SUBJECT: Submission Confirmation
FROM: Veterinary World <vetworld@ejmanager.com>
TO: kismiati59@gmail.com
DATE: 08/11/2019 13:38

Dear Sri Kismiati,

Your submission entitled **DIETARY SUPPLEMENTATION OF BINAHONG (Anredera Cordifolia) LEAF POWDER TO PRODUCE EGGS WITH LOW CHOLESTEROL** (Manuscript Number: VETWORLD-2019-11-571) has been received by **Veterinary World**.

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Best regards,

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SUBJECT: Article Revision Letter for Authors - (VETWORLD-2019-11-571)
FROM: Veterinary World <vetworld@ejmanager.com>
TO: kismiati59@gmail.com
DATE: 04/01/2020 09:49

Dear Sri Kismiati,

Your manuscript entitled \"DIETARY SUPPLEMENTATION OF BINAHONG (Anredera Cordifolia) LEAF POWDER TO PRODUCE EGGS WITH LOW CHOLESTEROL \" (Ms.Nr. VETWORLD-2019-11-571) was reviewed by reviewers of the Veterinary World. As initial decision, your manuscript was found interesting but some revisions have to be made before it can reach a publishable value. Please refer comments given at bottom.

You should send your revised manuscript via the online system of ScopeMed on my.ejmanager.com.

Sincerely yours,

Dr. Anjum Sherasiya
Editor-Veterinary World
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COMMENTS for Authors:

EDITORIAL COMMENTS:

- Highlight all corrections/additions in red color font in revised manuscript.
- Please answer all the comments below point-by-point in an accompanying response letter to your revised submission and include your responses at appropriate paragraphs in the revised word file.
- Include all authors name, affiliation and email address in revised Word file as per format and style of Veterinary World. Please check latest article from www.veterinaryworld.org for format of this section.
- All reference no. in the text must be in continuous no. as per style of Veterinary World and amend the reference section accordingly if you have not done it.
- Please divide the introduction into 3 paragraphs if you have already not done. Introduction must be divided into 3 paragraphs i.e., 1. introduction 2. significance of the study and 3. aim of the study.
- Include authors\' contributions (refer just below the conclusion section in latest article from www.veterinaryworld.org for format of this section) if you have not added.
- Include Acknowledgements along with source of fund for this study if you have not included.
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=> Reviewers\' Comments:

English needs to be corrected throughout the manuscript with the help of native English speaker as your manuscript needs extensive editing.

Other corrections are given in the attached file.

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ATTACHMENTS (20200206-150207-0000003): "2-1573190652_BYREV-59109 - Revision.doc"

Dear Prof. Dr. Anjum Sherasiya
Editor-in-Chief Veterinary World

Thank you very much indeed for giving us the opportunity to revise our submitted manuscript (VETWORLD-2019-11-571) to Veterinary World.

We have revised the manuscript according to the suggestions provided by the reviewers (in red colour) in the revised manuscript. The detail responses to the reviewers are listed in the bottom of this letter.

Once again, thank you very much indeed
Best regards,

Sri Kismiati, Ph.D.
Faculty of Animal and Agricultural Sciences, Diponegoro University
Semarang, Central Java Indonesia

ADDITION OF BINAHONG (*Anredera Cordifolia)* LEAF POWDER TO DIETS TO PRODUCE EGGS WITH LOW CHOLESTEROL

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Abstract

Aim: The study aimed to evaluate the addition of binahong leaf powder in the quail rations on the production and eggs quality.

Materials and methods: The research used 200 of 7-week-old quails and kept in 20 wire cages with a body weight of 123.77 ± 0.72 g. The quails were treated as follows: ration without binahong leaf powder (T0), addition 2% of binahong leaf powder (T1), addition 4% of binahong leaf powder (T2) and addition 6% of binahong leaf powder (T3) in rations. The study used a completely randomized design (CRD). The parameters measured were egg production, weight and characteristic of eggs, the content of cholesterol, triglyceride, HDL, LDL and egg protein content of yolk.

Results: The results showed that addition of binahong powder at 2 – 6% of ration did not significantly affect egg production, egg characteristic and protein, but significantly ($P<0.05$) affected the cholesterol, triglyceride, HDL and LDL content of yolk egg. The cholesterol, triglyceride and LDL decreased significantly

in 2% whereas HDL increased significantly at 4 and 6% of binahong leaf powder addition.

Conclusion: In conclusion, addition of 2% of binahong in ration was enough to get healthy quail eggs with the low cholesterol, triglyceride and LDL.

Keyword: binahong, cholesterol, HDL, LDL, triglyceride

Introduction

Quail egg contains high and complete nutrients, but also contains high cholesterol. Its cholesterol content is higher than that in chicken egg. The protein content in quail egg is about 13.30% [1] while the cholesterol content in yolk of egg quail is 6.79 mg/dl. In addition, the cholesterol content in chicken egg yolk is 4.03 mg/dl [2]. It has been known that overconsumption of cholesterol will increase blood cholesterol level, leading to vulnerability to heart disease. This makes some people afraid of consuming quail egg. Catapano and Wiklund [3] stated that blood cholesterol, particularly low-density lipoprotein (LDL)-cholesterol, has positive correlation with the occurrence of atherosclerosis. Previous study showed that consumption of high-density lipoprotein (HDL) derived from quail egg increases blood serum HDL and decrease atherosclerotic plaques of rabbit. Thus, it was concluded that egg HDL may be used as an anti-atherosclerotic for patient with Cardiovascular Disease/CVD in human [4]. Any attempt to obtain quail egg with low cholesterol and high HDL needs to be carried out.

Binahong (*Anredera cordifolia*) is a wild plant with rapid growth rate which requires no complicated cultivation, and thereby the availability is very abundant. Sutrisno *et al*. [5] and Leliqia *et al*. [6] revealed that the leaf of binahong contains bioactive compounds, such as flavonoid, tannin, saponin, phenol and steroid, while Astuti *et al*. [7] reported that the leaf of binahong also contains terpenoid which may potentially increase pancreas insulin secretion. Morever, Hasbullah [8] documented that binahong leaf exhibited hypolepidemic properties. With regard particularly to flavonoid, Kamboh *et al*. [9] showed that supplementation of bioflavonoid increased antioxidant and enzyme activity and decreased total cholesterol and triglyceride of serum and breast meat of broiler. The study by Quyang *et al*. [10] showed that supplementation of 15 mg/kg of alfalfa flavonoid increased HDL and decreases cholesterol, triglyceride, serum LDL and percentage of abdominal fat of broiler. In respect to saponin, feeding such component has been reported to decrease cholesterol synthesis, insulin and blood triglyceride and increase blood HDL. Saponin also decreased the content of cholesterol in broiler meat [11] and protein digestibility [12].

The present study aimed to evaluate the effect of addition of binahong leaf powder in the rations on the content of cholesterol, triglyceride, HDL, LDL and protein of quail eggs.

Materials and Methods

Ethical approvals

The procedure of using quail in this study has been approved by the animal ethics committee in the Faculty of Animal Sciences Diponegoro University, Semarang, Indonesia.

Materials

The study used 200 of 7-week old female quails with average body weight of 123.77 ± 0.72 g (means ± SD). The quails were kept in 20 units of wire cage, with cage size of 90 cm × 35 cm × 25 cm for 10 quails. The feed consisted of yellow corn, rice bran, pollard, poultry meat meal (PMM), soybean meal, $CaCO_3$, salt, premix and binahong leaf powder.

Methods

This present research was arranged according to a Completely Randomized Design (CRD) with 4 treatments and 5 repetitions (10 quails each). The treatments groups included T0: control ration (without binahong leaf powder), T1: control ration + 2% of binahong leaf powder, T2: control ration + 4% of binahong leaf powder and T3: control ration + 6% of binahong leaf powder in ration. The composition of the control ration and nutrient content of ration are presented in Table 1 and 2.

Recording of egg production was carried out every day during the research and using the formula: (number of eggs / number of chickens) × 100%. Egg weight and egg characteristic were measured every 3 days at the end of the week. Data of cholesterol, triglyceride, HLD, LDH and egg yolk protein were collected out by sampling 1 egg per experimental unit at the end of the study. Analysis of cholesterol and triglyceride was conducted based on cholesterol p-

amino phenazone method (CHOD-PAP), HDL and LDL analysis using enzymatic colorimetric method [13] and yolk protein analysis were using the Kjedahl method [14].

Result and Discussion

The Effect Addition of Binahong Leaf Powder on Egg Production

The results showed that addition of 2 - 6% of binahong leaf powder rich in flavonoid and saponin did not significantly influence the egg production of quail at 8 - 14 weeks of age (Table 3). This finding was similar with that of reported by Kusumanti and Murwani [15] showing that supplementation of binahong leaves powder did not significantly affect the egg production of laying hens. In line with this Iskender *et al*. [16] showed that flavonoids had no significant effect on egg production. In contrast, supplementation of quertecin flavonoid [17] and saponins derived of karaya increased egg production of laying hens [18]. These inconsistent data may be due to the differences in the level of flavonoid and saponin used in the study as well as the condition of the study.

The Effect of Binahong Leaf Powder on Egg Characteristic

Addition of binahong leaf powder did not significantly affect the characteristic of egg including egg weight, yolk weight, albumen weight and eggshell weight (Table 4). It has previously been hypothesized that the bioactive component of binahong leaf may positively affect the egg characteristic. However, data from this present study revealed differently. According to Leke *et al*. [19] flavonoid of papaya seeds increased egg quality (egg yolk, albumen and

egg shell weight) of the Indonesian local hens. Moreover, Afrose *et al*. [18] reported that supplementation of 25, 50 or 75mg/kg of karaya saponin increased egg weight, yolk egg weight and albumen weight of laying hens, while Ayasan *et al*. [20] reported that supplementation of Yucca schidigera powder equal to 120 ppm saponin increased egg weight but did not affect the eggshell weight. In accordance to our data, supplementation of 0.2-0.6g/kg flavonoid did not significantly influence egg quality in the study of [17]. Also, supplementation of flavonoid did not significantly influence eggshell weight as reported by Iskender *et al*. [16]. The differences in the nature and levels of bioactive compounds, the nutritional values of rations and the conditions of study may be responsibility for the above divergent result.

Cholesterol, triglyceride, HDL, LDL and protein content of Yolk

The data on cholesterol, triglyceride, HDL, LDL and yolk protein content due to binahong leaf powder supplementation are shown in Table 5. Addition of 2% of binahong leaf powder ($P<0.05$) decreased the level of cholesterol in the yolk of quail eggs. It seemed that flavonoid and saponin in the binahong leaf powder could inhibit cholesterol absorption, and hence the cholesterol deposition could be reduced [21,22,23]. This was confirmed by Lien *et al*. [24] that consumption of flavonoid increased the excretion of cholesterol through excreta. In addition, flavonoid may increase the reverse cholesterol transport (RCT) resulting in high cholesterol excretion and thus lower cholesterol level in yolk. It should, however, be noted that binahong leaf powder addition did not affect the cholesterol level, when supplemented on the level of 4 and 6%.

Table 5 showed that addition of binahong leaf powder decreased the triglyceride content of quail yolk egg. In line with our data, supplementation of flavonoid extracted from the root of Scutellaria baicalensis Georgi decreased triglyceride the serum of broiler [25]. Likewise, Kamboh and Zhu [21] and Ouyang *et al*. [10] reported that consumption of flavonoids decreased the level of triglyceride the serum and meat of broiler. According to Hsu and Yen [26] and Nagai *et al*. [27], flavonoid may inhibit the intracellular triglyceride synthesis, in the liver resulted in lower triglyceride deposition in quail egg yolk. In addition, flavonoid content in the binahong leaf powder may increase the expression of peroxisome proliferator-activated receptor α (PPARα) in the liver involving in lipid metabolism especially fatty acid oxidation [28,10].

It was apparent from the study that the content of yolk HDL increased ($P<0.05$) with the increased level of binahong leaf powder in the quail rations. In agreement to our finding, Afrose *et al.* [29] documented that saponin increased HDL content in broiler meat. Supplementation of karaya saponin also increased HDL content in quail egg [30]. Furthermore, Smith *et al*. [31] stated that supplementation of saponin extract increased HDL of blood, liver, kidney and heart tissues of white mouse. Kamboh and Zhu [21] reported that flavonoid increased HDL of serum dan HDL deposition in breast meat of broiler. The mechanism by which binahong leaf meal increased HDL concentration in quail egg yolk di not definitely know, but the role of binahong leaf powder in the reverse cholesterol transport (RCT) mechanism seemed to be attributable to the increased HDL content quail yolk. Millar *et al*. [23] and Marques *et al*. [32]

169 reported that the increased RCT mechanism due to binahong leaf powder
170 supplementation was accompanied by the increased level of HDL. This seemed to
171 be related to the contribution of HDL to RCT.

172 Table 5 showed that treatment with binahong leaf powder decreased the
173 level of LDL in the quail egg yolk. Similar to this, Afrose *et al.* [30] reported that
174 saponin decreased LDL content of quail egg. Likewise, Chaudhary *et al*. [33]
175 stated that saponin potentially decreased the LDL of meat. Also, supplementation
176 of saponin extract decreased LDL of blood, liver, kidney and heart tissues of
177 white mouse [31]. In addition to saponin, flavonoid has been reported to
178 decreased the LDL level in the blood of broiler [21, 10, 25]. Furthermore, Zhou *et*
179 *al*. [25] stated that the flavonoid supplementation also decreased the LDL content
180 of the breast meat of broiler. Binahong leaf contains saponin which works by
181 binding bile acid and forming large mixed-micelle, thus existing cholesterol in
182 micelle cannot be absorbed by microvilli on the surface of intestinal epithelial
183 cells, causing a decrease of total and LDL-cholesterol [34]. Saponin inhibited fat
184 metabolism through inhibiting lipase enzyme secretion, and thus decreases
185 cholesterol, LDL of blood [35] and increases HDL [36]. In respect to flavonoid,
186 this active compound has been documented to reduce the activity of fatty acid
187 synthase and thereby reduce the level of LDL in the animal tissues [10].

188 Addition of 2-6% binahong leaf powder did not significantly influence
189 protein content of egg yolk. The study was in contrast to previous study. Iskender
190 *et al*. [16] reported that supplementation of flavonoid (hesperidin, naringin or
191 quercetin) increases egg protein. Ahmad *et al*. [37] documented that bioactive

compounds from *Moringa Oleifera* leaf meal increased protein of laying hen yolk.

In general, protein content in egg is derived from feed. In this study the inclusion of binahong leaf powder was accompanied by the decreased crude protein in ration. Hence the protein-increasing effect by binahong leaf powder seemed to be inhibited by lower protein content in the quail ration due to binahong leaf inclusion.

Conclusion

The addition of 2% binahong leaf powder is best for lowering cholesterol, triglyceride and LDL levels of egg quail.

Authors' Contributions

SK conducted the research, data collection and drafting of the article. HIW developed the feeding concept and supervised the research, RM advised the experimental design, DS conducted data analysis and SM performed laboratory analysis.

Acknowledgements

The authors would like to thank Sugiharto S.Pt., M.Sc. Ph.D. for proofreading the manuscript.

Competing Interest

The authors declare that they have no competing interests.

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Table 1.

Feed Composition of the Control Ration

Feed ingredients	%
Yellow corn	48.00
Rice bran	6.00
Pollard	16.00
Poultry meat meal	14.00
Soybean meal	10.00
$CaCO_3$	5.50
Salt	0.25
Premix	0.25
Total	100.00

Table 2.

Nutrition Content of the Ration

Nutrient contents	Addition of binahong leaf powder			
	T0 (0%)	T1(2%)	T2(4%)	T3 (6%)
	----------%----------			
Energy Metabolic (kkal/kg)	2763.20	2694.12	2628.26	2565.40
Protein (%)	19.77	19.35	18.95	18.56
Fat (%)	4.66	4.58	4.51	4.43
Crude fibre (%)	4.29	4.95	5.56	6.12
Ca (%)	3.15	3.06	2.97	2.88
P (%)	0.79	0.76	0.74	0.71
Lysine (%)	0.96	0.93	0.89	0.86
Methionine (%)	0.48	0.46	0.44	0.42

Table 3.

Egg Production on **Addition** of Binahong Leaf Powder in Quail Rations

Age	Addition of Binahong Leaf Powder				p value
	T0 (0%)	T1(2%)	T2(4%)	T3 (6%)	
(weeks)	……%.....				
8	29.71±2.74	28.00±2.166	28.85±2.74	28.57±2.67	0.49
9	39.71±2.34	42.28±1.91	41.42±2.67	41.14±2.55	0.53
10	45.14±4.58	46.09±1.47	47.23±2.34	45.14±3.08	0.57
11	46.64±2.42	49.49±1.38	48.73±3.83	47.63±1.73	0.59
12	56.38±2.49	52.34±1.86	54.50±1.37	54.57±1.76	0.67
14	56.17±2.47	57.55±1.45	57.73±1.85	58.74±1.93	0.69

Table 4.

Influence of **Addition** of Binahong Leaf Powder on Quail Eggs Characteristic

Parameters	Addition of Binahong Leaf Powder				P value
	T0 (0%)	T1(2%)	T2(4%)	T3 (6%)	
Egg weight (g)	9.99±0.29	10.01±0.11	10.03±0.11	10.03±0.27	0.38
Yolk Weight (g)	3.65±0.13	3.92±0.25	3.68±0.12	3.93±0.17	0.57
Albumen Weight (g)	4.68±0.29	4.53±0.18	4.47±0.25	4.60±0.19	0.53
Eggshell Weight (g)	1.14±0.28	1.12±0.15	1.14±0.17	1.01±0.10	0.32
Yolk Weight (%)	38.77±2.34	41.80±2.72	40.01±1.39	41.13±0.79	0.51
Albumen Weight (%)	49.63±2.78	48.27±2.07	48.56±2.70	48.13±1.58	0.73
Eggshell Weight (%)	11.43±2.73	11.07±1.60	11.41±1.73	10.11±1.14	0.12

Table 5.

Influence of **Addition** of Binahong Leaf Powder on Cholesterol, Triglyceride, HDL, LDL and Protein of Quail Egg Yolk

Parameters	**Addition of Binahong Leaf Powder**				P value
	T0 (0%)	T1(2%)	T2(4%)	T3 (6%)	
Yolk Cholesterol (mg/g)	56.15±5.04[a]	23.77±3.75 [b]	51.97±4.63 [a]	51.35±7.55 [a]	<0.01
Yolk Triglyceride (mg/g)	903.22±58.21 [a]	608.62±22.46 [c]	778.34±53.59 [b]	811.63±104.01 [b]	<0.01
Yolk HDL (mg/g)	36.86±1.32[b]	34.8b±1.58 [b]	48.62±1.67 [a]	47.52±1.58 [a]	<0.01
Yolk LDL (mg/g)	29.18 ±1.59 [a]	16.06b±0.33 [b]	17.09±0.37 [b]	16.28±0.36 [b]	<0.01
Protein of yolk (%)	30.16±2.78	30.37±.82	29.15±0.81	30.19±2.55	0.00

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Addition of Binahong (*Anredera cordifolia)* leaf powder in diets to produce quail eggs with low cholesterol - Sri Kismiati, Hanny Indrat Wahyuni, Rina Muryani, Dwi Sunarti and Sri Sumarsih

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RESEARCH ARTICLE

Addition of binahong (*Anredera cordifolia*) leaf powder to diets to produce eggs with low cholesterol

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Abstract

Aim: The aim of this study was to evaluate the effect of the addition of binahong leaf powder to quail rations on the production and quality of eggs.

