]. Table 1 shows the composition and nutrient value

of the basal diet. Diets were offered at 125 g/bird/day, while drinking water was supplied *ad libitum*. Feed additives used in this study were virginiamycin antibiotic dan teak leaf extract.

The teak leaf extract was prepared with the following methods: First of all, teak leaf was air dried for 24 hours and then oven dried at 45^{o}C for 36 hours. Dried teak leaf was then ground into powder. Teak leaf powder was then macerated in a 70% ethanol solution for 36 hours. After that, the filtrate was evaporated at 90 RPM of speed, 60^{o}C of bath tube temperature, and 40^{o}C of tube temperature. Result of this evaporation process was thick extract which ready to be used as feed additive for laying hens.

The method used in this study was experiment in a completely randomized design. The treatments used were R0 = basal diet without any feed additive, R1 = basal diet + 0.015% of virginiamycin, R2 = basal diet + 0.4% of teak leaf extract, R3 = basal diet + 0.8% of teak leaf extract, R4 = basal diet + 1.2% of teak leaf extract, and R5 = basal diet + 1.6% of teak leaf extract.

Egg quality

Variables measured in this study were egg weight (g/egg), albumen weight (g/egg), yolk weight (g/egg), eggshell weight (g/egg), albumen percentage (%), yolk percentage (%), eggshell percentage (%), Haugh unit, shell thickness (mm), and yolk color score.

Data analysis

Data were analyzed by using analysis of variance and reported as means ± standard deviation. The significance of differences among treatment means were evaluated by using Duncan Multiple Range Test.

Table-1: Composition and nutrient value of basal diet

Feedstuff	Composition (%)*	Price (IDR/kg)
Corn	54.32	4,050
Rice bran	8.69	2,700
Soybean meal	18.16	7,800
Meat bone meal	8.00	7,500
Palm oil	1.50	7,200
Stone meal	2.88	500
Grit	5.76	500
Sodium bicarbonate	0.09	7,500
Antifungi	0.08	65,000
Salt	0.11	3,500
Choline chloride 60%	0.04	21,000
Lysine HCl	0.14	23,000
DL-methionine	0.17	47,000
Trace mineral	0.03	37,500
Vitamin HC	0.02	120,000
Total	100.00	4,821.34
Nutrient value	Calculated*	Analyzed**
Metabolizable energy (Kcal/kg)	2,829.93	2,853.60***
Dry matter (%)	88.38	88.78
Ash (%)	11.99	11.04
Crude protein (%)	17.88	17.74
Ether extract (%)	5.64	5.43
Crude fiber (%)	2.92	7.26
Calcium (%)	4.08	4.34
Total phosphorus (%)	0.78	0.78
Available phosphorus (%)	0.49	-
Lysine (%)	0.96	-
Digestible lysine (%)	0.83	-
Methionine (%)	0.44	-
Digestible methionine (%)	0.41	-
Sodium (%)	0.16	-
Chloride (%)	0.16	-

*Calculated by using WinFeed Program with the database of nutrient value of feedstuff from Guideline of Hy-Line Brown Laying Hens Management [8], ** Analyzed value of Laboratory of Animal Feed, Department of Animal Husbandry and Fisheries, Blitar Regency, *** Metabolizable energy was calculated from 80% gross energy [9].

RESULTS AND DISCUSSION

Nutrient value and bioactive compounds of teak leaf extract

Preliminary research to evaluate the characteristic of teak leaf extract in this study was done by analyzing its nutrient value and bioactive compounds. Table 2 shows the nutrient value of teak leaf powder and extract.

Table-2: Nutrient value of teak leaf powder and extract (100% dry matter)

Nutrient	Teak leaf powder	Teak leaf extract
Dry matter (%)	88.44	6.57
Ash (%)	13.00	13.55
Crude protein (%)	11.90	10.96
Ether extract (%)	1.7	-
Crude fiber (%)	35.91	-
Calcium (%)	1.87	2.44
Phosphorus (%)	0.25	0.46
Gross energy (Kcal/kg)	4,256	4,230

Analyzed value of Laboratory of Animal Feed, Department of Animal Husbandry and Fisheries, Blitar Regency

Proximate analysis in Table 2 shows that teak leaf extract had lower organic matter compared to teak leaf powder. This result can be seen on the crude protein content which decreased 0.94%, while metabolizable energy decreased 26 kcal/kg. On the other hand, inorganic matter of teak leaf extract was increase such as calcium, phosphorus, and ash with 0.87%, 0.21%, and 0.55% of increase, respectively, compared to teak leaf powder. Table 3 shows the bioactive compounds of teak leaf extract including flavonoid, anthocyanin, and antioxidant.