Materials and Methods: The study involved the use of two hundred 7-week-old quails housed evenly in 20 wire cages with a body weight of 123.77±0.72 g. The quails were treated as follows: Ration without binahong leaf powder (T0), addition 2% of binahong leaf powder (T1), addition 4% of binahong leaf powder (T2), and addition 6% of binahong leaf powder (T3). The study used a completely randomized design. The parameters measured were the production, weight, and characteristics of the eggs, as well as the cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and egg protein content in the yolk.

Results: The addition of 2-6% binahong powder did not significantly affect egg production, egg characteristics, or egg protein content, but significantly ($p<0.05$) affected the cholesterol, triglyceride, HDL, and LDL contents in yolk. The cholesterol, triglyceride, and LDL contents decreased significantly in T1, whereas HDL increased significantly in T2 and T3.

Conclusion: The addition of 2% binahong was enough to obtain healthy quail eggs with low levels of cholesterol, triglyceride, and LDL.

Commented [A1]: The addition of this word clarifies 10 quails per cage.

Please confirm.

Keywords: binahong, cholesterol, high-density lipoprotein, low-density lipoprotein, triglyceride.

<H1>Introduction

Quail egg contains many high-quality nutrients but also high cholesterol [1]. The protein content in a quail egg is approximately 13.30% [1]. The cholesterol content in a quail egg is higher than in a chicken egg. The cholesterol content in the yolk of a quail egg is 6.79 mg/dl, whereas the cholesterol content in chicken egg yolk is 4.03 mg/dl [2]. Overconsumption of cholesterol increases the blood cholesterol level, which leads to heart disease; thus, some people are afraid of consuming quail egg. Catapano and Wiklund [3] stated that blood cholesterol, particularly low-density lipoprotein (LDL) cholesterol, has a positive correlation with the occurrence of atherosclerosis. The consumption of high-density lipoprotein (HDL) derived from quail egg increases the blood serum HDL level and decreases atherosclerotic plaques in rabbits. Thus, egg HDL may be used as an anti-atherosclerotic agent for patients with cardiovascular disease [4]. An attempt to obtain quail egg with low cholesterol and high HDL contents needs to be carried out.

Binahong (*Anredera cordifolia*) is a wild plant with a rapid growth rate that requires no complicated cultivation; thus, binahong is abundant. Sutrisno *et al*. [5] and Leliqia *et al*. [6]

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revealed that the leaf of binahong contains bioactive compounds, such as flavonoids, tannins, saponins, phenols, and steroids, and Astuti *et al*. [7] reported that the leaf also contains terpenoid, which may potentially increase pancreatic insulin secretion. Furthermore, Hasbullah [8] documented that binahong leaf exhibits hypolipidemic properties. Kamboh *et al*. [9] showed that supplementation with a bioflavonoid increases antioxidant and enzyme activities and decreases total cholesterol and triglyceride levels in the serum and breast meat of broiler. A study by Ouyang *et al*. [10] showed that supplementation with 15 mg/kg of alfalfa flavonoid increases HDL levels and decreases the levels of cholesterol, triglyceride, and serum LDL and the percentage of abdominal fat of broiler. Feeding saponin decreases cholesterol, insulin, and blood triglyceride and increases blood HDL synthesis. Saponin also decreases the contents of cholesterol [11] and protein digestibility [12] of broiler meat.

The aim of this study was to evaluate the effect of the addition of binahong leaf powder to rations on the contents of cholesterol, triglyceride, HDL, LDL, and protein in quail eggs.

Commented [A3]: There should be a paragraph prior to this stating the significance of your study.

<H1>Materials and Methods

<H2>Ethical approval

The procedure of using quail in this study has been approved by the Animal Ethics Committee in the Faculty of Animal Sciences, Diponegoro University, Semarang, Indonesia.

<H2>Animals

The study used two hundred 7-week-old female quails with an average body weight of 123.77±0.72 g. The quails were housed in 20 wire cages. Each cage was 90 × 35 × 25 cm and housed 10 quails. The feed consisted of yellow corn, rice bran, pollard, poultry meat meal, soybean meal, $CaCO_3$, salt, premix, and binahong leaf powder.

<H2>Experimental design

This study was arranged according to a completely randomized design with four treatments and five repetitions (10 quails per group per repetition). The treatment groups included T0, control ration (without binahong leaf powder); T1, control ration + 2% binahong leaf powder; T2, control ration + 4% binahong leaf powder; and T3, control ration + 6% binahong leaf powder in ration. The composition and nutrient content of the ration are presented in Tables-1 and 2.

<H2>Tests and procedures

The recording of egg production was carried out every day during the study and the formula: Production = (number of eggs/number of chickens) × 100% was used. Egg weight and egg characteristics were measured every 3 days at the end of the week. The cholesterol, triglyceride, HDL, LDL, and protein contents in the egg yolk were collected by sampling one

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egg per experimental unit at the end of the study. The analyses of cholesterol and triglyceride contents were conducted based on the cholesterol p-aminophenazone method, HDL and LDL analysis was based on the enzymatic colorimetric method [13], and protein analysis was based on the Kjeldahl method [14].

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<H2>Statistical analysis

???

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<H1>Results and Discussion

<H2>Effect of the addition of binahong leaf powder on egg production

The addition of 2-6% of binahong leaf powder, rich in flavonoids and saponins, did not significantly influence quail egg production at 8-14 weeks of age (Table-3). This result was similar to that reported by Kusumanti and Murwani [15], which showed that supplementation of binahong leaf powder does not significantly affect the egg production of laying hens. In line with this, Iskender *et al*. [16] showed that flavonoids have no significant effect on egg production. In contrast, supplementation with the quercetin flavonoid [17] and saponins derived from karaya increases the egg production of laying hens [18]. These inconsistencies may be caused by the differences in the levels of flavonoids and saponins used in the study, as well as the conditions of the study.

<H2>Effect of binahong leaf powder on egg characteristics

The addition of binahong leaf powder did not significantly affect the characteristics of the egg, including egg weight, yolk weight, albumen weight, and eggshell weight (Table-4). It has been hypothesized that the bioactive component of binahong leaf may positively affect the egg characteristics. However, this study revealed different results. According to Leke *et al*. [19], flavonoids of papaya seeds increase egg quality (egg yolk, albumen, and eggshell weight) of Indonesian hens. Moreover, Afrose *et al*. [18] reported that supplementation with 25, 50, and 75 mg/kg of karaya saponin increases egg weight, yolk weight, and albumen weight of laying hens, whereas Ayasan *et al*. [20] reported that supplementation of *Yucca schidigera* powder, with 120 ppm of saponin, increases egg weight but does not affect the eggshell weight. As in our study, one study showed that supplementation with 0.2-0.6 g/kg of flavonoid does not significantly influence egg quality [17]. In addition, supplementation with flavonoids does not significantly influence eggshell weight, as reported by Iskender *et al*. [16]. The differences in the nature and levels of bioactive compounds, the nutritional values of rations, and the conditions of study may be responsible for these divergent results.

<H2>Cholesterol, triglyceride, HDL, LDL, and protein contents in yolk

The data on cholesterol, triglyceride, HDL, LDL, and protein contents in yolk after binahong leaf powder supplementation are shown in Table-5. The addition of 2% of binahong leaf powder decreased the level of cholesterol in the yolk of quail eggs ($p<0.05$). Flavonoids and saponins in the binahong leaf powder may inhibit cholesterol absorption; hence, cholesterol deposition may be reduced [21-23]. This is supported by Lien *et al*. [24] who showed that the consumption of flavonoids increases the excretion of cholesterol. In addition, flavonoids may increase reverse cholesterol transport (RCT), resulting in high cholesterol excretion; thus, there will be a lower cholesterol level in yolk. However, 4 and 6% binahong leaf powder did not affect the cholesterol level.

The addition of binahong leaf powder decreased the triglyceride content in quail yolk (Table-5). As shown in our study, supplementation with a flavonoid extracted from the root of *Scutellaria baicalensis* Georgi decreases triglyceride content in the serum of broiler [25]. Likewise, Kamboh and Zhu [21] and Ouyang *et al*. [10] reported that the consumption of flavonoids decreases the level of triglyceride in the serum and meat of broiler. According to Hsu and Yen [26] and Nagai *et al*. [27], flavonoids may inhibit intracellular triglyceride synthesis in the liver, which results in lower triglyceride deposition in quail egg yolk. In addition, flavonoid content in binahong leaf powder may increase the expression of

peroxisome proliferator-activated receptor α in the liver, which is involved in lipid metabolism, especially fatty acid oxidation [10,28].

The content of yolk HDL increased with the increased concentration of binahong leaf powder in the quail rations ($p<0.05$). Similar to the result of our study, Afrose *et al.* [29] documented that saponins increase HDL content in broiler meat. Supplementation with karaya saponin also increases HDL content in quail egg [30]. Furthermore, Smith *et al*. [31] stated that supplementation with saponin extract increases the HDL content in the blood, liver, kidneys, and heart tissues of white mice. Kamboh and Zhu [21] reported that flavonoids increase the HDL content in serum and HDL deposition in the breast meat of broiler. The mechanism by which binahong leaf increased HDL concentration in quail egg yolk is not known; however, the role of binahong leaf powder in the RCT mechanism seems to be attributable to the increased HDL content in quail yolk. Millar *et al*. [23] and Marques *et al*. [32] reported that the increase in the RCT mechanism due to binahong leaf powder supplementation is accompanied by an increase in the level of HDL. Thus, this mechanism seems to be related to the contribution of HDL on RCT.

Treatment with binahong leaf powder decreased the level of LDL in the quail egg yolk (Table-5). Afrose *et al.* [30] reported that saponins decrease the LDL content of quail egg.

Likewise, Chaudhary *et al*. [33] stated that saponins potentially decrease the LDL content of meat. In addition, supplementation with saponin extract decreases LDL content in the blood, liver, kidneys, and heart tissues of white mice [31]. Flavonoids have been reported to decrease the LDL level in the blood of broiler [10,21,25]. Furthermore, Zhou *et al*. [25] stated that flavonoid supplementation also decreases the LDL content of the breast meat of broiler. Binahong leaf extract contains saponins, which bind bile acid and form large mixed micelles. Cholesterol in the micelles cannot be absorbed by microvilli on the surface of intestinal epithelial cells, which causes a decrease in total and LDL cholesterol levels [34]. Saponins inhibit fat metabolism through the inhibition of lipase secretion and decrease cholesterol and LDL [35], but increase HDL [36], contents in the blood. Flavonoids are active compounds that reduce the activity of fatty acid synthase and reduce the level of LDL in animal tissues [10].

The addition of 2-6% binahong leaf powder did not significantly influence the protein content of egg yolk. This contrasted with a previous study. Iskender *et al*. [16] reported that supplementation with flavonoids (hesperidin, naringin, and quercetin) increases egg protein. Ahmad *et al*. [37] documented that bioactive compounds from *Moringa oleifera* leaf extracts increase the protein content in yolk.

In general, the protein content in an egg is derived from the feed. In this study, the inclusion of binahong leaf powder was accompanied by the decreased crude protein in the ration. Hence, the protein-increasing effect by binahong leaf powder seemed to be inhibited by lower protein content in the quail ration due to the binahong leaf inclusion.

<H1>Conclusion

The addition of 2% binahong leaf powder is best for lowering cholesterol, triglyceride, and LDL levels in quail egg.

<H1>Authors' Contributions

SK conducted the research, data collection, and drafting of the article. HIW developed the feeding concept and supervised the research, RM advised the experimental design, DS conducted data analysis, and SS performed laboratory analysis. All authors read and approved the final manuscript.

<H1>Acknowledgments

The authors would like to thank Sugiharto S.Pt., M.Sc. Ph.D. for proofreading the manuscript.

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<H1>Competing Interests

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<H1>References

1. Thomas, K.S., Jagatheesan, P.N.R., Reetha, T.L. and Rajendran, D. (2016) Nutrient composition of Japanese quail eggs. *Int. J. Sci. Environ.*, 5(3): 1293-1295.
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Kajian aktivitas penyembuhan luka dan antibakteri binahong (*Anredera cordifolia* (Ten.) steenis, pegagan (*Centella asiatica* (L.) Urban) serta kombinasinya terhadap bakteri *Staphylococcus aureus* dan *Pseudomonas aeruginosa* dari pasien luka kaki diabetes. *Bionat. J. Ilmu Hayati Fisik*, 16(2): 78-82.

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Tables

Table-1: Feed composition of the control ration.	
Feed ingredients	%
Yellow corn	48.00
Rice bran	6.00
Pollard	16.00
Poultry meat meal	14.00
Soybean meal	10.00
$CaCO_3$	5.50
Salt	0.25
Premix	0.25
Total	100.00

Table-2: Nutrition content of the ration.				
Nutrient contents	Addition of binahong leaf powder			
	T0 (0%)	T1 (2%)	T2 (4%)	T3 (6%)

Energy metabolic (kkal/kg)	2763.20	2694.12	2628.26	2565.40
Protein (%)	19.77	19.35	18.95	18.56
Fat (%)	4.66	4.58	4.51	4.43
Crude fiber (%)	4.29	4.95	5.56	6.12
Ca (%)	3.15	3.06	2.97	2.88
P (%)	0.79	0.76	0.74	0.71
Lysine (%)	0.96	0.93	0.89	0.86
Methionine (%)	0.48	0.46	0.44	0.42

Table-3: Egg production on addition of binahong leaf powder in quail rations.

Age (weeks)	Addition of binahong leaf powder				p-value
	T0 (0%)	T1 (2%)	T2 (4%)	T3 (6%)	
8	29.71±2.74	28.00±2.166	28.85±2.74	28.57±2.67	0.49

9	39.71±2.34	42.28±1.91	41.42±2.67	41.14±2.55	0.53
10	45.14±4.58	46.09±1.47	47.23±2.34	45.14±3.08	0.57
11	46.64±2.42	49.49±1.38	48.73±3.83	47.63±1.73	0.59
12	56.38±2.49	52.34±1.86	54.50±1.37	54.57±1.76	0.67
14	56.17±2.47	57.55±1.45	57.73±1.85	58.74±1.93	0.69

Table-4: Influence of addition of binahong leaf powder on quail eggs characteristic.

Parameters	Addition of binahong leaf powder				p-value
	T0 (0%)	T1 (2%)	T2(4%)	T3 (6%)	
Egg weight (g)	9.99±0.29	10.01±0.11	10.03±0.11	10.03±0.27	0.38
Yolk weight (g)	3.65±0.13	3.92±0.25	3.68±0.12	3.93±0.17	0.57
Albumen weight (g)	4.68±0.29	4.53±0.18	4.47±0.25	4.60±0.19	0.53
Eggshell weight (g)	1.14±0.28	1.12±0.15	1.14±0.17	1.01±0.10	0.32
Yolk weight (%)	38.77±2.34	41.80±2.72	40.01±1.39	41.13±0.79	0.51
Albumen weight (%)	49.63±2.78	48.27±2.07	48.56±2.70	48.13±1.58	0.73

Eggshell weight (%)	11.43±2.73	11.07±1.60	11.41±1.73	10.11±1.14	0.12

Table-5: Influence of the addition of binahong leaf powder on cholesterol, triglyceride, HDL, LDL, and protein of quail egg yolk.

Parameters	Addition of binahong leaf powder				p-value
	T0 (0%)	T1 (2%)	T2 (4%)	T3 (6%)	
Yolk cholesterol (mg/g)	56.15±5.04[a]	23.77±3.75[b]	51.97±4.63[a]	51.35±7.55[a]	<0.01
Yolk triglyceride (mg/g)	903.22±58.21[a]	608.62±22.46 c	778.34±53.59 b	811.63±104.01 b	<0.01
Yolk HDL (mg/g)	36.86±1.32[b]	34.8b±1.58[b]	48.62±1.67[a]	47.52±1.58[a]	<0.01
Yolk LDL	29.18 ±1.59 [a]	16.06b±0.33[b]	17.09±0.37[b]	16.28±0.36[b]	<0.0

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(mg/g)					1
Protein of yolk (%)	30.16±2.78	30.37±.82	29.15±0.81	30.19±2.55	0.00

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RESEARCH ARTICLE

Addition of binahong (*Anredera cordifolia*) leaf powder to diets to produce eggs with low cholesterol

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Abstract

Aim: The aim of this study was to evaluate the effect of the addition of binahong leaf powder to quail rations on the production and quality of eggs.

Materials and Methods: The study involved the use of two hundred 7-week-old quails housed evenly in 20 wire cages with a body weight of 123.77±0.72 g. The quails were treated as follows: Ration without binahong leaf powder (T0), addition 2% of binahong leaf powder (T1), addition 4% of binahong leaf powder (T2), and addition 6% of binahong leaf powder (T3). The study used a completely randomized design. The parameters measured were the production, weight, and characteristics of the eggs, as well as the cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and egg protein content in the yolk.

Results: The addition of 2-6% binahong powder did not significantly affect egg production, egg characteristics, or egg protein content, but significantly ($p<0.05$) affected the cholesterol, triglyceride, HDL, and LDL contents in yolk. The cholesterol, triglyceride, and LDL contents decreased significantly in T1, whereas HDL increased significantly in T2 and T3.

Conclusion: The addition of 2% binahong was enough to obtain healthy quail eggs with low levels of cholesterol, triglyceride, and LDL.

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Keywords: binahong, cholesterol, high-density lipoprotein, low-density lipoprotein, triglyceride.

<H1>Introduction

Quail egg contains many high-quality nutrients but also high cholesterol [1]. The protein content in a quail egg is approximately 13.30% [1]. The cholesterol content in a quail egg is higher than in a chicken egg. The cholesterol content in the yolk of a quail egg is 6.79 mg/dl, whereas the cholesterol content in chicken egg yolk is 4.03 mg/dl [2]. Overconsumption of cholesterol increases the blood cholesterol level, which leads to heart disease; thus, some people are afraid of consuming quail egg. Catapano and Wiklund [3] stated that blood cholesterol, particularly low-density lipoprotein (LDL) cholesterol, has a positive correlation with the occurrence of atherosclerosis. The consumption of high-density lipoprotein (HDL) derived from quail egg increases the blood serum HDL level and decreases atherosclerotic plaques in rabbits. Thus, egg HDL may be used as an anti-atherosclerotic agent for patients with cardiovascular disease [4]. An attempt to obtain quail egg with low cholesterol and high HDL contents needs to be carried out.

Binahong (*Anredera cordifolia*) is a wild plant with a rapid growth rate that requires no complicated cultivation; thus, binahong is abundant. Sutrisno *et al*. [5] and Leliqia *et al*. [6]

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revealed that the leaf of binahong contains bioactive compounds, such as flavonoids, tannins, saponins, phenols, and steroids, and Astuti *et al*. [7] reported that the leaf also contains terpenoid, which may potentially increase pancreatic insulin secretion. Furthermore, Hasbullah [8] documented that binahong leaf exhibits hypolipidemic properties. Kamboh *et al*. [9] showed that supplementation with a bioflavonoid increases antioxidant and enzyme activities and decreases total cholesterol and triglyceride levels in the serum and breast meat of broiler. A study by Ouyang *et al*. [10] showed that supplementation with 15 mg/kg of alfalfa flavonoid increases HDL levels and decreases the levels of cholesterol, triglyceride, and serum LDL and the percentage of abdominal fat of broiler. Feeding saponin decreases cholesterol, insulin, and blood triglyceride and increases blood HDL synthesis. Saponin also decreases the contents of cholesterol [11] and protein digestibility [12] of broiler meat.

The aim of this study was to evaluate the effect of the addition of binahong leaf powder to rations on the contents of cholesterol, triglyceride, HDL, LDL, and protein in quail eggs.

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<H1>Materials and Methods

<H2>Ethical approval

The procedure of using quail in this study has been approved by the Animal Ethics Committee in the Faculty of Animal Sciences, Diponegoro University, Semarang, Indonesia.

<H2>Animals

The study used two hundred 7-week-old female quails with an average body weight of 123.77±0.72 g. The quails were housed in 20 wire cages. Each cage was 90 × 35 × 25 cm and housed 10 quails. The feed consisted of yellow corn, rice bran, pollard, poultry meat meal, soybean meal, $CaCO_3$, salt, premix, and binahong leaf powder.

<H2>Experimental design

This study was arranged according to a completely randomized design with four treatments and five repetitions (10 quails per group per repetition). The treatment groups included T0, control ration (without binahong leaf powder); T1, control ration + 2% binahong leaf powder; T2, control ration + 4% binahong leaf powder; and T3, control ration + 6% binahong leaf powder in ration. The composition and nutrient content of the ration are presented in Tables-1 and 2.

<H2>Tests and procedures

The recording of egg production was carried out every day during the study and the formula: Production = (number of eggs/number of chickens) × 100% was used. Egg weight and egg characteristics were measured every 3 days at the end of the week. The cholesterol, triglyceride, HDL, LDL, and protein contents in the egg yolk were collected by sampling one

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egg per experimental unit at the end of the study. The analyses of cholesterol and triglyceride contents were conducted based on the cholesterol p-aminophenazone method, HDL and LDL analysis was based on the enzymatic colorimetric method [13], and protein analysis was based on the Kjeldahl method [14].

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<H2>Statistical analysis

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<H1>Results and Discussion

<H2>Effect of the addition of binahong leaf powder on egg production

The addition of 2-6% of binahong leaf powder, rich in flavonoids and saponins, did not significantly influence quail egg production at 8-14 weeks of age (Table-3). This result was similar to that reported by Kusumanti and Murwani [15], which showed that supplementation of binahong leaf powder does not significantly affect the egg production of laying hens. In line with this, Iskender *et al*. [16] showed that flavonoids have no significant effect on egg production. In contrast, supplementation with the quercetin flavonoid [17] and saponins derived from karaya increases the egg production of laying hens [18]. These inconsistencies may be caused by the differences in the levels of flavonoids and saponins used in the study, as well as the conditions of the study.

<H2>Effect of binahong leaf powder on egg characteristics

The addition of binahong leaf powder did not significantly affect the characteristics of the egg, including egg weight, yolk weight, albumen weight, and eggshell weight (Table-4). It has been hypothesized that the bioactive component of binahong leaf may positively affect the egg characteristics. However, this study revealed different results. According to Leke *et al*. [19], flavonoids of papaya seeds increase egg quality (egg yolk, albumen, and eggshell weight) of Indonesian hens. Moreover, Afrose *et al*. [18] reported that supplementation with 25, 50, and 75 mg/kg of karaya saponin increases egg weight, yolk weight, and albumen weight of laying hens, whereas Ayasan *et al*. [20] reported that supplementation of *Yucca schidigera* powder, with 120 ppm of saponin, increases egg weight but does not affect the eggshell weight. As in our study, one study showed that supplementation with 0.2-0.6 g/kg of flavonoid does not significantly influence egg quality [17]. In addition, supplementation with flavonoids does not significantly influence eggshell weight, as reported by Iskender *et al*. [16]. The differences in the nature and levels of bioactive compounds, the nutritional values of rations, and the conditions of study may be responsible for these divergent results.

<H2>Cholesterol, triglyceride, HDL, LDL, and protein contents in yolk

The data on cholesterol, triglyceride, HDL, LDL, and protein contents in yolk after binahong leaf powder supplementation are shown in Table-5. The addition of 2% of binahong leaf powder decreased the level of cholesterol in the yolk of quail eggs ($p<0.05$). Flavonoids and saponins in the binahong leaf powder may inhibit cholesterol absorption; hence, cholesterol deposition may be reduced [21-23]. This is supported by Lien *et al*. [24] who showed that the consumption of flavonoids increases the excretion of cholesterol. In addition, flavonoids may increase reverse cholesterol transport (RCT), resulting in high cholesterol excretion; thus, there will be a lower cholesterol level in yolk. However, 4 and 6% binahong leaf powder did not affect the cholesterol level.

The addition of binahong leaf powder decreased the triglyceride content in quail yolk (Table-5). As shown in our study, supplementation with a flavonoid extracted from the root of *Scutellaria baicalensis* Georgi decreases triglyceride content in the serum of broiler [25]. Likewise, Kamboh and Zhu [21] and Ouyang *et al*. [10] reported that the consumption of flavonoids decreases the level of triglyceride in the serum and meat of broiler. According to Hsu and Yen [26] and Nagai *et al*. [27], flavonoids may inhibit intracellular triglyceride synthesis in the liver, which results in lower triglyceride deposition in quail egg yolk. In addition, flavonoid content in binahong leaf powder may increase the expression of

peroxisome proliferator-activated receptor α in the liver, which is involved in lipid metabolism, especially fatty acid oxidation [10,28].

The content of yolk HDL increased with the increased concentration of binahong leaf powder in the quail rations ($p<0.05$). Similar to the result of our study, Afrose *et al.* [29] documented that saponins increase HDL content in broiler meat. Supplementation with karaya saponin also increases HDL content in quail egg [30]. Furthermore, Smith *et al*. [31] stated that supplementation with saponin extract increases the HDL content in the blood, liver, kidneys, and heart tissues of white mice. Kamboh and Zhu [21] reported that flavonoids increase the HDL content in serum and HDL deposition in the breast meat of broiler. The mechanism by which binahong leaf increased HDL concentration in quail egg yolk is not known; however, the role of binahong leaf powder in the RCT mechanism seems to be attributable to the increased HDL content in quail yolk. Millar *et al*. [23] and Marques *et al*. [32] reported that the increase in the RCT mechanism due to binahong leaf powder supplementation is accompanied by an increase in the level of HDL. Thus, this mechanism seems to be related to the contribution of HDL on RCT.

Treatment with binahong leaf powder decreased the level of LDL in the quail egg yolk (Table-5). Afrose *et al.* [30] reported that saponins decrease the LDL content of quail egg.

Likewise, Chaudhary *et al*. [33] stated that saponins potentially decrease the LDL content of meat. In addition, supplementation with saponin extract decreases LDL content in the blood, liver, kidneys, and heart tissues of white mice [31]. Flavonoids have been reported to decrease the LDL level in the blood of broiler [10,21,25]. Furthermore, Zhou *et al*. [25] stated that flavonoid supplementation also decreases the LDL content of the breast meat of broiler. Binahong leaf extract contains saponins, which bind bile acid and form large mixed micelles. Cholesterol in the micelles cannot be absorbed by microvilli on the surface of intestinal epithelial cells, which causes a decrease in total and LDL cholesterol levels [34]. Saponins inhibit fat metabolism through the inhibition of lipase secretion and decrease cholesterol and LDL [35], but increase HDL [36], contents in the blood. Flavonoids are active compounds that reduce the activity of fatty acid synthase and reduce the level of LDL in animal tissues [10].

The addition of 2-6% binahong leaf powder did not significantly influence the protein content of egg yolk. This contrasted with a previous study. Iskender *et al*. [16] reported that supplementation with flavonoids (hesperidin, naringin, and quercetin) increases egg protein. Ahmad *et al*. [37] documented that bioactive compounds from *Moringa oleifera* leaf extracts increase the protein content in yolk.

In general, the protein content in an egg is derived from the feed. In this study, the inclusion of binahong leaf powder was accompanied by the decreased crude protein in the ration. Hence, the protein-increasing effect by binahong leaf powder seemed to be inhibited by lower protein content in the quail ration due to the binahong leaf inclusion.

<H1>Conclusion

The addition of 2% binahong leaf powder is best for lowering cholesterol, triglyceride, and LDL levels in quail egg.

<H1>Authors' Contributions

SK conducted the research, data collection, and drafting of the article. HIW developed the feeding concept and supervised the research, RM advised the experimental design, DS conducted data analysis, and SS performed laboratory analysis. All authors read and approved the final manuscript.

<H1>Acknowledgments

The authors would like to thank Sugiharto S.Pt., M.Sc. Ph.D. for proofreading the manuscript.

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<H1>Competing Interests

The authors declare that they have no competing interests.

<H1>Publisher's Note

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Kajian aktivitas penyembuhan luka dan antibakteri binahong (*Anredera cordifolia* (Ten.) steenis, pegagan (*Centella asiatica* (L.) Urban) serta kombinasinya terhadap bakteri *Staphylococcus aureus* dan *Pseudomonas aeruginosa* dari pasien luka kaki diabetes. *Bionat. J. Ilmu Hayati Fisik*, 16(2): 78-82.

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Tables

Table-1: Feed composition of the control ration.	
Feed ingredients	%
Yellow corn	48.00
Rice bran	6.00
Pollard	16.00
Poultry meat meal	14.00
Soybean meal	10.00
$CaCO_3$	5.50
Salt	0.25
Premix	0.25
Total	100.00

Table-2: Nutrition content of the ration.				
Nutrient contents	Addition of binahong leaf powder			
	T0 (0%)	T1 (2%)	T2 (4%)	T3 (6%)

Energy metabolic (kkal/kg)	2763.20	2694.12	2628.26	2565.40
Protein (%)	19.77	19.35	18.95	18.56
Fat (%)	4.66	4.58	4.51	4.43
Crude fiber (%)	4.29	4.95	5.56	6.12
Ca (%)	3.15	3.06	2.97	2.88
P (%)	0.79	0.76	0.74	0.71
Lysine (%)	0.96	0.93	0.89	0.86
Methionine (%)	0.48	0.46	0.44	0.42

Table-3: Egg production on addition of binahong leaf powder in quail rations.

Age (weeks)	Addition of binahong leaf powder				p-value
	T0 (0%)	T1 (2%)	T2 (4%)	T3 (6%)	
8	29.71±2.74	28.00±2.166	28.85±2.74	28.57±2.67	0.49

9	39.71±2.34	42.28±1.91	41.42±2.67	41.14±2.55	0.53
10	45.14±4.58	46.09±1.47	47.23±2.34	45.14±3.08	0.57
11	46.64±2.42	49.49±1.38	48.73±3.83	47.63±1.73	0.59
12	56.38±2.49	52.34±1.86	54.50±1.37	54.57±1.76	0.67
14	56.17±2.47	57.55±1.45	57.73±1.85	58.74±1.93	0.69

Table-4: Influence of addition of binahong leaf powder on quail eggs characteristic.

Parameters	Addition of binahong leaf powder				p-value
	T0 (0%)	T1 (2%)	T2(4%)	T3 (6%)	
Egg weight (g)	9.99±0.29	10.01±0.11	10.03±0.11	10.03±0.27	0.38
Yolk weight (g)	3.65±0.13	3.92±0.25	3.68±0.12	3.93±0.17	0.57
Albumen weight (g)	4.68±0.29	4.53±0.18	4.47±0.25	4.60±0.19	0.53
Eggshell weight (g)	1.14±0.28	1.12±0.15	1.14±0.17	1.01±0.10	0.32
Yolk weight (%)	38.77±2.34	41.80±2.72	40.01±1.39	41.13±0.79	0.51
Albumen weight (%)	49.63±2.78	48.27±2.07	48.56±2.70	48.13±1.58	0.73

Eggshell weight (%)	11.43±2.73	11.07±1.60	11.41±1.73	10.11±1.14	0.12

Table-5: Influence of the addition of binahong leaf powder on cholesterol, triglyceride, HDL, LDL, and protein of quail egg yolk.

Parameters	Addition of binahong leaf powder				p-value
	T0 (0%)	T1 (2%)	T2 (4%)	T3 (6%)	
Yolk cholesterol (mg/g)	56.15±5.04[a]	23.77±3.75[b]	51.97±4.63[a]	51.35±7.55[a]	<0.01
Yolk triglyceride (mg/g)	903.22±58.21[a]	608.62±22.46 c	778.34±53.59 b	811.63±104.01 b	<0.01
Yolk HDL (mg/g)	36.86±1.32[b]	34.8b±1.58[b]	48.62±1.67[a]	47.52±1.58[a]	<0.01
Yolk LDL	29.18 ±1.59 [a]	16.06b±0.33[b]	17.09±0.37[b]	16.28±0.36[b]	<0.0

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(mg/g)					1
Protein of yolk (%)	30.16±2.78	30.37±.82	29.15±0.81	30.19±2.55	0.00

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RESEARCH ARTICLE

Addition of binahong (*Anredera cordifolia*) leaf powder to diets to produce eggs with low cholesterol

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Abstract

Aim: The aim of this study was to evaluate the effect of the addition of binahong leaf powder to quail rations on the production and quality of eggs.

Materials and Methods: The study involved the use of two hundred 7-week-old quails housed evenly in 20 wire cages with a body weight of 123.77±0.72 g. The quails were treated as follows: Ration without binahong leaf powder (T0), addition 2% of binahong leaf powder (T1), addition 4% of binahong leaf powder (T2), and addition 6% of binahong leaf powder (T3). The study used a completely randomized design. The parameters measured were the production, weight, and characteristics of the eggs, as well as the cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and egg protein content in the yolk.

Results: The addition of 2-6% binahong powder did not significantly affect egg production, egg characteristics, or egg protein content, but significantly ($p<0.05$) affected the cholesterol, triglyceride, HDL, and LDL contents in yolk. The cholesterol, triglyceride, and LDL contents decreased significantly in T1, whereas HDL increased significantly in T2 and T3.

Conclusion: The addition of 2% binahong was enough to obtain healthy quail eggs with low levels of cholesterol, triglyceride, and LDL.

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Keywords: binahong, cholesterol, high-density lipoprotein, low-density lipoprotein, triglyceride.

<H1>Introduction

Quail egg contains many high-quality nutrients but also high cholesterol [1]. The protein content in a quail egg is approximately 13.30% [1]. The cholesterol content in a quail egg is higher than in a chicken egg. The cholesterol content in the yolk of a quail egg is 6.79 mg/dl, whereas the cholesterol content in chicken egg yolk is 4.03 mg/dl [2]. Overconsumption of cholesterol increases the blood cholesterol level, which leads to heart disease; thus, some people are afraid of consuming quail egg. Catapano and Wiklund [3] stated that blood cholesterol, particularly low-density lipoprotein (LDL) cholesterol, has a positive correlation with the occurrence of atherosclerosis. The consumption of high-density lipoprotein (HDL) derived from quail egg increases the blood serum HDL level and decreases atherosclerotic plaques in rabbits. Thus, egg HDL may be used as an anti-atherosclerotic agent for patients with cardiovascular disease [4]. An attempt to obtain quail egg with low cholesterol and high HDL contents needs to be carried out.

Binahong (*Anredera cordifolia*) is a wild plant with a rapid growth rate that requires no complicated cultivation; thus, binahong is abundant [5]. Sutrisno *et al*. [5] and Leliqia *et al*.

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That sentence can be found in Sutrisno *et al.* (2014).

[6] revealed that the leaf of binahong contains bioactive compounds, such as flavonoids, tannins, saponins, phenols, and steroids, and Astuti *et al*. [7] reported that the leaf also contains terpenoid, which may potentially increase pancreatic insulin secretion. Furthermore, Hasbullah [8] documented that binahong leaf exhibits hypolipidemic properties. Kamboh *et al*. [9] showed that supplementation with a bioflavonoid increases antioxidant and enzyme activities and decreases total cholesterol and triglyceride levels in the serum and breast meat of broiler. A study by Ouyang *et al*. [10] showed that supplementation with 15 mg/kg of alfalfa flavonoid increases HDL levels and decreases the levels of cholesterol, triglyceride, and serum LDL and the percentage of abdominal fat of broiler. Feeding saponin decreases cholesterol, insulin, and blood triglyceride and increases blood HDL synthesis. Saponin also decreases the contents of cholesterol [11] and protein digestibility [12] of broiler meat.

Based on the facts that binahong contain flavonoids, tannins, saponins, phenols, and steroids and it has potentially reduced cholesterol, triglyceride, LDL serum and increased HDL. Hopefully, it will produce a healthy egg product. Therefore, the aim of this study was to evaluate the effect of the addition of binahong leaf powder to rations on the contents of cholesterol, triglyceride, HDL, LDL, and protein in quail eggs.

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<H1>Materials and Methods

<H2>Ethical approval

The procedure of using quail in this study has been approved by the Animal Ethics Committee in the Faculty of Animal Sciences, Diponegoro University, Semarang, Indonesia.

<H2>Animals

The study used two hundred 7-week-old female quails with an average body weight of 123.77±0.72 g. The quails were housed in 20 wire cages. Each cage was 90 × 35 × 25 cm and housed 10 quails. The feed consisted of yellow corn, rice bran, pollard, poultry meat meal, soybean meal, $CaCO_3$, salt, premix, and binahong leaf powder.

<H2>Experimental design

This study was arranged according to a completely randomized design with four treatments and five repetitions (10 quails per group per repetition). The treatment groups included T0, control ration (without binahong leaf powder); T1, control ration + 2% binahong leaf powder; T2, control ration + 4% binahong leaf powder; and T3, control ration + 6% binahong leaf powder in ration. The composition and nutrient content of the ration are presented in Tables-1 and 2.

<H2>Tests and procedures

The recording of egg production was carried out every day during the study and the formula: Production = (number of eggs/number of chickens) × 100% was used. Egg weight and egg characteristics were measured ~~every 3 days at the end of the week~~ every 3 consecutive days weekly. The cholesterol, triglyceride, HDL, LDL, and protein contents in the egg yolk were collected by sampling one egg per experimental unit at the end of the study. The analyses of cholesterol and triglyceride contents were conducted based on the cholesterol p-aminophenazone method [13], HDL and LDL analysis was based on the enzymatic colorimetric method [13], and protein analysis was based on the Kjeldahl method [14].

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I measured 3 consecutive days every week.

Egg weight and egg characteristics were measured every 3 consecutive days weekly

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<H2>Statistical analysis

??? Data were analyzed as a completely randomized design using one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test

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<H1>Results and Discussion

<H2>Effect of the addition of binahong leaf powder on egg production

The addition of 2-6% of binahong leaf powder, rich in flavonoids and saponins, did not significantly influence quail egg production at 8-14 weeks of age (Table-3). This result was similar to that reported by Kusumanti and Murwani [15], which showed that supplementation of binahong leaf powder does not significantly affect the egg production of laying hens. In

line with this, Iskender *et al*. [16] showed that flavonoids have no significant effect on egg production. In contrast, supplementation with the quercetin flavonoid [17] and saponins derived from karaya increases the egg production of laying hens [18]. These inconsistencies may be caused by the differences in the levels of flavonoids and saponins used in the study, as well as the conditions of the study.