Table-3: Bioactive compounds of teak leaf extract

Bioactive compound	Analyzed value
Flavonoid	128.69 mg/100g
Anthocyanin	83.89 ppm
Antioxidant	47.61%

Analyzed value of Laboratory of Food and Nutrition Studies Center, Gadjah Mada University

Egg weight

Table 4 shows that the use of teak leaf extract had a highly significant effect (P<0.01) on the egg weight. Laying hens fed diet containing teak leaf extract had higher egg weight compared to control. This finding showed that the addition of teak leaf extract could increase egg weight.

Table-4: Effect of dietary teak leaf extract on egg quality of laying hens

Variable	R0	R1	R2	R3	R4	R5
Egg weight (g/egg)	58.17±0.62^{a}	62.03±0.43^{c}	58.56±0.30^{a}	59.75±1.00^{ab}	61.94±0.84^{c}	61.06±1.06^{bc}
Albumen weight (g/egg)	35.36±0.66^{a}	37.67±0.77^{c}	35.08±0.64^{a}	35.61±0.80^{ab}	37.44±1.25^{bc}	36.61±1.16^{abc}
Albumen percentage (%)	60.77±1.07	60.70±0.88	59.90±0.79	59.57±0.53	60.39±1.43	59.93±0.98
Yolk weight (g/egg)	15.94±0.69	16.39±0.48	16.08±0.31	16.19±0.23	16.25±0.31	16.28±0.28
Yolk percentage (%)	27.44±1.14	26.44±0.97	27.48±0.61	27.13±0.23	26.27±0.67	26.67±0.39
Shell weight (g/egg)	6.86±0.17^{a}	7.97±0.36^{bc}	7.39±0.23^{ab}	7.94±0.19^{bc}	8.25±0.55^{c}	8.17±0.28^{c}
Shell percentage (%)	11.80±0.22^{a}	12.86±0,53^{ab}	12.63±0.44^{ab}	13.31±0.31^{b}	13.34±0.97^{b}	13.40±0.67^{b}
Haugh Unit	70.64±3.23^{a}	78.80±1.08^{b}	75.95±1.49^{a}	83.64±0.86^{b}	85.51±4.63^{b}	87.53±2.11^{b}
Shell thickness (mm)	0.33±0,00^{a}	0.36±0.00^{c}	0.34±0.00^{a}	0.35±0.00^{b}	0.36±0.00^{c}	0.36±0.00^{c}
Yolk color score	7.03±0.14^{a}	7.17±0.11^{a}	7.28±0.11^{ab}	7.28±0.14^{ab}	7.22±0.00^{ab}	7.44±0.16^{b}

$^{a-c}$ means value followed by different superscript showed a highly significant differences (P<0.01), R0 = basal diet without any feed additive, R1 = basal diet + 0.015% of virginiamycin, R2 = basal diet + 0.4% of teak leaf extract, R3 = basal diet + 0.8% of teak leaf extract, R4 = basal diet + 1.2% of teak leaf extract, and R5 = basal diet + 1.6% of teak leaf extract

Duncan Multiple Range Test analysis showed that the lowest egg weight (58.17±0.62 g/egg) was found in the control diet. The addition of teak leaf extract until the level of 0.8% did not significantly increase egg weight. The significant increase in egg weight was found after the addition of teak leaf extract

at the level of 1.2 and 1.6%. Both of those treatments had statistically similar effect to the addition of virginiamycin at the level of 0.015%.

The increase in egg weight which was found in this study may be due to the teak leaf extract containing flavonoids in the amount of 128.69 mg/100 g. According to Khera and Bhargava [7], flavonoids had antibacterial activity. Brisbin *et al*. [10] Stated that the reduction of pathogenic bacteria in the digestive tract of chickens could increase feed digestibility, optimize nutrient absorption and increase the beneficial bacteria. This mechanisms then may increase the egg weight.