<H2>Effect of binahong leaf powder on egg characteristics

The addition of binahong leaf powder did not significantly affect the characteristics of the egg, including egg weight, yolk weight, albumen weight, and eggshell weight (Table-4). It has been hypothesized that the bioactive component of binahong leaf may positively affect the egg characteristics. However, this study revealed different results. According to Leke *et al*. [19], flavonoids of papaya seeds increase egg quality (egg yolk, albumen, and eggshell weight) of Indonesian hens. Moreover, Afrose *et al*. [18] reported that supplementation with 25, 50, and 75 mg/kg of karaya saponin increases egg weight, yolk weight, and albumen weight of laying hens, whereas Ayasan *et al*. [20] reported that supplementation of *Yucca schidigera* powder, with 120 ppm of saponin, increases egg weight but does not affect the eggshell weight. As in our study, one study showed that supplementation with 0.2-0.6 g/kg of flavonoid does not significantly influence egg quality [17]. In addition, supplementation with

flavonoids does not significantly influence eggshell weight, as reported by Iskender *et al*. [16]. The differences in the nature and levels of bioactive compounds, the nutritional values of rations, and the conditions of study may be responsible for these divergent results.

<H2>Cholesterol, triglyceride, HDL, LDL, and protein contents in yolk

The data on cholesterol, triglyceride, HDL, LDL, and protein contents in yolk after binahong leaf powder supplementation are shown in Table-5. The addition of 2% of binahong leaf powder decreased the level of cholesterol in the yolk of quail eggs ($p<0.05$). Flavonoids and saponins in the binahong leaf powder may inhibit cholesterol absorption; hence, cholesterol deposition may be reduced [21-23]. This is supported by Lien *et al*. [24] who showed that the consumption of flavonoids increases the excretion of cholesterol. In addition, flavonoids may increase reverse cholesterol transport (RCT), resulting in high cholesterol excretion; thus, there will be a lower cholesterol level in yolk. However, 4 and 6% binahong leaf powder did not affect the cholesterol level.

The addition of binahong leaf powder decreased the triglyceride content in quail yolk (Table-5). As shown in our study, supplementation with a flavonoid extracted from the root of *Scutellaria baicalensis* Georgi decreases triglyceride content in the serum of broiler [25]. Likewise, Kamboh and Zhu [21] and Ouyang *et al*. [10] reported that the consumption of

flavonoids decreases the level of triglyceride in the serum and meat of broiler. According to Hsu and Yen [26] and Nagai *et al*. [27], flavonoids may inhibit intracellular triglyceride synthesis in the liver, which results in lower triglyceride deposition in quail egg yolk. In addition, flavonoid content in binahong leaf powder may increase the expression of peroxisome proliferator-activated receptor α in the liver, which is involved in lipid metabolism, especially fatty acid oxidation [10,28].

The content of yolk HDL increased with the increased concentration of binahong leaf powder in the quail rations ($p<0.05$). Similar to the result of our study, Afrose *et al.* [29] documented that saponins increase HDL content in broiler meat. Supplementation with karaya saponin also increases HDL content in quail egg [30]. Furthermore, Smith *et al*. [31] stated that supplementation with saponin extract increases the HDL content in the blood, liver, kidneys, and heart tissues of white mice. Kamboh and Zhu [21] reported that flavonoids increase the HDL content in serum and HDL deposition in the breast meat of broiler. The mechanism by which binahong leaf increased HDL concentration in quail egg yolk is not known; however, the role of binahong leaf powder in the RCT mechanism seems to be attributable to the increased HDL content in quail yolk. Millar *et al*. [23] and Marques *et al*. [32] reported that the increase in the RCT mechanism due to binahong leaf powder supplementation is

accompanied by an increase in the level of HDL. Thus, this mechanism seems to be related to the contribution of HDL on RCT.

Treatment with binahong leaf powder decreased the level of LDL in the quail egg yolk (Table-5). Afrose *et al.* [30] reported that saponins decrease the LDL content of quail egg. Likewise, Chaudhary *et al*. [33] stated that saponins potentially decrease the LDL content of meat. In addition, supplementation with saponin extract decreases LDL content in the blood, liver, kidneys, and heart tissues of white mice [31]. Flavonoids have been reported to decrease the LDL level in the blood of broiler [10,21,25]. Furthermore, Zhou *et al*. [25] stated that flavonoid supplementation also decreases the LDL content of the breast meat of broiler. Binahong leaf extract contains saponins, which bind bile acid and form large mixed micelles. Cholesterol in the micelles cannot be absorbed by microvilli on the surface of intestinal epithelial cells, which causes a decrease in total and LDL cholesterol levels [34]. Saponins inhibit fat metabolism through the inhibition of lipase secretion and decrease cholesterol and LDL [35], but increase HDL [36], contents in the blood. Flavonoids are active compounds that reduce the activity of fatty acid synthase and reduce the level of LDL in animal tissues [10].

The addition of 2-6% binahong leaf powder did not significantly influence the protein content of egg yolk. This contrasted with a previous study. Iskender *et al*. [16] reported that supplementation with flavonoids (hesperidin, naringin, and quercetin) increases egg protein. Ahmad *et al*. [37] documented that bioactive compounds from *Moringa oleifera* leaf extracts increase the protein content in yolk.

In general, the protein content in an egg is derived from the feed. In this study, the inclusion of binahong leaf powder was accompanied by the decreased crude protein in the ration. Hence, the protein-increasing effect by binahong leaf powder seemed to be inhibited by lower protein content in the quail ration due to the binahong leaf inclusion.

<H1>Conclusion

The addition of 2% binahong leaf powder is best for lowering cholesterol, triglyceride, and LDL levels in quail egg.

<H1>Authors' Contributions

SK conducted the research, data collection, and drafting of the article. HIW developed the feeding concept and supervised the research, RM advised the experimental design, DS conducted data analysis, and SS performed laboratory analysis. All authors read and approved the final manuscript.

<H1>Acknowledgments

The authors are very grateful for the support of Poultry Production Laboratory, Faculty of Animal and Agricultural Sciences, Diponegoro University. The authors also would like to thank Sugiharto S.Pt., M.Sc. Ph.D. for proofreading the manuscript.

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<H1>Competing Interests

The authors declare that they have no competing interests.

<H1>Publisher's Note

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Archives • 2

OVERVIEW OF EFFICACY, SAFETY A

ANREDERA CORDIFO

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Abstract

Anredera cordifolia (Ten.) Steenis is used for medical pur
content, pharmacology activity and toxicity test result of
glycoside, flavonoids, saponins and alkaloids were found i
such as ursolic acid, ancordin, apigenin, etc. were isolat
benefits in repairing kidney function, as antibacterial, ant
inhibitor, antidiabetic, antihypertensive, vasodilator,

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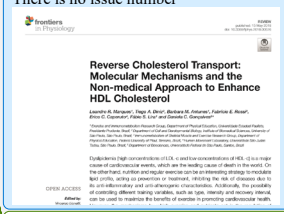

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Tables

Table-1: Feed composition of the control ration.	
Feed ingredients	%
Yellow corn	48.00

Rice bran	6.00
Pollard	16.00
Poultry meat meal	14.00
Soybean meal	10.00
$CaCO_3$	5.50
Salt	0.25
Premix	0.25
Total	100.00

Table-2: Nutrition content of the ration.

Nutrient contents	Addition of binahong leaf powder			
	T0 (0%)	T1 (2%)	T2 (4%)	T3 (6%)
Energy metabolic (kkal/kg)	2763.20	2694.12	2628.26	2565.40
Protein (%)	19.77	19.35	18.95	18.56

Fat (%)	4.66	4.58	4.51	4.43
Crude fiber (%)	4.29	4.95	5.56	6.12
Ca (%)	3.15	3.06	2.97	2.88
P (%)	0.79	0.76	0.74	0.71
Lysine (%)	0.96	0.93	0.89	0.86
Methionine (%)	0.48	0.46	0.44	0.42

Table-3: Egg production on addition of binahong leaf powder in quail rations.

Age (weeks)	Addition of binahong leaf powder				p-value
	T0 (0%)	T1 (2%)	T2 (4%)	T3 (6%)	
8	29.71±2.74	28.00±2.166	28.85±2.74	28.57±2.67	0.49
9	39.71±2.34	42.28±1.91	41.42±2.67	41.14±2.55	0.53
10	45.14±4.58	46.09±1.47	47.23±2.34	45.14±3.08	0.57
11	46.64±2.42	49.49±1.38	48.73±3.83	47.63±1.73	0.59

12	56.38±2.49	52.34±1.86	54.50±1.37	54.57±1.76	0.67
14	56.17±2.47	57.55±1.45	57.73±1.85	58.74±1.93	0.69

Table-4: Influence of addition of binahong leaf powder on quail eggs characteristic.

Parameters	Addition of binahong leaf powder				p-value
	T0 (0%)	T1 (2%)	T2(4%)	T3 (6%)	
Egg weight (g)	9.99±0.29	10.01±0.11	10.03±0.11	10.03±0.27	0.38
Yolk weight (g)	3.65±0.13	3.92±0.25	3.68±0.12	3.93±0.17	0.57
Albumen weight (g)	4.68±0.29	4.53±0.18	4.47±0.25	4.60±0.19	0.53
Eggshell weight (g)	1.14±0.28	1.12±0.15	1.14±0.17	1.01±0.10	0.32
Yolk weight (%)	38.77±2.34	41.80±2.72	40.01±1.39	41.13±0.79	0.51
Albumen weight (%)	49.63±2.78	48.27±2.07	48.56±2.70	48.13±1.58	0.73
Eggshell weight (%)	11.43±2.73	11.07±1.60	11.41±1.73	10.11±1.14	0.12

Table-5: Influence of the addition of binahong leaf powder on cholesterol, triglyceride, HDL, LDL, and protein of quail egg yolk.

Parameters	Addition of binahong leaf powder				p-value
	T0 (0%)	T1 (2%)	T2 (4%)	T3 (6%)	
Yolk cholesterol (mg/g)	56.15±5.04[a]	23.77±3.75[b]	51.97±4.63[a]	51.35±7.55[a]	<0.01
Yolk triglyceride (mg/g)	903.22±58.21[a]	608.62±22.46 [c]	778.34±53.59 [b]	811.63±104.01 [b]	<0.01
Yolk HDL (mg/g)	36.86±1.32[b]	34.8b±1.58[b]	48.62±1.67[a]	47.52±1.58[a]	<0.01
Yolk LDL (mg/g)	29.18 ±1.59 [a]	16.06b±0.33[b]	17.09±0.37[b]	16.28±0.36[b]	<0.01
Protein of yolk (%)	30.16±2.78	30.37±.82	29.15±0.81	30.19±2.55	0.00

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Mean within a row for each parameter with different superscripts are significantly different ($p<0.01$) ~~→ jurnal lainyang lain~~

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RESEARCH ARTICLE

Addition of binahong (*Anredera cordifolia*) leaf powder to diets to produce eggs with low cholesterol

Sri Kismiati {ORCID:0000-0002-0259-1575}, Hanny Indrat Wahyuni, Rina Muryani, Dwi Sunarti and Sri Sumarsih

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Abstract

Aim: The aim of this study was to evaluate the effect of the addition of binahong leaf powder to quail rations on the production and quality of eggs.

Materials and Methods: The study involved the use of two hundred 7-week-old quails housed evenly in 20 wire cages with a body weight of 123.77±0.72 g. The quails were treated as follows: Ration without binahong leaf powder (T0), addition 2% of binahong leaf powder (T1), addition 4% of binahong leaf powder (T2), and addition 6% of binahong leaf powder (T3). The study used a completely randomized design. The parameters measured were the production, weight, and characteristics of the eggs, as well as the cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and egg protein content in the yolk.

Results: The addition of 2-6% binahong powder did not significantly affect egg production, egg characteristics, or egg protein content, but significantly ($p<0.05$) affected the cholesterol, triglyceride, HDL, and LDL contents in yolk. The cholesterol, triglyceride, and LDL contents decreased significantly in T1, whereas HDL increased significantly in T2 and T3.

Conclusion: The addition of 2% binahong was enough to obtain healthy quail eggs with low levels of cholesterol, triglyceride, and LDL.

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Yes, I am agree with this

Keywords: binahong, cholesterol, high-density lipoprotein, low-density lipoprotein, triglyceride.

<H1>Introduction

Quail egg contains many high-quality nutrients but also high cholesterol [1]. The protein content in a quail egg is approximately 13.30% [1]. The cholesterol content in a quail egg is higher than in a chicken egg. The cholesterol content in the yolk of a quail egg is 6.79 mg/dl, whereas the cholesterol content in chicken egg yolk is 4.03 mg/dl [2]. Overconsumption of cholesterol increases the blood cholesterol level, which leads to heart disease; thus, some people are afraid of consuming quail egg. Catapano and Wiklund [3] stated that blood cholesterol, particularly low-density lipoprotein (LDL) cholesterol, has a positive correlation with the occurrence of atherosclerosis. The consumption of high-density lipoprotein (HDL) derived from quail egg increases the blood serum HDL level and decreases atherosclerotic plaques in rabbits. Thus, egg HDL may be used as an anti-atherosclerotic agent for patients with cardiovascular disease [4]. An attempt to obtain quail egg with low cholesterol and high HDL contents needs to be carried out.

Binahong (*Anredera cordifolia*) is a wild plant with a rapid growth rate that requires no complicated cultivation; thus, binahong is abundant [5]. Sutrisno *et al*. [5] and Leliqia *et al*.

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That sentence can be found in Sutrisno *et al*. (2014).

[6] revealed that the leaf of binahong contains bioactive compounds, such as flavonoids, tannins, saponins, phenols, and steroids, and Astuti *et al*. [7] reported that the leaf also contains terpenoid, which may potentially increase pancreatic insulin secretion. Furthermore, Hasbullah [8] documented that binahong leaf exhibits hypolipidemic properties. Kamboh *et al*. [9] showed that supplementation with a bioflavonoid increases antioxidant and enzyme activities and decreases total cholesterol and triglyceride levels in the serum and breast meat of broiler. A study by Ouyang *et al*. [10] showed that supplementation with 15 mg/kg of alfalfa flavonoid increases HDL levels and decreases the levels of cholesterol, triglyceride, and serum LDL and the percentage of abdominal fat of broiler. Feeding saponin decreases cholesterol, insulin, and blood triglyceride and increases blood HDL synthesis. Saponin also decreases the contents of cholesterol [11] and protein digestibility [12] of broiler meat.

Based on the facts that binahong contain flavonoids, tannins, saponins, phenols, and steroids and it has potentially reduced cholesterol, triglyceride, LDL serum and increased HDL. Hopefully, it will produce a healthy egg product. Therefore, t~~T~~he aim of this study was to evaluate the effect of the addition of binahong leaf powder to rations on the contents of cholesterol, triglyceride, HDL, LDL, and protein in quail eggs.

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<H1>Materials and Methods

<H2>Ethical approval

The procedure of using quail in this study has been approved by the Animal Ethics Committee in the Faculty of Animal Sciences, Diponegoro University, Semarang, Indonesia.

<H2>Animals

The study used two hundred 7-week-old female quails with an average body weight of 123.77±0.72 g. The quails were housed in 20 wire cages. Each cage was 90 × 35 × 25 cm and housed 10 quails. The feed consisted of yellow corn, rice bran, pollard, poultry meat meal, soybean meal, $CaCO_3$, salt, premix, and binahong leaf powder.

<H2>Experimental design

This study was arranged according to a completely randomized design with four treatments and five repetitions (10 quails per group per repetition). The treatment groups included T0, control ration (without binahong leaf powder); T1, control ration + 2% binahong leaf powder; T2, control ration + 4% binahong leaf powder; and T3, control ration + 6% binahong leaf powder in ration. The composition and nutrient content of the ration are presented in Tables-1 and 2.

<H2>Tests and procedures

The recording of egg production was carried out every day during the study and the formula: Production = (number of eggs/number of chickens) × 100% was used. Egg weight and egg characteristics were measured ~~every 3 days at the end of the week~~ every 3 consecutive days weekly. The cholesterol, triglyceride, HDL, LDL, and protein contents in the egg yolk were collected by sampling one egg per experimental unit at the end of the study. The analyses of cholesterol and triglyceride contents were conducted based on the cholesterol p-aminophenazone method [13], HDL and LDL analysis was based on the enzymatic colorimetric method [13], and protein analysis was based on the Kjeldahl method [14].

Comment [A4]: Please note that if you measure every 3 days, not all measurements will be at the end of the week. Please respecify when these parameters were measured.

I measured 3 consecutive days every week.

Egg weight and egg characteristics were measured every 3 consecutive days weekly

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<H2>Statistical analysis

??? Data were analyzed as a completely randomized design using one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test

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<H1>Results and Discussion

<H2>Effect of the addition of binahong leaf powder on egg production

The addition of 2-6% of binahong leaf powder, rich in flavonoids and saponins, did not significantly influence quail egg production at 8-14 weeks of age (Table-3). This result was similar to that reported by Kusumanti and Murwani [15], which showed that supplementation of binahong leaf powder does not significantly affect the egg production of laying hens. In

line with this, Iskender *et al*. [16] showed that flavonoids have no significant effect on egg production. In contrast, supplementation with the quercetin flavonoid [17] and saponins derived from karaya increases the egg production of laying hens [18]. These inconsistencies may be caused by the differences in the levels of flavonoids and saponins used in the study, as well as the conditions of the study.

<H2>Effect of binahong leaf powder on egg characteristics

The addition of binahong leaf powder did not significantly affect the characteristics of the egg, including egg weight, yolk weight, albumen weight, and eggshell weight (Table-4). It has been hypothesized that the bioactive component of binahong leaf may positively affect the egg characteristics. However, this study revealed different results. According to Leke *et al*. [19], flavonoids of papaya seeds increase egg quality (egg yolk, albumen, and eggshell weight) of Indonesian hens. Moreover, Afrose *et al*. [18] reported that supplementation with 25, 50, and 75 mg/kg of karaya saponin increases egg weight, yolk weight, and albumen weight of laying hens, whereas Ayasan *et al*. [20] reported that supplementation of *Yucca schidigera* powder, with 120 ppm of saponin, increases egg weight but does not affect the eggshell weight. As in our study, one study showed that supplementation with 0.2-0.6 g/kg of flavonoid does not significantly influence egg quality [17]. In addition, supplementation with

flavonoids does not significantly influence eggshell weight, as reported by Iskender *et al*. [16]. The differences in the nature and levels of bioactive compounds, the nutritional values of rations, and the conditions of study may be responsible for these divergent results.

<H2>Cholesterol, triglyceride, HDL, LDL, and protein contents in yolk

The data on cholesterol, triglyceride, HDL, LDL, and protein contents in yolk after binahong leaf powder supplementation are shown in Table-5. The addition of 2% of binahong leaf powder decreased the level of cholesterol in the yolk of quail eggs ($p<0.05$). Flavonoids and saponins in the binahong leaf powder may inhibit cholesterol absorption; hence, cholesterol deposition may be reduced [21-23]. This is supported by Lien *et al*. [24] who showed that the consumption of flavonoids increases the excretion of cholesterol. In addition, flavonoids may increase reverse cholesterol transport (RCT), resulting in high cholesterol excretion; thus, there will be a lower cholesterol level in yolk. However, 4 and 6% binahong leaf powder did not affect the cholesterol level.

The addition of binahong leaf powder decreased the triglyceride content in quail yolk (Table-5). As shown in our study, supplementation with a flavonoid extracted from the root of *Scutellaria baicalensis* Georgi decreases triglyceride content in the serum of broiler [25]. Likewise, Kamboh and Zhu [21] and Ouyang *et al*. [10] reported that the consumption of

flavonoids decreases the level of triglyceride in the serum and meat of broiler. According to Hsu and Yen [26] and Nagai *et al*. [27], flavonoids may inhibit intracellular triglyceride synthesis in the liver, which results in lower triglyceride deposition in quail egg yolk. In addition, flavonoid content in binahong leaf powder may increase the expression of peroxisome proliferator-activated receptor α in the liver, which is involved in lipid metabolism, especially fatty acid oxidation [10,28].

The content of yolk HDL increased with the increased concentration of binahong leaf powder in the quail rations ($p<0.05$). Similar to the result of our study, Afrose *et al.* [29] documented that saponins increase HDL content in broiler meat. Supplementation with karaya saponin also increases HDL content in quail egg [30]. Furthermore, Smith *et al*. [31] stated that supplementation with saponin extract increases the HDL content in the blood, liver, kidneys, and heart tissues of white mice. Kamboh and Zhu [21] reported that flavonoids increase the HDL content in serum and HDL deposition in the breast meat of broiler. The mechanism by which binahong leaf increased HDL concentration in quail egg yolk is not known; however, the role of binahong leaf powder in the RCT mechanism seems to be attributable to the increased HDL content in quail yolk. Millar *et al.* [23] and Marques *et al.* [32] reported that the increase in the RCT mechanism due to binahong leaf powder supplementation is

accompanied by an increase in the level of HDL. Thus, this mechanism seems to be related to the contribution of HDL on RCT.

Treatment with binahong leaf powder decreased the level of LDL in the quail egg yolk (Table-5). Afrose *et al.* [30] reported that saponins decrease the LDL content of quail egg. Likewise, Chaudhary *et al*. [33] stated that saponins potentially decrease the LDL content of meat. In addition, supplementation with saponin extract decreases LDL content in the blood, liver, kidneys, and heart tissues of white mice [31]. Flavonoids have been reported to decrease the LDL level in the blood of broiler [10,21,25]. Furthermore, Zhou *et al*. [25] stated that flavonoid supplementation also decreases the LDL content of the breast meat of broiler. Binahong leaf extract contains saponins, which bind bile acid and form large mixed micelles. Cholesterol in the micelles cannot be absorbed by microvilli on the surface of intestinal epithelial cells, which causes a decrease in total and LDL cholesterol levels [34]. Saponins inhibit fat metabolism through the inhibition of lipase secretion and decrease cholesterol and LDL [35], but increase HDL [36], contents in the blood. Flavonoids are active compounds that reduce the activity of fatty acid synthase and reduce the level of LDL in animal tissues [10].

The addition of 2-6% binahong leaf powder did not significantly influence the protein content of egg yolk. This contrasted with a previous study. Iskender *et al*. [16] reported that supplementation with flavonoids (hesperidin, naringin, and quercetin) increases egg protein. Ahmad *et al*. [37] documented that bioactive compounds from *Moringa oleifera* leaf extracts increase the protein content in yolk.

In general, the protein content in an egg is derived from the feed. In this study, the inclusion of binahong leaf powder was accompanied by the decreased crude protein in the ration. Hence, the protein-increasing effect by binahong leaf powder seemed to be inhibited by lower protein content in the quail ration due to the binahong leaf inclusion.

<H1>Conclusion

The addition of 2% binahong leaf powder is best for lowering cholesterol, triglyceride, and LDL levels in quail egg.

<H1>Authors' Contributions

SK conducted the research, data collection, and drafting of the article. HIW developed the feeding concept and supervised the research, RM advised the experimental design, DS conducted data analysis, and SS performed laboratory analysis. All authors read and approved the final manuscript.

<H1>Acknowledgments

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<H1>Competing Interests

The authors declare that they have no competing interests.

<H1>Publisher's Note

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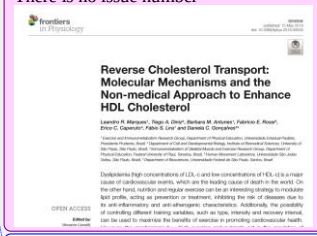

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Tables

Table-1: Feed composition of the control ration.	
Feed ingredients	%
Yellow corn	48.00

Rice bran	6.00
Pollard	16.00
Poultry meat meal	14.00
Soybean meal	10.00
$CaCO_3$	5.50
Salt	0.25
Premix	0.25
Total	100.00

Table-2: Nutrition content of the ration.

Nutrient contents	Addition of binahong leaf powder			
	T0 (0%)	T1 (2%)	T2 (4%)	T3 (6%)
Energy metabolic (kkal/kg)	2763.20	2694.12	2628.26	2565.40
Protein (%)	19.77	19.35	18.95	18.56

Fat (%)	4.66	4.58	4.51	4.43
Crude fiber (%)	4.29	4.95	5.56	6.12
Ca (%)	3.15	3.06	2.97	2.88
P (%)	0.79	0.76	0.74	0.71
Lysine (%)	0.96	0.93	0.89	0.86
Methionine (%)	0.48	0.46	0.44	0.42

Table-3: Egg production on addition of binahong leaf powder in quail rations.

Age (weeks)	Addition of binahong leaf powder				p-value
	T0 (0%)	T1 (2%)	T2 (4%)	T3 (6%)	
8	29.71±2.74	28.00±2.166	28.85±2.74	28.57±2.67	0.49
9	39.71±2.34	42.28±1.91	41.42±2.67	41.14±2.55	0.53
10	45.14±4.58	46.09±1.47	47.23±2.34	45.14±3.08	0.57
11	46.64±2.42	49.49±1.38	48.73±3.83	47.63±1.73	0.59

12	56.38±2.49	52.34±1.86	54.50±1.37	54.57±1.76	0.67
14	56.17±2.47	57.55±1.45	57.73±1.85	58.74±1.93	0.69

Table-4: Influence of addition of binahong leaf powder on quail eggs characteristic.

Parameters	Addition of binahong leaf powder				p-value
	T0 (0%)	T1 (2%)	T2(4%)	T3 (6%)	
Egg weight (g)	9.99±0.29	10.01±0.11	10.03±0.11	10.03±0.27	0.38
Yolk weight (g)	3.65±0.13	3.92±0.25	3.68±0.12	3.93±0.17	0.57
Albumen weight (g)	4.68±0.29	4.53±0.18	4.47±0.25	4.60±0.19	0.53
Eggshell weight (g)	1.14±0.28	1.12±0.15	1.14±0.17	1.01±0.10	0.32
Yolk weight (%)	38.77±2.34	41.80±2.72	40.01±1.39	41.13±0.79	0.51
Albumen weight (%)	49.63±2.78	48.27±2.07	48.56±2.70	48.13±1.58	0.73
Eggshell weight (%)	11.43±2.73	11.07±1.60	11.41±1.73	10.11±1.14	0.12

Table-5: Influence of the addition of binahong leaf powder on cholesterol, triglyceride, HDL, LDL, and protein of quail egg yolk.

Parameters	Addition of binahong leaf powder				p-value
	T0 (0%)	T1 (2%)	T2 (4%)	T3 (6%)	
Yolk cholesterol (mg/g)	56.15±5.04[a]	23.77±3.75[b]	51.97±4.63[a]	51.35±7.55[a]	<0.01
Yolk triglyceride (mg/g)	903.22±58.21[a]	608.62±22.46 c	778.34±53.59 b	811.63±104.01 b	<0.01
Yolk HDL (mg/g)	36.86±1.32[b]	34.8b±1.58[b]	48.62±1.67[a]	47.52±1.58[a]	<0.01
Yolk LDL (mg/g)	29.18 ±1.59 [a]	16.06b±0.33[b]	17.09±0.37[b]	16.28±0.36[b]	<0.01
Protein of yolk (%)	30.16±2.78	30.37±.82	29.15±0.81	30.19±2.55	0.00

~~Mean bearing different superscripts between the treatments differ significantly. Data represented as mean±SE (p<0.01) → vw~~

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Mean within a row for each parameter with different superscripts are significantly different (p<0.01)

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Mean within a row for each parameter with different superscripts are significantly different ($p<0.01$) ~~-> jurnal lainyang lain~~

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Technical/Copyediting by Sinjore – 12/03/2020

RESEARCH ARTICLE

Addition of binahong (*Anredera cordifolia*) leaf powder to diets to produce eggs with low cholesterol

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Abstract

Aim: The aim of this study was to evaluate the effect of the addition of binahong leaf powder to quail rations on the production and quality of eggs.

Materials and Methods: The study involved the use of two hundred 7-week-old quails housed evenly in 20 wire cages with a body weight of 123.77±0.72 g. The quails were treated as follows: Ration without binahong leaf powder (T0), addition 2% of binahong leaf powder (T1), addition 4% of binahong leaf powder (T2), and addition 6% of binahong leaf powder (T3). The study used a completely randomized design. The parameters measured were the production, weight, and characteristics of the eggs, as well as the cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and egg protein content in the yolk.

Results: The addition of 2-6% binahong powder did not significantly affect egg production, egg characteristics, or egg protein content, but significantly ($p<0.05$) affected the cholesterol, triglyceride, HDL, and LDL contents in yolk. The cholesterol, triglyceride, and LDL contents decreased significantly in T1, whereas HDL increased significantly in T2 and T3.

Conclusion: The addition of 2% binahong was enough to obtain healthy quail eggs with low levels of cholesterol, triglyceride, and LDL.

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Keywords: binahong, cholesterol, high-density lipoprotein, low-density lipoprotein, triglyceride.

<H1>Introduction

Quail egg contains many high-quality nutrients but also high cholesterol [1]. The protein content in a quail egg is approximately 13.30% [1]. The cholesterol content in a quail egg is higher than in a chicken egg. The cholesterol content in the yolk of a quail egg is 6.79 mg/dl, whereas the cholesterol content in chicken egg yolk is 4.03 mg/dl [2]. Overconsumption of cholesterol increases the blood cholesterol level, which leads to heart disease; thus, some people are afraid of consuming quail egg. Catapano and Wiklund [3] stated that blood cholesterol, particularly low-density lipoprotein (LDL) cholesterol, has a positive correlation with the occurrence of atherosclerosis. The consumption of high-density lipoprotein (HDL) derived from quail egg increases the blood serum HDL level and decreases atherosclerotic plaques in rabbits. Thus, egg HDL may be used as an anti-atherosclerotic agent for patients with cardiovascular disease [4]. An attempt to obtain quail egg with low cholesterol and high HDL contents needs to be carried out.

Binahong (*Anredera cordifolia*) is a wild plant with a rapid growth rate that requires no complicated cultivation; thus, binahong is abundant [5]. Sutrisno *et al*. [5] and Leliqia *et al*.

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That sentence can be found in Sutrisno *et al.* (2014).

[6] revealed that the leaf of binahong contains bioactive compounds, such as flavonoids, tannins, saponins, phenols, and steroids, and Astuti *et al*. [7] reported that the leaf also contains terpenoid, which may potentially increase pancreatic insulin secretion. Furthermore, Hasbullah [8] documented that binahong leaf exhibits hypolipidemic properties. Kamboh *et al*. [9] showed that supplementation with a bioflavonoid increases antioxidant and enzyme activities and decreases total cholesterol and triglyceride levels in the serum and breast meat of broiler. A study by Ouyang *et al*. [10] showed that supplementation with 15 mg/kg of alfalfa flavonoid increases HDL levels and decreases the levels of cholesterol, triglyceride, and serum LDL and the percentage of abdominal fat of broiler. Feeding saponin decreases cholesterol, insulin, and blood triglyceride and increases blood HDL synthesis. Saponin also decreases the contents of cholesterol [11] and protein digestibility [12] of broiler meat.

Based on the facts that binahong contain flavonoids, tannins, saponins, phenols, and steroids and it has potentially reduced cholesterol, triglyceride, LDL serum and increased HDL. Hopefully, it will produce a healthy egg product. Therefore, t~~T~~he aim of this study was to evaluate the effect of the addition of binahong leaf powder to rations on the contents of cholesterol, triglyceride, HDL, LDL, and protein in quail eggs.

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<H1>Materials and Methods

<H2>Ethical approval

The procedure of using quail in this study has been approved by the Animal Ethics Committee in the Faculty of Animal Sciences, Diponegoro University, Semarang, Indonesia.

<H2>Animals

The study used two hundred 7-week-old female quails with an average body weight of 123.77±0.72 g. The quails were housed in 20 wire cages. Each cage was 90 × 35 × 25 cm and housed 10 quails. The feed consisted of yellow corn, rice bran, pollard, poultry meat meal, soybean meal, $CaCO_3$, salt, premix, and binahong leaf powder.

<H2>Experimental design

This study was arranged according to a completely randomized design with four treatments and five repetitions (10 quails per group per repetition). The treatment groups included T0, control ration (without binahong leaf powder); T1, control ration + 2% binahong leaf powder; T2, control ration + 4% binahong leaf powder; and T3, control ration + 6% binahong leaf powder in ration. The composition and nutrient content of the ration are presented in Tables-1 and 2.

<H2>Tests and procedures

The recording of egg production was carried out every day during the study and the formula: Production = (number of eggs/number of chickens) × 100% was used. Egg weight and egg characteristics were measured ~~every 3 days at the end of the week~~ every 3 consecutive days weekly. The cholesterol, triglyceride, HDL, LDL, and protein contents in the egg yolk were collected by sampling one egg per experimental unit at the end of the study. The analyses of cholesterol and triglyceride contents were conducted based on the cholesterol p-aminophenazone method [13], HDL and LDL analysis was based on the enzymatic colorimetric method [13], and protein analysis was based on the Kjeldahl method [14].

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Egg weight and egg characteristics were measured every 3 consecutive days weekly

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<H2>Statistical analysis

??? Data were analyzed as a completely randomized design using one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test

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<H1>Results and Discussion

<H2>Effect of the addition of binahong leaf powder on egg production

The addition of 2-6% of binahong leaf powder, rich in flavonoids and saponins, did not significantly influence quail egg production at 8-14 weeks of age (Table-3). This result was similar to that reported by Kusumanti and Murwani [15], which showed that supplementation of binahong leaf powder does not significantly affect the egg production of laying hens. In

line with this, Iskender *et al*. [16] showed that flavonoids have no significant effect on egg production. In contrast, supplementation with the quercetin flavonoid [17] and saponins derived from karaya increases the egg production of laying hens [18]. These inconsistencies may be caused by the differences in the levels of flavonoids and saponins used in the study, as well as the conditions of the study.

<H2>Effect of binahong leaf powder on egg characteristics

The addition of binahong leaf powder did not significantly affect the characteristics of the egg, including egg weight, yolk weight, albumen weight, and eggshell weight (Table-4). It has been hypothesized that the bioactive component of binahong leaf may positively affect the egg characteristics. However, this study revealed different results. According to Leke *et al*. [19], flavonoids of papaya seeds increase egg quality (egg yolk, albumen, and eggshell weight) of Indonesian hens. Moreover, Afrose *et al*. [18] reported that supplementation with 25, 50, and 75 mg/kg of karaya saponin increases egg weight, yolk weight, and albumen weight of laying hens, whereas Ayasan *et al*. [20] reported that supplementation of *Yucca schidigera* powder, with 120 ppm of saponin, increases egg weight but does not affect the eggshell weight. As in our study, one study showed that supplementation with 0.2-0.6 g/kg of flavonoid does not significantly influence egg quality [17]. In addition, supplementation with

flavonoids does not significantly influence eggshell weight, as reported by Iskender *et al*. [16]. The differences in the nature and levels of bioactive compounds, the nutritional values of rations, and the conditions of study may be responsible for these divergent results.

<H2>Cholesterol, triglyceride, HDL, LDL, and protein contents in yolk

The data on cholesterol, triglyceride, HDL, LDL, and protein contents in yolk after binahong leaf powder supplementation are shown in Table-5. The addition of 2% of binahong leaf powder decreased the level of cholesterol in the yolk of quail eggs ($p<0.05$). Flavonoids and saponins in the binahong leaf powder may inhibit cholesterol absorption; hence, cholesterol deposition may be reduced [21-23]. This is supported by Lien *et al*. [24] who showed that the consumption of flavonoids increases the excretion of cholesterol. In addition, flavonoids may increase reverse cholesterol transport (RCT), resulting in high cholesterol excretion; thus, there will be a lower cholesterol level in yolk. However, 4 and 6% binahong leaf powder did not affect the cholesterol level.

The addition of binahong leaf powder decreased the triglyceride content in quail yolk (Table-5). As shown in our study, supplementation with a flavonoid extracted from the root of *Scutellaria baicalensis* Georgi decreases triglyceride content in the serum of broiler [25]. Likewise, Kamboh and Zhu [21] and Ouyang *et al*. [10] reported that the consumption of

flavonoids decreases the level of triglyceride in the serum and meat of broiler. According to Hsu and Yen [26] and Nagai *et al*. [27], flavonoids may inhibit intracellular triglyceride synthesis in the liver, which results in lower triglyceride deposition in quail egg yolk. In addition, flavonoid content in binahong leaf powder may increase the expression of peroxisome proliferator-activated receptor α in the liver, which is involved in lipid metabolism, especially fatty acid oxidation [10,28].

The content of yolk HDL increased with the increased concentration of binahong leaf powder in the quail rations ($p<0.05$). Similar to the result of our study, Afrose *et al.* [29] documented that saponins increase HDL content in broiler meat. Supplementation with karaya saponin also increases HDL content in quail egg [30]. Furthermore, Smith *et al*. [31] stated that supplementation with saponin extract increases the HDL content in the blood, liver, kidneys, and heart tissues of white mice. Kamboh and Zhu [21] reported that flavonoids increase the HDL content in serum and HDL deposition in the breast meat of broiler. The mechanism by which binahong leaf increased HDL concentration in quail egg yolk is not known; however, the role of binahong leaf powder in the RCT mechanism seems to be attributable to the increased HDL content in quail yolk. Millar *et al*. [23] and Marques *et al*. [32] reported that the increase in the RCT mechanism due to binahong leaf powder supplementation is

accompanied by an increase in the level of HDL. Thus, this mechanism seems to be related to the contribution of HDL on RCT.

Treatment with binahong leaf powder decreased the level of LDL in the quail egg yolk (Table-5). Afrose *et al.* [30] reported that saponins decrease the LDL content of quail egg. Likewise, Chaudhary *et al*. [33] stated that saponins potentially decrease the LDL content of meat. In addition, supplementation with saponin extract decreases LDL content in the blood, liver, kidneys, and heart tissues of white mice [31]. Flavonoids have been reported to decrease the LDL level in the blood of broiler [10,21,25]. Furthermore, Zhou *et al*. [25] stated that flavonoid supplementation also decreases the LDL content of the breast meat of broiler. Binahong leaf extract contains saponins, which bind bile acid and form large mixed micelles. Cholesterol in the micelles cannot be absorbed by microvilli on the surface of intestinal epithelial cells, which causes a decrease in total and LDL cholesterol levels [34]. Saponins inhibit fat metabolism through the inhibition of lipase secretion and decrease cholesterol and LDL [35], but increase HDL [36], contents in the blood. Flavonoids are active compounds that reduce the activity of fatty acid synthase and reduce the level of LDL in animal tissues [10].

The addition of 2-6% binahong leaf powder did not significantly influence the protein content of egg yolk. This contrasted with a previous study. Iskender *et al*. [16] reported that supplementation with flavonoids (hesperidin, naringin, and quercetin) increases egg protein. Ahmad *et al*. [37] documented that bioactive compounds from *Moringa oleifera* leaf extracts increase the protein content in yolk.

In general, the protein content in an egg is derived from the feed. In this study, the inclusion of binahong leaf powder was accompanied by the decreased crude protein in the ration. Hence, the protein-increasing effect by binahong leaf powder seemed to be inhibited by lower protein content in the quail ration due to the binahong leaf inclusion.

<H1>Conclusion

The addition of 2% binahong leaf powder is best for lowering cholesterol, triglyceride, and LDL levels in quail egg.