The increase in egg weight maybe also related to the antioxidant and antifungal activity of teak leaf extract, which can improve the function of the digestive tract [7, 10], can inhibit the growth of fungi, especially *A. flavus* and *A. fumigatus* [11]. According to Siloto *et al*. [12] The fungal contamination, especially aflatoxin in feed, can affect egg weight, albumen weight and yolk weight, because aflatoxin can suppress protein and fat metabolism in the liver, while protein and fat were the main components of egg.

In line with this current finding, Iskander *et al*. [13] also reported that adding flavonoids in the form of quercetin as much as 0.5 g/kg diets could increase the total protein of egg from 14.28 to 14.85 g/100 g. Bintang *et al*. [14] also reported that the addition of noni pulp (containing flavonoids) as much as 5 g/kg diets could increase egg weight from 53.97 to 56.34 g/egg. Gumus *et al*. [15] Reported that the addition of 0.5% herbal ingredients of turmeric (*Curcuma longa*) which has antibacterial activity in laying hens diet could increase egg weight from 56.38 to 59.37 g/egg. Whereas, in this study, the egg weight increased from 58.17 to 61.96 g/egg.

Albumen weight and percentage

Table 4 shows that the addition of teak leaf extract had a highly significant effect ($P<0.01$) on the albumen weight, but did not significantly affect ($P> 0.05$) albumen percentage. The addition of teak leaf extract increased the egg white weight compared to the control but did not increase the percentage of egg white. This result showed that the increase in albumen weight was in line with the increase in egg weight.

Duncan Multiple Range Test analysis showed that the addition of teak leaf extract at the level of 0.4 and 0.8% did not significantly increase albumen weight. However, the significant increase in albumen weight was found after the addition of teak leaf extract at the level of 1.2 and 1.6%. Both of those treatments had similar albumen weight compared to the addition of virginiamycin at the level of 0.015%.

The increase in albumen weight from 35.08 to 37.67 g/egg may be connected to the increase in protein digestibility affected by the presence of flavonoids in teak leaf extract. Protein will affect albumen synthesis [16]. Figueiredo *et al*. [17] Explained that the essential amino acids content in the diet, especially lysine and threonine, could affect the egg characteristics such as egg size, yolk, albumen deposition and internal egg quality. Iskander *et al*. [13] Reported that flavonoid supplementation in the form of quercetin at the level of 0.5 g/kg could increase the total egg protein from 14.28 to 14.85 g/100 g.

The increase in albumen weight can be caused by the increase in egg weight. According to Alfiyah *et al*. [18], egg weight can affect the quality of the egg contents, the weight of the egg contents tended to follow the pattern of the increase in egg weight. The increase in egg weight may also increase the weight of the egg contents. In addition to these factors, there were major factors that can affect egg size, namely nutrition and types of poultry.

The albumen weight and albumen percentage of laying hens in this present study were 35.08 to 37.67 g/egg and 59.57 to 60.77%, respectively. This result was relatively lower than the results of Santoso and Fenita [19], which reported that the albumen weight and albumen percentage of laying hens were 41.55 to 42.21 g/egg and 62.04 to 64.26%, respectively. In another study, Pelicia *et al*. [20] found that the albumen percentage of laying hens was ranged from 64.67 to 65.45%. Siloto *et al*. [12] Reported that albumen percentage of laying hens was 67.51%. The relatively lower of albumen weight may be due to the age of laying hens used in this study. Chukwuka *et al*. [21] Noted that the age of birds could affect the weight of albumen, yolk, and shell.

Yolk weight and percentage

The results of statistical analysis in Table 4 show that the addition of teak leaf extract did not significantly affect ($P> 0.05$) yolk weight and percentage. These findings showed that the teak leaf extract treatment until the level of 1.6% could not increase yolk weight and percentage. This result may be because of the similar fat content for yolk synthesizes in all dietary treatment. Alfiyah *et al*. [18] explained that fat content in the diet plays a role in the egg yolk formation and fat synthesis that occurs in the liver and then transferred to the ovaries through the bloodstream.

The results that were not significantly different were also caused by quinones, steroids, glycosides, phenolic acids, flavonoids, carbohydrates, alkoloid, tannins, saponins content in teak leaf extract [7]. Budiarto *et al*. [22] explained that alkaloid content of *Guazuma ulmifolia* leaves can inhibit the lipase activity in the gastrointestinal tract so that the fat absorption in the body decreases. Narita [23] explained that the flavonoid was included in natural phenolic compounds which can inhibit intestinal micelle formation where

absorption of bile acids was one of its functions to dissolve fat through the bile ducts into the intestine. Saponin inhibit cholesterol absorption in the intestine by forming complex insoluble bonds with cholesterol, binds to bile acids to form micelles and increases the binding of cholesterol by fiber, while tannin can inhibit absorption of fat in the intestine by reacting with mucosal proteins and intestinal epithelial cells. The reduction of fat absorption resulting in the decrease of fat deposition for egg yolks synthesize.