<H1>Authors' Contributions

SK conducted the research, data collection, and drafting of the article. HIW developed the feeding concept and supervised the research, RM advised the experimental design, DS conducted data analysis, and SS performed laboratory analysis. All authors read and approved the final manuscript.

<H1>Acknowledgments

The authors are very grateful for the support of Poultry Production Laboratory, Faculty of Animal and Agricultural Sciences, Diponegoro University. The authors also would like to thank Sugiharto S.Pt., M.Sc. Ph.D. for proofreading the manuscript.

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<H1>Competing Interests

The authors declare that they have no competing interests.

<H1>Publisher's Note

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Archives • 2

OVERVIEW OF EFFICACY, SAFETY A

ANREDERA CORDIFO

Ni Putu Eka Leliqia[1,3*], Elin Yulin

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Indon

[2]Pharmaceutical Biology Research Group, School of Ph

[3]Pharmacy Department, Faculty of Mathematics and

*leliqia@g

Abstract

Anredera cordifolia (Ten.) Steenis is used for medical pu content, pharmacology activity and toxicity test result o glycoside, flavonoids, saponins and alkaloids were found such as ursolic acid, ancordin, apigenin, etc. were isola benefits in repairing kidney function, as antibacterial, an inhibitor, antidiabetic, antihypertensive, vasodilator,

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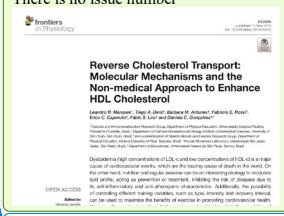

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Tables

Table-1: Feed composition of the control ration.	
Feed ingredients	%
Yellow corn	48.00

Rice bran	6.00
Pollard	16.00
Poultry meat meal	14.00
Soybean meal	10.00
$CaCO_3$	5.50
Salt	0.25
Premix	0.25
Total	100.00

Table-2: Nutrition content of the ration.

Nutrient contents	Addition of binahong leaf powder			
	T0 (0%)	T1 (2%)	T2 (4%)	T3 (6%)
Energy metabolic (kkal/kg)	2763.20	2694.12	2628.26	2565.40
Protein (%)	19.77	19.35	18.95	18.56

Fat (%)	4.66	4.58	4.51	4.43
Crude fiber (%)	4.29	4.95	5.56	6.12
Ca (%)	3.15	3.06	2.97	2.88
P (%)	0.79	0.76	0.74	0.71
Lysine (%)	0.96	0.93	0.89	0.86
Methionine (%)	0.48	0.46	0.44	0.42

Table-3: Egg production on addition of binahong leaf powder in quail rations.

Age (weeks)	Addition of binahong leaf powder				p-value
	T0 (0%)	T1 (2%)	T2 (4%)	T3 (6%)	
8	29.71±2.74	28.00±2.166	28.85±2.74	28.57±2.67	0.49
9	39.71±2.34	42.28±1.91	41.42±2.67	41.14±2.55	0.53
10	45.14±4.58	46.09±1.47	47.23±2.34	45.14±3.08	0.57
11	46.64±2.42	49.49±1.38	48.73±3.83	47.63±1.73	0.59

12	56.38±2.49	52.34±1.86	54.50±1.37	54.57±1.76	0.67
14	56.17±2.47	57.55±1.45	57.73±1.85	58.74±1.93	0.69

Table-4: Influence of addition of binahong leaf powder on quail eggs characteristic.

Parameters	Addition of binahong leaf powder				p-value
	T0 (0%)	T1 (2%)	T2(4%)	T3 (6%)	
Egg weight (g)	9.99±0.29	10.01±0.11	10.03±0.11	10.03±0.27	0.38
Yolk weight (g)	3.65±0.13	3.92±0.25	3.68±0.12	3.93±0.17	0.57
Albumen weight (g)	4.68±0.29	4.53±0.18	4.47±0.25	4.60±0.19	0.53
Eggshell weight (g)	1.14±0.28	1.12±0.15	1.14±0.17	1.01±0.10	0.32
Yolk weight (%)	38.77±2.34	41.80±2.72	40.01±1.39	41.13±0.79	0.51
Albumen weight (%)	49.63±2.78	48.27±2.07	48.56±2.70	48.13±1.58	0.73
Eggshell weight (%)	11.43±2.73	11.07±1.60	11.41±1.73	10.11±1.14	0.12

Table-5: Influence of the addition of binahong leaf powder on cholesterol, triglyceride, HDL, LDL, and protein of quail egg yolk.

Parameters	Addition of binahong leaf powder				p-value
	T0 (0%)	T1 (2%)	T2 (4%)	T3 (6%)	
Yolk cholesterol (mg/g)	56.15±5.04[a]	23.77±3.75[b]	51.97±4.63[a]	51.35±7.55[a]	<0.01
Yolk triglyceride (mg/g)	903.22±58.21[a]	608.62±22.46 c	778.34±53.59 b	811.63±104.01 b	<0.01
Yolk HDL (mg/g)	36.86±1.32[b]	34.8b±1.58[b]	48.62±1.67[a]	47.52±1.58[a]	<0.01
Yolk LDL (mg/g)	29.18 ±1.59 [a]	16.06b±0.33[b]	17.09±0.37[b]	16.28±0.36[b]	<0.01
Protein of yolk (%)	30.16±2.78	30.37±.82	29.15±0.81	30.19±2.55	0.00

~~Mean bearing different superscripts between the treatments differ significantly. Data represented as mean±SE (p<0.01) → vw~~

Commented [s16]: Kindly provide significance value for "a", "b", "c" in the footnote.

Mean within a row for each parameter with different superscripts are significantly different (p<0.01)

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Mean within a row for each parameter with different superscripts are significantly different ($p<0.01$) ~~→ jurnal lainyang lain~~

The correction of the Sri Kismiati *et al.* 's manuscript entitle:

Addition of binahong (*Anredera cordifolia*) leaf powder to diets to produce eggs with low cholesterol

1. **Abstract** → the sentence is ok
2. **Introduction**:

   Page 3, Comment A2 → That sentence can be found in Sutrisno *et al.* (2014).

   Page 4, Comment A3 → We added sentences prior to the aim of the study.
   Based on the facts that binahong contain flavonoids, tannins, saponins, phenols, and steroids and it has potentially reduced cholesterol, triglyceride, LDL serum and increased HDL. Hopefully, it will produce a healthy egg product. Therefore, the aim of this study was .....

   Commented [A1]: There should be a paragraph prior to this stating the significance of your study.

3. **Materials and Methods**:

   Page 6, Comment A4 → Egg weight and egg characteristics were measured every 3 consecutive days weekly.

   Page 6, Comment A5 → The citation of the sentence is the same as no 13.

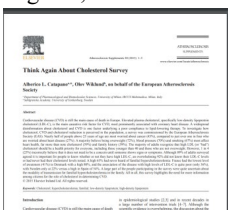
   Page 6, Comment S6 → We added a sentence.
   Data were analyzed as a completely randomized design using one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test

4. **Acknowledgments**:
   Page 12, Comment S7 → We added a sentence.
   The authors are very grateful for the support of Poultry Production Laboratory, Faculty of Animal and Agricultural Sciences, Diponegoro University. The authors also ....

5. **Reference:**
   Page 12, Comment S8 → Nwankwo, N.

   Commented [s2]: Kindly provide author initial

   Page 13, Comment S9 → There is no issue no, 20(2015):1-5



Think Again About Cholesterol Survey

Alberto L. Catapano*, Otto Wiklund†, on behalf of the European Atherosclerosis Society

   Page 13, Comment S10 → Reference no [5] in English is

Sutrisno, E., Adnyana, I.K., Sukandar, E.Y., Fidrianny, I. and dan Lestari, T. (2014). The study of Antibacterial activity tests that were performed by the leaves extract of binahong (*Anredera cordifolia* (Ten.) Steenis, pegagan (*Centella asiatica* (L.) Urban) and its combination on the *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria of diabetes patients feet wound. *Bionat. J. Ilmu Hayati Fisik*, 16(2): 78-82.

Page 13, Comment S11 → There is no issue number

April 30, 2017

PharmacologyOnLine

Archives • 2017 • vol.1 • 124-131

OVERVIEW OF EFFICACY, SAFETY AND PHYTOCHEMICAL STUDY OF ANREDERA CORDIFOLIA (TEN.) STEENIS

Ni Putu Eka Leliqia[1,2*], Elin Yulinah Sukandar[2], Irda Fidrianny[3],

[1]Pharmacology-Clinical Pharmacy Research Group, School of Pharmacy, Bandung Institute of Technology, Indonesia

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Abstract

Anredera cordifolia (Ten.) Steenis is used for medical purposes. In this article, traditional usage, phytochemical content, pharmacology activity and toxicity test result of *A. cordifolia* will be summarized. Terpenoids, steroid, glycoside, flavonoids, saponins and alkaloids were found in *A. cordifolia*. Apart from that, some pure compounds such as ursolic acid, ancordin, apigenin, etc. were isolated from this plant. *A. cordifolia* was proven to have benefits in repairing kidney function, as antibacterial, antifungal, antivirus, protease inhibitor, xanthine oxidase inhibitor, antidiabetic, antihypertensive, vasodilator, diuretic, anti-obesity, hypolipidemic, antioxidant,

Page 13, Comment S12 → last accessed details:

1. http://www.pharmacologyonline.silae.it. 15 August 2019

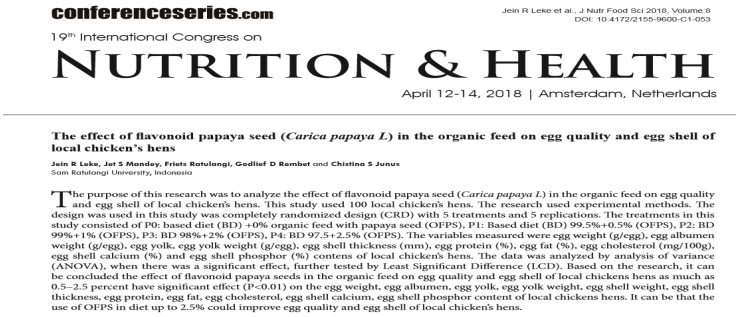
Commented [s3]: Kindly provide last accessed details

1.http://www.pharmacologyonline.silae.it. 15 August 2019

Page 14, Comment S13 → Reference no [8]

Hasbullah, U.H.A. (2016) The content of saponin compounds in the leaves, stems and tubers of the Binahong plant (Anredera cordifolia (Ten) Steenis. *Planta Trop. J. Agro Sci*., 4(1): 20-24.

Page 15, Comment S14 → There is no issue number



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Jein R Leke et al., J Nutr Food Sci 2018, Volume:8
DOI: 10.4172/2155-9600-C1-053

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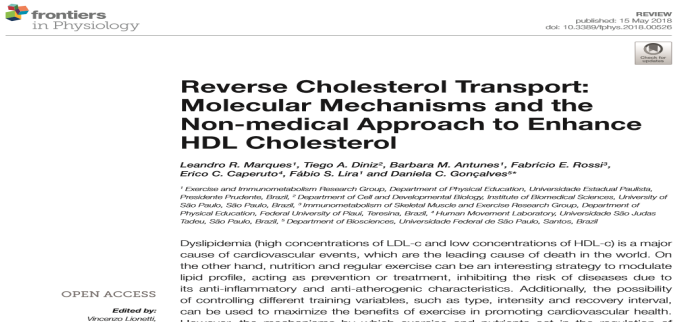
The effect of flavonoid papaya seed (*Carica papaya L*) in the organic feed on egg quality and egg shell of local chicken's hens

Jein R Leke, Jet S Mandey, Frietz Ratulangi, Godlief D Rembet and **Chistina S Junus**
Sam Ratulangi University, Indonesia

The purpose of this research was to analyze the effect of flavonoid papaya seed (*Carica papaya L*) in the organic feed on egg quality and egg shell of local chicken's hens. This study used 100 local chicken's hens. The research used experimental methods. The design was used in this study was completely randomized design (CRD) with 5 treatments and 5 replications. The treatments in this study consisted of P0: based diet (BD) +0% organic feed with papaya seed (OFPS), P1: Based diet (BD) 99.5%+0.5% (OFPS), P2: BD 99%+1% (OFPS), P3: BD 98%+2% (OFPS), P4: BD 97.5+2.5% (OFPS). The variables measured were egg weight (g/egg), egg albumen weight (g/egg), egg yolk, egg yolk weight (g/egg), egg shell thickness (mm), egg protein (%), egg fat (%), egg cholesterol (mg/100g), egg shell calcium (%) and egg shell phosphor (%) contens of local chicken's hens. The data was analyzed by analysis of variance (ANOVA), when there was a significant effect, further tested by Least Significant Difference (LCD). Based on the research, it can be concluded the effect of flavonoid papaya seeds in the organic feed on egg quality and egg shell of local chickens hens as much as 0.5–2.5 percent have significant effect (P<0.01) on the egg weight, egg albumen, egg yolk, egg yolk weight, egg shell weight, egg shell thickness, egg protein, egg fat, egg cholesterol, egg shell calcium, egg shell phosphor content of local chickens hens. It can be that the use of OFPS in diet up to 2.5% could improve egg quality and egg shell of local chicken's hens.

Page 18, Comment S15 → There is no issue number



frontiers in Physiology

REVIEW
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Reverse Cholesterol Transport: Molecular Mechanisms and the Non-medical Approach to Enhance HDL Cholesterol

*Leandro R. Marques[1], Tiego A. Diniz[2], Barbara M. Antunes[1], Fabrício E. Rossi[3], Erico C. Caperuto[4], Fábio S. Lira[1] and Daniela C. Gonçalves[5]**

[1] Exercise and Immunometabolism Research Group, Department of Physical Education, Universidade Estadual Paulista, Presidente Prudente, Brazil, [2] Department of Cell and Developmental Biology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil, [3] Immunometabolism of Skeletal Muscle and Exercise Research Group, Department of Physical Education, Federal University of Piauí, Teresina, Brazil, [4] Human Movement Laboratory, Universidade São Judas Tadeu, São Paulo, Brazil, [5] Department of Biosciences, Universidade Federal de São Paulo, Santos, Brazil

Dyslipidemia (high concentrations of LDL-c and low concentrations of HDL-c) is a major cause of cardiovascular events, which are the leading cause of death in the world. On the other hand, nutrition and regular exercise can be an interesting strategy to modulate lipid profile, acting as prevention or treatment, inhibiting the risk of diseases due to its anti-inflammatory and anti-atherogenic characteristics. Additionally, the possibility of controlling different training variables, such as type, intensity and recovery interval, can be used to maximize the benefits of exercise in promoting cardiovascular health.

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Page 22, Comment S16 → Mean within a row for each parameter with different superscripts are significantly different ($p<0.01$)

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RESEARCH ARTICLE
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Addition of binahong (*Anredera cordifolia*) leaf powder to diets to produce eggs with low cholesterol

Sri Kismiati, Hanny Indrat Wahyuni, Rina Muryani, Dwi Sunarti and Sri Sumarsih

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Abstract

Aim: The aim of this study was to evaluate the effect of the addition of binahong leaf powder to quail rations on the production and quality of eggs.

Materials and Methods: The study involved the use of two hundred 7-week-old quails housed evenly in 20 wire cages with a body weight of 123.77±0.72 g. The quails were treated as follows: Ration without binahong leaf powder (T0), addition 2% of binahong leaf powder (T1), addition 4% of binahong leaf powder (T2), and addition 6% of binahong leaf powder (T3). The study used a completely randomized design. The parameters measured were the production, weight, and characteristics of the eggs, as well as the cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and egg protein content in the yolk.

Results: The addition of 2-6% binahong powder did not significantly affect egg production, egg characteristics, or egg protein content, but significantly ($p<0.05$) affected the cholesterol, triglyceride, HDL, and LDL contents in yolk. The cholesterol, triglyceride, and LDL contents decreased significantly in T1, whereas HDL increased significantly in T2 and T3.

Conclusion: The addition of 2% binahong was enough to obtain healthy quail eggs with low levels of cholesterol, triglyceride, and LDL.

Keywords: binahong, cholesterol, high-density lipoprotein, low-density lipoprotein, triglyceride.

Introduction

Quail egg contains many high-quality nutrients but also high cholesterol [1]. The protein content in a quail egg is approximately 13.30% [1]. The cholesterol content in a quail egg is higher than in a chicken egg. The cholesterol content in the yolk of a quail egg is 6.79 mg/dl, whereas the cholesterol content in chicken egg yolk is 4.03 mg/dl [2]. Overconsumption of cholesterol increases the blood cholesterol level, which leads to heart disease; thus, some people are afraid of consuming quail egg. Catapano and Wiklund [3] stated that blood cholesterol, particularly low-density lipoprotein (LDL) cholesterol, has a positive correlation with the occurrence of atherosclerosis. The consumption of high-density lipoprotein (HDL) derived from quail egg increases the blood serum HDL level and decreases atherosclerotic plaques in rabbits. Thus, egg HDL may be used as an anti-atherosclerotic agent for patients with cardiovascular disease [4]. An attempt to obtain quail egg with low cholesterol and high HDL contents needs to be carried out.

Binahong (*Anredera cordifolia*) is a wild plant with a rapid growth rate that requires no complicated cultivation; thus, binahong is abundant [5]. Sutrisno *et al*. [5] and Leliqia *et al*. [6] revealed that the leaf of binahong contains bioactive compounds, such as flavonoids, tannins, saponins, phenols, and steroids, and Astuti *et al*. [7] reported that the leaf also contains terpenoid, which may potentially increase pancreatic insulin secretion. Furthermore, Hasbullah [8] documented that binahong leaf exhibits hypolipidemic properties. Kamboh *et al*. [9] showed that supplementation with a bioflavonoid increases antioxidant and enzyme activities and decreases total cholesterol and triglyceride levels in the serum and breast meat of broiler. A study by Ouyang *et al*. [10] showed that supplementation with 15 mg/kg of alfalfa flavonoid increases HDL levels and decreases the levels of cholesterol, triglyceride, and serum LDL and the percentage of abdominal fat of broiler. Feeding saponin decreases cholesterol, insulin, and blood triglyceride and increases blood HDL synthesis. Saponin also decreases the contents of cholesterol [11] and protein digestibility [12] of broiler meat.

Based on the facts that binahong contain flavonoids, tannins, saponins, phenols, and steroids and it has potentially reduced cholesterol, triglyceride, LDL

serum and increased HDL. Hopefully, it will produce a healthy egg product. Therefore, the aim of this study was to evaluate the effect of the addition of binahong leaf powder to rations on the contents of cholesterol, triglyceride, HDL, LDL, and protein in quail eggs.

Materials and Methods

Ethical approval

The procedure of using quail in this study has been approved by the Animal Ethics Committee in the Faculty of Animal Sciences, Diponegoro University, Semarang, Indonesia.

Animals

The study used two hundred 7-week-old female quails with an average body weight of 123.77±0.72 g. The quails were housed in 20 wire cages. Each cage was 90 × 35 × 25 cm and housed 10 quails. The feed consisted of yellow corn, rice bran, pollard, poultry meat meal, soybean meal, $CaCO_3$, salt, premix, and binahong leaf powder.

Experimental design

This study was arranged according to a completely randomized design with four treatments and five repetitions (10 quails per group per repetition). The treatment groups included T0, control ration (without binahong leaf powder); T1, control ration + 2% binahong leaf powder; T2, control ration + 4% binahong leaf powder; and T3, control ration + 6% binahong leaf powder in ration. The composition and nutrient content of the ration are presented in Tables-1 and 2.

Tests and procedures

The recording of egg production was carried out every day during the study and the formula: Production = (number of eggs/number of chickens) × 100% was used. Egg weight and egg characteristics were measured every 3 consecutive days weekly. The cholesterol, triglyceride, HDL, LDL, and protein contents in the egg yolk were collected by sampling one egg per experimental unit at the end of the study. The analyses of cholesterol and triglyceride contents were conducted based on the cholesterol p-aminophenazone method [13], HDL and LDL analysis was based on the enzymatic colorimetric method [13], and protein analysis was based on the Kjeldahl method [14].

Table-1: Feed composition of the control ration.

Feed ingredients	%
Yellow corn	48.00
Rice bran	6.00
Pollard	16.00
Poultry meat meal	14.00
Soybean meal	10.00
$CaCO_3$	5.50
Salt	0.25
Premix	0.25
Total	100.00

Table-2: Nutrition content of the ration.

Nutrient contents	Addition of binahong leaf powder			
	T0 (0%)	T1 (2%)	T2 (4%)	T3 (6%)
Energy metabolic (kkal/kg)	2763.20	2694.12	2628.26	2565.40
Protein (%)	19.77	19.35	18.95	18.56
Fat (%)	4.66	4.58	4.51	4.43
Crude fiber (%)	4.29	4.95	5.56	6.12
Ca (%)	3.15	3.06	2.97	2.88
P (%)	0.79	0.76	0.74	0.71
Lysine (%)	0.96	0.93	0.89	0.86
Methionine (%)	0.48	0.46	0.44	0.42

Statistical analysis

Data were analyzed as a completely randomized design using one-way analysis of variance and Duncan's Multiple Range Test.

Results and Discussion

Effect of the addition of binahong leaf powder on egg production

The addition of 2-6% of binahong leaf powder, rich in flavonoids and saponins, did not significantly influence quail egg production at 8-14 weeks of age (Table-3). This result was similar to that reported by Kusumanti and Murwani [15], which showed that supplementation of binahong leaf powder does not significantly affect the egg production of laying hens. In line with this, Iskender *et al*. [16] showed that flavonoids have no significant effect on egg production. In contrast, supplementation with the quercetin flavonoid [17] and saponins derived from karaya increases the egg production of laying hens [18]. These inconsistencies may be caused by the differences in the levels of flavonoids and saponins used in the study, as well as the conditions of the study.

Effect of binahong leaf powder on egg characteristics

The addition of binahong leaf powder did not significantly affect the characteristics of the egg, including egg weight, yolk weight, albumen weight, and eggshell weight (Table-4). It has been hypothesized that the bioactive component of binahong leaf may positively affect the egg characteristics. However, this study revealed different results. According to Leke *et al*. [19], flavonoids of papaya seeds increase egg quality (egg yolk, albumen, and eggshell weight) of Indonesian hens. Moreover, Afrose *et al*. [18] reported that supplementation with 25, 50, and 75 mg/kg of karaya saponin increases egg weight, yolk weight, and albumen weight of laying hens, whereas Ayasan *et al*. [20] reported that supplementation of *Yucca schidigera* powder, with 120 ppm of saponin, increases egg weight but does not affect the eggshell weight. As in our study, one study showed that supplementation with 0.2-0.6 g/kg of flavonoid does not significantly influence egg quality [17]. In addition, supplementation with flavonoids does not significantly influence eggshell weight, as reported by Iskender *et al*. [16]. The differences in the nature and levels of bioactive compounds, the nutritional values of rations, and the conditions of study may be responsible for these divergent results.

Table-3: Egg production on addition of binahong leaf powder in quail rations.

Age (weeks)	Addition of binahong leaf powder				p-value
	T0 (0%)	T1 (2%)	T2 (4%)	T3 (6%)	
8	29.71±2.74	28.00±2.166	28.85±2.74	28.57±2.67	0.49
9	39.71±2.34	42.28±1.91	41.42±2.67	41.14±2.55	0.53
10	45.14±4.58	46.09±1.47	47.23±2.34	45.14±3.08	0.57
11	46.64±2.42	49.49±1.38	48.73±3.83	47.63±1.73	0.59
12	56.38±2.49	52.34±1.86	54.50±1.37	54.57±1.76	0.67
14	56.17±2.47	57.55±1.45	57.73±1.85	58.74±1.93	0.69

Table-4: Influence of addition of binahong leaf powder on quail eggs characteristic.

Parameters	Addition of binahong leaf powder				p-value
	T0 (0%)	T1 (2%)	T2(4%)	T3 (6%)	
Egg weight (g)	9.99±0.29	10.01±0.11	10.03±0.11	10.03±0.27	0.38
Yolk weight (g)	3.65±0.13	3.92±0.25	3.68±0.12	3.93±0.17	0.57
Albumen weight (g)	4.68±0.29	4.53±0.18	4.47±0.25	4.60±0.19	0.53
Eggshell weight (g)	1.14±0.28	1.12±0.15	1.14±0.17	1.01±0.10	0.32
Yolk weight (%)	38.77±2.34	41.80±2.72	40.01±1.39	41.13±0.79	0.51
Albumen weight (%)	49.63±2.78	48.27±2.07	48.56±2.70	48.13±1.58	0.73
Eggshell weight (%)	11.43±2.73	11.07±1.60	11.41±1.73	10.11±1.14	0.12

Cholesterol, triglyceride, HDL, LDL, and protein contents in yolk

The data on cholesterol, triglyceride, HDL, LDL, and protein contents in yolk after binahong leaf powder supplementation are shown in Table-5. The addition of 2% of binahong leaf powder decreased the level of cholesterol in the yolk of quail eggs ($p<0.05$). Flavonoids and saponins in the binahong leaf powder may inhibit cholesterol absorption; hence, cholesterol deposition may be reduced [21-23]. This is supported by Lien *et al*. [24] who showed that the consumption of flavonoids increases the excretion of cholesterol. In addition, flavonoids may increase reverse cholesterol transport (RCT), resulting in high cholesterol excretion; thus, there will be a lower cholesterol level in yolk. However, 4 and 6% binahong leaf powder did not affect the cholesterol level.

The addition of binahong leaf powder decreased the triglyceride content in quail yolk (Table-5). As shown in our study, supplementation with a flavonoid extracted from the root of *Scutellaria baicalensis* Georgi decreases triglyceride content in the serum of broiler [25]. Likewise, Kamboh and Zhu [21] and Ouyang *et al*. [10] reported that the consumption of flavonoids decreases the level of triglyceride in the serum and meat of broiler. According to Hsu and Yen [26] and Nagai *et al*. [27], flavonoids may inhibit intracellular triglyceride synthesis in the liver, which results in lower triglyceride deposition in quail egg yolk. In addition, flavonoid content in binahong leaf powder may increase the expression of peroxisome proliferator-activated receptor α in the liver, which is involved in lipid metabolism, especially fatty acid oxidation [10,28].

The content of yolk HDL increased with the increased concentration of binahong leaf powder in the quail rations ($p<0.05$). Similar to the result of our study, Afrose *et al*. [29] documented that saponins increase HDL content in broiler meat. Supplementation with karaya saponin also increases HDL content in quail egg [30]. Furthermore, Smith *et al*. [31] stated that supplementation with saponin extract increases the HDL content in the blood, liver, kidneys, and heart tissues of white mice. Kamboh and Zhu [21] reported that flavonoids increase the HDL content in serum and HDL deposition in the breast meat of broiler. The mechanism by which binahong leaf increased HDL concentration in quail egg yolk is not known; however, the role of binahong leaf powder in the RCT mechanism seems to be attributable to the increased HDL content in quail yolk. Millar *et al*. [23] and Marques *et al*. [32] reported that the increase in the RCT mechanism due to binahong leaf powder supplementation is accompanied by an increase in the level of HDL. Thus, this mechanism seems to be related to the contribution of HDL on RCT.

Treatment with binahong leaf powder decreased the level of LDL in the quail egg yolk (Table-5). Afrose *et al*. [30] reported that saponins decrease the LDL content of quail egg. Likewise, Chaudhary *et al*. [33] stated that saponins potentially decrease the LDL content of meat. In addition, supplementation with saponin extract decreases LDL content in the blood, liver, kidneys, and heart tissues of white mice [31]. Flavonoids have been reported to decrease the LDL level in the blood of broiler [10,21,25]. Furthermore, Zhou *et al*. [25] stated that flavonoid supplementation also decreases the LDL content of the breast meat of broiler. Binahong leaf extract contains saponins, which bind bile acid and form large mixed micelles. Cholesterol in the micelles cannot be absorbed by microvilli on the surface of intestinal epithelial cells, which causes a decrease in total and LDL cholesterol levels [34]. Saponins inhibit fat metabolism through

Table-5: Influence of the addition of binahong leaf powder on cholesterol, triglyceride, HDL, LDL, and protein of quail egg yolk.

Parameters	Addition of binahong leaf powder				p-value
	T0 (0%)	T1 (2%)	T2 (4%)	T3 (6%)	
Yolk cholesterol (mg/g)	56.15±5.04[a]	23.77±3.75[b]	51.97±4.63[a]	51.35±7.55[a]	<0.01
Yolk triglyceride (mg/g)	903.22±58.21[a]	608.62±22.46[c]	778.34±53.59[b]	811.63±104.01[b]	<0.01
Yolk HDL (mg/g)	36.86±1.32[b]	34.8b±1.58[b]	48.62±1.67[a]	47.52±1.58[a]	<0.01
Yolk LDL (mg/g)	29.18 ±1.59 [a]	16.06b±0.33[b]	17.09±0.37[b]	16.28±0.36[b]	<0.01
Protein of yolk (%)	30.16±2.78	30.37±.82	29.15±0.81	30.19±2.55	0.00

Mean within a row for each parameter with different superscripts are significantly different (p<0.01)

the inhibition of lipase secretion and decrease cholesterol and LDL [35], but increase HDL [36], contents in the blood. Flavonoids are active compounds that reduce the activity of fatty acid synthase and reduce the level of LDL in animal tissues [10].

The addition of 2-6% binahong leaf powder did not significantly influence the protein content of egg yolk. This contrasted with a previous study. Iskender *et al*. [16] reported that supplementation with flavonoids (hesperidin, naringin, and quercetin) increases egg protein. Ahmad *et al*. [37] documented that bioactive compounds from *Moringa oleifera* leaf extracts increase the protein content in yolk.

In general, the protein content in an egg is derived from the feed. In this study, the inclusion of binahong leaf powder was accompanied by the decreased crude protein in the ration. Hence, the protein-increasing effect by binahong leaf powder seemed to be inhibited by lower protein content in the quail ration due to the binahong leaf inclusion.

Conclusion

The addition of 2% binahong leaf powder is best for lowering cholesterol, triglyceride, and LDL levels in quail egg.

Authors' Contributions

SK conducted the research, data collection, and drafting of the article. HIW developed the feeding concept and supervised the research, RM advised the experimental design, DS conducted data analysis, and SS performed laboratory analysis. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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RESEARCH ARTICLE
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Addition of binahong (*Anredera cordifolia*) leaf powder to diets to produce eggs with low cholesterol

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Abstract

Aim: The aim of this study was to evaluate the effect of the addition of binahong leaf powder to quail rations on the production and quality of eggs.

Materials and Methods: The study involved the use of two hundred 7-week-old quails housed evenly in 20 wire cages with a body weight of 123.77±0.72 g. The quails were treated as follows: Ration without binahong leaf powder (T0), addition 2% of binahong leaf powder (T1), addition 4% of binahong leaf powder (T2), and addition 6% of binahong leaf powder (T3). The study used a completely randomized design. The parameters measured were the production, weight, and characteristics of the eggs, as well as the cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and egg protein content in the yolk.

Results: The addition of 2-6% binahong powder did not significantly affect egg production, egg characteristics, or egg protein content, but significantly ($p<0.05$) affected the cholesterol, triglyceride, HDL, and LDL contents in yolk. The cholesterol, triglyceride, and LDL contents decreased significantly in T1, whereas HDL increased significantly in T2 and T3.

Conclusion: The addition of 2% binahong was enough to obtain healthy quail eggs with low levels of cholesterol, triglyceride, and LDL.

Keywords: binahong, cholesterol, high-density lipoprotein, low-density lipoprotein, triglyceride.

Introduction

Quail egg contains many high-quality nutrients but also high cholesterol [1]. The protein content in a quail egg is approximately 13.30% [1]. The cholesterol content in a quail egg is higher than in a chicken egg. The cholesterol content in the yolk of a quail egg is 6.79 mg/dl, whereas the cholesterol content in chicken egg yolk is 4.03 mg/dl [2]. Overconsumption of cholesterol increases the blood cholesterol level, which leads to heart disease; thus, some people are afraid of consuming quail egg. Catapano and Wiklund [3] stated that blood cholesterol, particularly low-density lipoprotein (LDL) cholesterol, has a positive correlation with the occurrence of atherosclerosis. The consumption of high-density lipoprotein (HDL) derived from quail egg increases the blood serum HDL level and decreases atherosclerotic plaques in rabbits. Thus, egg HDL may be used as an anti-atherosclerotic agent for patients with cardiovascular disease [4]. An attempt to obtain quail egg with low cholesterol and high HDL contents needs to be carried out.

Binahong (*Anredera cordifolia*) is a wild plant with a rapid growth rate that requires no complicated cultivation; thus, binahong is abundant [5]. Sutrisno *et al*. [5] and Leliqia *et al*. [6] revealed that the leaf of binahong contains bioactive compounds, such as flavonoids, tannins, saponins, phenols, and steroids, and Astuti *et al*. [7] reported that the leaf also contains terpenoid, which may potentially increase pancreatic insulin secretion. Furthermore, Hasbullah [8] documented that binahong leaf exhibits hypolipidemic properties. Kamboh *et al*. [9] showed that supplementation with a bioflavonoid increases antioxidant and enzyme activities and decreases total cholesterol and triglyceride levels in the serum and breast meat of broiler. A study by Ouyang *et al*. [10] showed that supplementation with 15 mg/kg of alfalfa flavonoid increases HDL levels and decreases the levels of cholesterol, triglyceride, and serum LDL and the percentage of abdominal fat of broiler. Feeding saponin decreases cholesterol, insulin, and blood triglyceride and increases blood HDL synthesis. Saponin also decreases the contents of cholesterol [11] and protein digestibility [12] of broiler meat.

Based on the facts that binahong contain flavonoids, tannins, saponins, phenols, and steroids and it has potentially reduced cholesterol, triglyceride, LDL

serum and increased HDL. Hopefully, it will produce a healthy egg product. Therefore, the aim of this study was to evaluate the effect of the addition of binahong leaf powder to rations on the contents of cholesterol, triglyceride, HDL, LDL, and protein in quail eggs.

Materials and Methods

Ethical approval

The procedure of using quail in this study has been approved by the Animal Ethics Committee in the Faculty of Animal Sciences, Diponegoro University, Semarang, Indonesia.

Animals

The study used two hundred 7-week-old female quails with an average body weight of 123.77±0.72 g. The quails were housed in 20 wire cages. Each cage was 90 × 35 × 25 cm and housed 10 quails. The feed consisted of yellow corn, rice bran, pollard, poultry meat meal, soybean meal, $CaCO_3$, salt, premix, and binahong leaf powder.

Experimental design

This study was arranged according to a completely randomized design with four treatments and five repetitions (10 quails per group per repetition). The treatment groups included T0, control ration (without binahong leaf powder); T1, control ration + 2% binahong leaf powder; T2, control ration + 4% binahong leaf powder; and T3, control ration + 6% binahong leaf powder in ration. The composition and nutrient content of the ration are presented in Tables-1 and 2.

Tests and procedures

The recording of egg production was carried out every day during the study and the formula: Production = (number of eggs/number of chickens) × 100% was used. Egg weight and egg characteristics were measured every 3 consecutive days weekly. The cholesterol, triglyceride, HDL, LDL, and protein contents in the egg yolk were collected by sampling one egg per experimental unit at the end of the study. The analyses of cholesterol and triglyceride contents were conducted based on the cholesterol p-aminophenazone method [13], HDL and LDL analysis was based on the enzymatic colorimetric method [13], and protein analysis was based on the Kjeldahl method [14].

Table-1: Feed composition of the control ration.

Feed ingredients	%
Yellow corn	48.00
Rice bran	6.00
Pollard	16.00
Poultry meat meal	14.00
Soybean meal	10.00
$CaCO_3$	5.50
Salt	0.25
Premix	0.25
Total	100.00

Table-2: Nutrition content of the ration.

Nutrient contents	Addition of binahong leaf powder			
	T0 (0%)	T1 (2%)	T2 (4%)	T3 (6%)
Energy metabolic (kkal/kg)	2763.20	2694.12	2628.26	2565.40
Protein (%)	19.77	19.35	18.95	18.56
Fat (%)	4.66	4.58	4.51	4.43
Crude fiber (%)	4.29	4.95	5.56	6.12
Ca (%)	3.15	3.06	2.97	2.88
P (%)	0.79	0.76	0.74	0.71
Lysine (%)	0.96	0.93	0.89	0.86
Methionine (%)	0.48	0.46	0.44	0.42

Statistical analysis

Data were analyzed as a completely randomized design using one-way analysis of variance and Duncan's Multiple Range Test.

Results and Discussion

Effect of the addition of binahong leaf powder on egg production

The addition of 2-6% of binahong leaf powder, rich in flavonoids and saponins, did not significantly influence quail egg production at 8-14 weeks of age (Table-3). This result was similar to that reported by Kusumanti and Murwani [15], which showed that supplementation of binahong leaf powder does not significantly affect the egg production of laying hens. In line with this, Iskender *et al*. [16] showed that flavonoids have no significant effect on egg production. In contrast, supplementation with the quercetin flavonoid [17] and saponins derived from karaya increases the egg production of laying hens [18]. These inconsistencies may be caused by the differences in the levels of flavonoids and saponins used in the study, as well as the conditions of the study.

Effect of binahong leaf powder on egg characteristics

The addition of binahong leaf powder did not significantly affect the characteristics of the egg, including egg weight, yolk weight, albumen weight, and eggshell weight (Table-4). It has been hypothesized that the bioactive component of binahong leaf may positively affect the egg characteristics. However, this study revealed different results. According to Leke *et al*. [19], flavonoids of papaya seeds increase egg quality (egg yolk, albumen, and eggshell weight) of Indonesian hens. Moreover, Afrose *et al*. [18] reported that supplementation with 25, 50, and 75 mg/kg of karaya saponin increases egg weight, yolk weight, and albumen weight of laying hens, whereas Ayasan *et al*. [20] reported that supplementation of *Yucca schidigera* powder, with 120 ppm of saponin, increases egg weight but does not affect the eggshell weight. As in our study, one study showed that supplementation with 0.2-0.6 g/kg of flavonoid does not significantly influence egg quality [17]. In addition, supplementation with flavonoids does not significantly influence eggshell weight, as reported by Iskender *et al*. [16]. The differences in the nature and levels of bioactive compounds, the nutritional values of rations, and the conditions of study may be responsible for these divergent results.

Table-3: Egg production on addition of binahong leaf powder in quail rations.

Age (weeks)	Addition of binahong leaf powder				p-value
	T0 (0%)	T1 (2%)	T2 (4%)	T3 (6%)	
8	29.71±2.74	28.00±2.166	28.85±2.74	28.57±2.67	0.49
9	39.71±2.34	42.28±1.91	41.42±2.67	41.14±2.55	0.53
10	45.14±4.58	46.09±1.47	47.23±2.34	45.14±3.08	0.57
11	46.64±2.42	49.49±1.38	48.73±3.83	47.63±1.73	0.59
12	56.38±2.49	52.34±1.86	54.50±1.37	54.57±1.76	0.67
14	56.17±2.47	57.55±1.45	57.73±1.85	58.74±1.93	0.69

Table-4: Influence of addition of binahong leaf powder on quail eggs characteristic.