Fayyaz *et al*. [24] Reported that the addition of flavonoids in the form of quercetin could increase laying rate, shell strength, egg protein content and Haugh unit, and decrease fat content in the yolk. Iskander *et al*. [13] reported that supplementation of 0.5 g/kg of hesperidin and quercetin could reduce yolk cholesterol from 16.08 to 15.15%.

Yolk weight of laying hens in this study ranged from 15.94 to 16.39 g/egg, while the yolk percentage ranged from 26.27 to 27.48%. In another study, Siloto *et al*. [12] found that the yolk weight and percentage of laying hens were 15.78 g/egg and 23.35%, respectively. Padhi *et al*. [25] Noted that yolk weight and percentage of laying hens were 13.05 g/egg and 27.50%, respectively.

Shell weight and percentage

Table 4 shows that the teak leaf extract supplementation had a highly significant effect ($P<0.01$) on the shell weight and percentage. This results statistically showed that the teak leaf extract treatment could increase the shell weight and percentage compared to control. Duncan's Multiple Range Test analysis showed that teak leaf extract supplementation at the level of 0.4% did not increase the shell weight and percentage, the increase of these variables was visible when teak leaf extract was supplemented at the level of at least 0.8%. Moreover, the use of teak leaf extract at the level of 0.8% had a similar effect to those supplemented at the level of 1.6% and also the same as virginiamycin treatment.

Mathematically, the increase in shell percentage occurred because of the increase in shell weight was higher than the increase in egg weight, because the shell percentage was inversely proportional to the egg weight. The increase in shell weight may be due to the increase in calcium and phosphorus absorption in the digestive tract caused by bioactive compound in teak leaf extract, especially flavonoids. Hence, the availability of calcium and phosphorus for calcification was more optimal. Iskander *et al.* [13] Reported the supplementation of 0.5 g/kg of flavonoids in the form of hesperidin, naringin and quercetin could increase calcium metabolism. Nys [26] stated that eggshell quality was influenced by the calcium, phosphorus, microminerals, vitamin D3 and particle size of mineral sources. Chukwuka *et al*. [21] Explained that the calcium and phosphorus were essential macro minerals for calcification in eggshells. Świątkiewicz *et al*. [27] Explained that the factors that influence mineralization and eggshell quality were genetic, environmental, nutrition and animal health. Świątkiewicz *et al*. [3] Reported that supplementation of herbal extracts, probiotics, and chitosan could increase calcium absorption and supplementation of 2000 mg/kg of herbal extracts in 56-week old laying hens could increase the shell percentage from 10.76 to 10.99%. A similar result was also reported by Sharma *et al*. [1], who found that supplementation of laying hens diet with calcium, phosphorus, and herbs could improve eggshell quality.

In this study, the shell weight of laying hens ranged from 6.86 to 8.25 g/egg, while the shell percentage ranged from 11.80 to 13.40%. This result was relatively higher than the report of Siloto *et al*. [12] who found that the shell weight and percentage of laying hens were 6.17 g/egg and 9.13%, respectively. In another study, Iskander *et al*. [13] Reported that the shell weight of laying hens ranged from 8.18 to 8.64 g/egg, while the shell percentage ranged from 12.22 to 12.80%.

Haugh Unit

Table 4 shows that the use of dietary teak leaf extract had a highly significant ($P<0.01$) effect on Haugh unit. This finding statistically showed that the addition of teak leaf extract could increase Haugh units compared to control. The increase in Haugh units occurred along with an increase in the supplementation level of teak leaf extract. Duncan's Multiple Range Test analysis showed that the addition of teak leaf extract up to 0.4% level did not improve the Haugh unit. The increase in Haugh unit began to be seen in the addition of teak leaf extract of at least 0.8%. Statistically, the addition of teak leaf extract at the level of 0.8, 1.2, and 1.6% had a similar effect on Haugh unit compared to the addition of virginiamycin at the level of 0.015%. The highest value of Haugh unit was 87.53 which recorded in the 1.6% of teak leaf extract treatment.

The increase in Haugh unit was mathematically related to the increase in egg weight and albumen height. According to Mampioper *et al*. [28] Haugh unit was the basis for measuring the egg quality index that connected the albumen height and egg weight. In this study, the increase in Haugh unit was in line with the increase in egg and albumen weight. In the previous study, it was stated that Haugh unit was influenced by the feed quality [29], egg storage length [30], microminerals (Fe, Co, Cu, Zn, and Cr) and vitamins (A, B_1 , B_{12}, and C) [31, 32], and the age of laying hens [25]. The bioactive compounds of teak leaf extract could improve feed digestibility [10] so that the nutrients which affect Haught unit will be more easily absorbed.

Shell thickness

Table 4 shows that the addition of teak leaf extract had a highly significant effect (P<0.01) on shell thickness. This finding statistically showed that the treatment of teak leaf extract could increase shell thickness compared to control. The increase in shell thickness was in line with the increase in shell weight, shell percentage and the supplementation level of teak leaf extract.

Duncan Multiple Range Test analysis showed that the teak leaf extract supplementation up to 0.8% had no significant effect on the shell thickness, whereas the use of 1.2 and 1.6% of teak leaf extract could increase shell thickness compared to control. Results also showed that the use of 1.2% dietary teak leaf extract had a similar effect on shell thickness compared to the use of 1.6% dietary teak leaf extract and virginiamycin treatments.

The increase in shell thickness in this current study may be because of antibacterial activity of teak leaf extract [7], which could suppress pathogenic bacteria and increase non-pathogenic bacteria in the digestive tract, as well as optimizing the function of the digestive tract [33]. Świątkiewicz *et al*. [27] explained that non-pathogenic bacterial fermentation could optimize intestinal mucosa function and feed absorption, increase phytate degradation, and increase calcium-binding-protein resulting increase in calcium absorption. Park *et al*. [34] Reported that phytogenic feed additive could balance the intestinal microflora, increase absorption of nutrients such as ash, calcium, phosphorus and in line with the increase in egg weight, hen day production, and eggshell breaking. Sharma *et al*. [1] And Hunton [35] stated that calcium and phosphorus were important factors in the shell structure formation.

Shell thickness of laying hens in this current study ranged from 0.331 to 0.358 mm. This result was relatively lower than those reported by Iskander *et al*. [13] who found that shell thickness of laying hens fed diet containing 0.5 g/kg flavonoid ranged from 0.40 to 0.41 mm. However, this current finding was in agreement with Sakroni *et al*. [36] Who noted that shell thickness of Isa Brown laying hens was 0.35±0.01 mm.

Yolk color score

The results of statistical analysis in Table 4 showed that the addition of teak leaf extract had a highly significant effect (P<0.01) on the yolk color score. This finding statistically showed that the treatment of adding teak leaf extract could increase the yolk score compared to the control. Duncan Multiple Range Test analysis showed that the inclusion of teak leaf extract with a level of 1.6% could increase the yolk color score compared to the control and antibiotic treatments.

In this study, teak leaf extract contained flavonoid and anthocyanin in the amount of 128.69 mg/100 g and 83.89 ppm, respectively (Table 3). Iskander *et al*. [13] Reported that flavonoids could increase plasma yellow fluid in the yolks which bounded to protein. Anthocyanin was a pigment which gave blue, purple, violet, magenta, red, and orange colors to the plants [37]. This pigment was classified as flavonoid compounds and has antioxidant activity [38]. Fayyaz *et al*. [24] Explained that the flavonoids content in the feed in the form of quercetin could accumulate into the yolk and albumen with protein because the quercetin was a good protein binding. In Fayyaz *et al*. [24] study, the yolk contained 6.36 to 7.41 µg/ml. So that with the increase in the supplementation levels of teak leaf extract will be in line with the increase in anthocyanin and flavonoids consumed and absorbed, resulting in increase in the yolk score.

CONCLUSION

Based on the results above, it could be concluded that the use of 1.2% of dietary teak leaf extract could improve egg quality of laying hens (egg weight, albumen weight, shell weight, shell percentage, Haugh unit, shell thickness, and yolk color score). It is recommended to use 1.2% of teak leaf extract to replace antibiotic in laying hens diet.

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