Parameters	Addition of binahong leaf powder				p-value
	T0 (0%)	T1 (2%)	T2(4%)	T3 (6%)	
Egg weight (g)	9.99±0.29	10.01±0.11	10.03±0.11	10.03±0.27	0.38
Yolk weight (g)	3.65±0.13	3.92±0.25	3.68±0.12	3.93±0.17	0.57
Albumen weight (g)	4.68±0.29	4.53±0.18	4.47±0.25	4.60±0.19	0.53
Eggshell weight (g)	1.14±0.28	1.12±0.15	1.14±0.17	1.01±0.10	0.32
Yolk weight (%)	38.77±2.34	41.80±2.72	40.01±1.39	41.13±0.79	0.51
Albumen weight (%)	49.63±2.78	48.27±2.07	48.56±2.70	48.13±1.58	0.73
Eggshell weight (%)	11.43±2.73	11.07±1.60	11.41±1.73	10.11±1.14	0.12

Cholesterol, triglyceride, HDL, LDL, and protein contents in yolk

The data on cholesterol, triglyceride, HDL, LDL, and protein contents in yolk after binahong leaf powder supplementation are shown in Table-5. The addition of 2% of binahong leaf powder decreased the level of cholesterol in the yolk of quail eggs ($p<0.05$). Flavonoids and saponins in the binahong leaf powder may inhibit cholesterol absorption; hence, cholesterol deposition may be reduced [21-23]. This is supported by Lien *et al*. [24] who showed that the consumption of flavonoids increases the excretion of cholesterol. In addition, flavonoids may increase reverse cholesterol transport (RCT), resulting in high cholesterol excretion; thus, there will be a lower cholesterol level in yolk. However, 4 and 6% binahong leaf powder did not affect the cholesterol level.

The addition of binahong leaf powder decreased the triglyceride content in quail yolk (Table-5). As shown in our study, supplementation with a flavonoid extracted from the root of *Scutellaria baicalensis* Georgi decreases triglyceride content in the serum of broiler [25]. Likewise, Kamboh and Zhu [21] and Ouyang *et al*. [10] reported that the consumption of flavonoids decreases the level of triglyceride in the serum and meat of broiler. According to Hsu and Yen [26] and Nagai *et al*. [27], flavonoids may inhibit intracellular triglyceride synthesis in the liver, which results in lower triglyceride deposition in quail egg yolk. In addition, flavonoid content in binahong leaf powder may increase the expression of peroxisome proliferator-activated receptor α in the liver, which is involved in lipid metabolism, especially fatty acid oxidation [10,28].

The content of yolk HDL increased with the increased concentration of binahong leaf powder in the quail rations ($p<0.05$). Similar to the result of our study, Afrose *et al*. [29] documented that saponins increase HDL content in broiler meat. Supplementation with karaya saponin also increases HDL content in quail egg [30]. Furthermore, Smith *et al*. [31] stated that supplementation with saponin extract increases the HDL content in the blood, liver, kidneys, and heart tissues of white mice. Kamboh and Zhu [21] reported that flavonoids increase the HDL content in serum and HDL deposition in the breast meat of broiler. The mechanism by which binahong leaf increased HDL concentration in quail egg yolk is not known; however, the role of binahong leaf powder in the RCT mechanism seems to be attributable to the increased HDL content in quail yolk. Millar *et al*. [23] and Marques *et al*. [32] reported that the increase in the RCT mechanism due to binahong leaf powder supplementation is accompanied by an increase in the level of HDL. Thus, this mechanism seems to be related to the contribution of HDL on RCT.

Treatment with binahong leaf powder decreased the level of LDL in the quail egg yolk (Table-5). Afrose *et al*. [30] reported that saponins decrease the LDL content of quail egg. Likewise, Chaudhary *et al*. [33] stated that saponins potentially decrease the LDL content of meat. In addition, supplementation with saponin extract decreases LDL content in the blood, liver, kidneys, and heart tissues of white mice [31]. Flavonoids have been reported to decrease the LDL level in the blood of broiler [10,21,25]. Furthermore, Zhou *et al*. [25] stated that flavonoid supplementation also decreases the LDL content of the breast meat of broiler. Binahong leaf extract contains saponins, which bind bile acid and form large mixed micelles. Cholesterol in the micelles cannot be absorbed by microvilli on the surface of intestinal epithelial cells, which causes a decrease in total and LDL cholesterol levels [34]. Saponins inhibit fat metabolism through

Table-5: Influence of the addition of binahong leaf powder on cholesterol, triglyceride, HDL, LDL, and protein of quail egg yolk.

Parameters	Addition of binahong leaf powder				p-value
	T0 (0%)	T1 (2%)	T2 (4%)	T3 (6%)	
Yolk cholesterol (mg/g)	56.15±5.04[a]	23.77±3.75[b]	51.97±4.63[a]	51.35±7.55[a]	<0.01
Yolk triglyceride (mg/g)	903.22±58.21[a]	608.62±22.46[c]	778.34±53.59[b]	811.63±104.01[b]	<0.01
Yolk HDL (mg/g)	36.86±1.32[b]	34.8b±1.58[b]	48.62±1.67[a]	47.52±1.58[a]	<0.01
Yolk LDL (mg/g)	29.18 ±1.59[a]	16.06b±0.33[b]	17.09±0.37[b]	16.28±0.36[b]	<0.01
Protein of yolk (%)	30.16±2.78	30.37±.82	29.15±0.81	30.19±2.55	0.00

Mean within a row for each parameter with different superscripts are significantly different (p<0.01)

the inhibition of lipase secretion and decrease cholesterol and LDL [35], but increase HDL [36], contents in the blood. Flavonoids are active compounds that reduce the activity of fatty acid synthase and reduce the level of LDL in animal tissues [10].

The addition of 2-6% binahong leaf powder did not significantly influence the protein content of egg yolk. This contrasted with a previous study. Iskender *et al*. [16] reported that supplementation with flavonoids (hesperidin, naringin, and quercetin) increases egg protein. Ahmad *et al*. [37] documented that bioactive compounds from *Moringa oleifera* leaf extracts increase the protein content in yolk.

In general, the protein content in an egg is derived from the feed. In this study, the inclusion of binahong leaf powder was accompanied by the decreased crude protein in the ration. Hence, the protein-increasing effect by binahong leaf powder seemed to be inhibited by lower protein content in the quail ration due to the binahong leaf inclusion.

Conclusion

The addition of 2% binahong leaf powder is best for lowering cholesterol, triglyceride, and LDL levels in quail egg.

Authors' Contributions

SK conducted the research, data collection, and drafting of the article. HIW developed the feeding concept and supervised the research, RM advised the experimental design, DS conducted data analysis, and SS performed laboratory analysis. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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RESEARCH ARTICLE
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Addition of binahong (*Anredera cordifolia*) leaf powder to diets to produce eggs with low cholesterol

Sri Kismiati, Hanny Indrat Wahyuni, Rina Muryani, Dwi Sunarti and Sri Sumarsih

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Abstract

Aim: The aim of this study was to evaluate the effect of the addition of binahong leaf powder to quail rations on the production and quality of eggs.

Materials and Methods: The study involved the use of two hundred 7-week-old quails housed evenly in 20 wire cages with a body weight of 123.77±0.72 g. The quails were treated as follows: Ration without binahong leaf powder (T0), addition 2% of binahong leaf powder (T1), addition 4% of binahong leaf powder (T2), and addition 6% of binahong leaf powder (T3). The study used a completely randomized design. The parameters measured were the production, weight, and characteristics of the eggs, as well as the cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and egg protein content in the yolk.

Results: The addition of 2-6% binahong powder did not significantly affect egg production, egg characteristics, or egg protein content, but significantly ($p<0.05$) affected the cholesterol, triglyceride, HDL, and LDL contents in yolk. The cholesterol, triglyceride, and LDL contents decreased significantly in T1, whereas HDL increased significantly in T2 and T3.

Conclusion: The addition of 2% binahong was enough to obtain healthy quail eggs with low levels of cholesterol, triglyceride, and LDL.

Keywords: binahong, cholesterol, high-density lipoprotein, low-density lipoprotein, triglyceride.

Introduction

Quail egg contains many high-quality nutrients but also high cholesterol [1]. The protein content in a quail egg is approximately 13.30% [1]. The cholesterol content in a quail egg is higher than in a chicken egg. The cholesterol content in the yolk of a quail egg is 6.79 mg/dl, whereas the cholesterol content in chicken egg yolk is 4.03 mg/dl [2]. Overconsumption of cholesterol increases the blood cholesterol level, which leads to heart disease; thus, some people are afraid of consuming quail egg. Catapano and Wiklund [3] stated that blood cholesterol, particularly low-density lipoprotein (LDL) cholesterol, has a positive correlation with the occurrence of atherosclerosis. The consumption of high-density lipoprotein (HDL) derived from quail egg increases the blood serum HDL level and decreases atherosclerotic plaques in rabbits. Thus, egg HDL may be used as an anti-atherosclerotic agent for patients with cardiovascular disease [4]. An attempt to obtain quail egg with low cholesterol and high HDL contents needs to be carried out.

Binahong (*Anredera cordifolia*) is a wild plant with a rapid growth rate that requires no complicated cultivation; thus, binahong is abundant [5]. Sutrisno *et al*. [5] and Leliqia *et al*. [6] revealed that the leaf of binahong contains bioactive compounds, such as flavonoids, tannins, saponins, phenols, and steroids, and Astuti *et al*. [7] reported that the leaf also contains terpenoid, which may potentially increase pancreatic insulin secretion. Furthermore, Hasbullah [8] documented that binahong leaf exhibits hypolipidemic properties. Kamboh *et al*. [9] showed that supplementation with a bioflavonoid increases antioxidant and enzyme activities and decreases total cholesterol and triglyceride levels in the serum and breast meat of broiler. A study by Ouyang *et al*. [10] showed that supplementation with 15 mg/kg of alfalfa flavonoid increases HDL levels and decreases the levels of cholesterol, triglyceride, and serum LDL and the percentage of abdominal fat of broiler. Feeding saponin decreases cholesterol, insulin, and blood triglyceride and increases blood HDL synthesis. Saponin also decreases the contents of cholesterol [11] and protein digestibility [12] of broiler meat.

Based on the facts that binahong contain flavonoids, tannins, saponins, phenols, and steroids and it has potentially reduced cholesterol, triglyceride, LDL

serum and increased HDL. Hopefully, it will produce a healthy egg product. Therefore, the aim of this study was to evaluate the effect of the addition of binahong leaf powder to rations on the contents of cholesterol, triglyceride, HDL, LDL, and protein in quail eggs.

Materials and Methods

Ethical approval

The procedure of using quail in this study has been approved by the Animal Ethics Committee in the Faculty of Animal Sciences, Diponegoro University, Semarang, Indonesia.

Animals

The study used two hundred 7-week-old female quails with an average body weight of 123.77±0.72 g. The quails were housed in 20 wire cages. Each cage was 90 × 35 × 25 cm and housed 10 quails. The feed consisted of yellow corn, rice bran, pollard, poultry meat meal, soybean meal, $CaCO_3$, salt, premix, and binahong leaf powder.

Experimental design

This study was arranged according to a completely randomized design with four treatments and five repetitions (10 quails per group per repetition). The treatment groups included T0, control ration (without binahong leaf powder); T1, control ration + 2% binahong leaf powder; T2, control ration + 4% binahong leaf powder; and T3, control ration + 6% binahong leaf powder in ration. The composition and nutrient content of the ration are presented in Tables-1 and 2.

Tests and procedures

The recording of egg production was carried out every day during the study and the formula: Production = (number of eggs/number of chickens) × 100% was used. Egg weight and egg characteristics were measured every 3 consecutive days weekly. The cholesterol, triglyceride, HDL, LDL, and protein contents in the egg yolk were collected by sampling one egg per experimental unit at the end of the study. The analyses of cholesterol and triglyceride contents were conducted based on the cholesterol p-aminophenazone method [13], HDL and LDL analysis was based on the enzymatic colorimetric method [13], and protein analysis was based on the Kjeldahl method [14].

Table-1: Feed composition of the control ration.

Feed ingredients	%
Yellow corn	48.00
Rice bran	6.00
Pollard	16.00
Poultry meat meal	14.00
Soybean meal	10.00
$CaCO_3$	5.50
Salt	0.25
Premix	0.25
Total	100.00

Table-2: Nutrition content of the ration.

Nutrient contents	Addition of binahong leaf powder			
	T0 (0%)	T1 (2%)	T2 (4%)	T3 (6%)
Energy metabolic (kkal/kg)	2763.20	2694.12	2628.26	2565.40
Protein (%)	19.77	19.35	18.95	18.56
Fat (%)	4.66	4.58	4.51	4.43
Crude fiber (%)	4.29	4.95	5.56	6.12
Ca (%)	3.15	3.06	2.97	2.88
P (%)	0.79	0.76	0.74	0.71
Lysine (%)	0.96	0.93	0.89	0.86
Methionine (%)	0.48	0.46	0.44	0.42

Statistical analysis

Data were analyzed as a completely randomized design using one-way analysis of variance and Duncan's Multiple Range Test.

Results and Discussion

Effect of the addition of binahong leaf powder on egg production

The addition of 2-6% of binahong leaf powder, rich in flavonoids and saponins, did not significantly influence quail egg production at 8-14 weeks of age (Table-3). This result was similar to that reported by Kusumanti and Murwani [15], which showed that supplementation of binahong leaf powder does not significantly affect the egg production of laying hens. In line with this, Iskender *et al*. [16] showed that flavonoids have no significant effect on egg production. In contrast, supplementation with the quercetin flavonoid [17] and saponins derived from karaya increases the egg production of laying hens [18]. These inconsistencies may be caused by the differences in the levels of flavonoids and saponins used in the study, as well as the conditions of the study.

Effect of binahong leaf powder on egg characteristics

The addition of binahong leaf powder did not significantly affect the characteristics of the egg, including egg weight, yolk weight, albumen weight, and eggshell weight (Table-4). It has been hypothesized that the bioactive component of binahong leaf may positively affect the egg characteristics. However, this study revealed different results. According to Leke *et al*. [19], flavonoids of papaya seeds increase egg quality (egg yolk, albumen, and eggshell weight) of Indonesian hens. Moreover, Afrose *et al*. [18] reported that supplementation with 25, 50, and 75 mg/kg of karaya saponin increases egg weight, yolk weight, and albumen weight of laying hens, whereas Ayasan *et al*. [20] reported that supplementation of *Yucca schidigera* powder, with 120 ppm of saponin, increases egg weight but does not affect the eggshell weight. As in our study, one study showed that supplementation with 0.2-0.6 g/kg of flavonoid does not significantly influence egg quality [17]. In addition, supplementation with flavonoids does not significantly influence eggshell weight, as reported by Iskender *et al*. [16]. The differences in the nature and levels of bioactive compounds, the nutritional values of rations, and the conditions of study may be responsible for these divergent results.

Table-3: Egg production on addition of binahong leaf powder in quail rations.

Age (weeks)	Addition of binahong leaf powder				p-value
	T0 (0%)	T1 (2%)	T2 (4%)	T3 (6%)	
8	29.71±2.74	28.00±2.166	28.85±2.74	28.57±2.67	0.49
9	39.71±2.34	42.28±1.91	41.42±2.67	41.14±2.55	0.53
10	45.14±4.58	46.09±1.47	47.23±2.34	45.14±3.08	0.57
11	46.64±2.42	49.49±1.38	48.73±3.83	47.63±1.73	0.59
12	56.38±2.49	52.34±1.86	54.50±1.37	54.57±1.76	0.67
14	56.17±2.47	57.55±1.45	57.73±1.85	58.74±1.93	0.69

Table-4: Influence of addition of binahong leaf powder on quail eggs characteristic.

Parameters	Addition of binahong leaf powder				p-value
	T0 (0%)	T1 (2%)	T2(4%)	T3 (6%)	
Egg weight (g)	9.99±0.29	10.01±0.11	10.03±0.11	10.03±0.27	0.38
Yolk weight (g)	3.65±0.13	3.92±0.25	3.68±0.12	3.93±0.17	0.57
Albumen weight (g)	4.68±0.29	4.53±0.18	4.47±0.25	4.60±0.19	0.53
Eggshell weight (g)	1.14±0.28	1.12±0.15	1.14±0.17	1.01±0.10	0.32
Yolk weight (%)	38.77±2.34	41.80±2.72	40.01±1.39	41.13±0.79	0.51
Albumen weight (%)	49.63±2.78	48.27±2.07	48.56±2.70	48.13±1.58	0.73
Eggshell weight (%)	11.43±2.73	11.07±1.60	11.41±1.73	10.11±1.14	0.12

Cholesterol, triglyceride, HDL, LDL, and protein contents in yolk

The data on cholesterol, triglyceride, HDL, LDL, and protein contents in yolk after binahong leaf powder supplementation are shown in Table-5. The addition of 2% of binahong leaf powder decreased the level of cholesterol in the yolk of quail eggs ($p<0.05$). Flavonoids and saponins in the binahong leaf powder may inhibit cholesterol absorption; hence, cholesterol deposition may be reduced [21-23]. This is supported by Lien *et al*. [24] who showed that the consumption of flavonoids increases the excretion of cholesterol. In addition, flavonoids may increase reverse cholesterol transport (RCT), resulting in high cholesterol excretion; thus, there will be a lower cholesterol level in yolk. However, 4 and 6% binahong leaf powder did not affect the cholesterol level.

The addition of binahong leaf powder decreased the triglyceride content in quail yolk (Table-5). As shown in our study, supplementation with a flavonoid extracted from the root of *Scutellaria baicalensis* Georgi decreases triglyceride content in the serum of broiler [25]. Likewise, Kamboh and Zhu [21] and Ouyang *et al*. [10] reported that the consumption of flavonoids decreases the level of triglyceride in the serum and meat of broiler. According to Hsu and Yen [26] and Nagai *et al*. [27], flavonoids may inhibit intracellular triglyceride synthesis in the liver, which results in lower triglyceride deposition in quail egg yolk. In addition, flavonoid content in binahong leaf powder may increase the expression of peroxisome proliferator-activated receptor α in the liver, which is involved in lipid metabolism, especially fatty acid oxidation [10,28].

The content of yolk HDL increased with the increased concentration of binahong leaf powder in the quail rations ($p<0.05$). Similar to the result of our study, Afrose *et al*. [29] documented that saponins increase HDL content in broiler meat. Supplementation with karaya saponin also increases HDL content in quail egg [30]. Furthermore, Smith *et al*. [31] stated that supplementation with saponin extract increases the HDL content in the blood, liver, kidneys, and heart tissues of white mice. Kamboh and Zhu [21] reported that flavonoids increase the HDL content in serum and HDL deposition in the breast meat of broiler. The mechanism by which binahong leaf increased HDL concentration in quail egg yolk is not known; however, the role of binahong leaf powder in the RCT mechanism seems to be attributable to the increased HDL content in quail yolk. Millar *et al*. [23] and Marques *et al*. [32] reported that the increase in the RCT mechanism due to binahong leaf powder supplementation is accompanied by an increase in the level of HDL. Thus, this mechanism seems to be related to the contribution of HDL on RCT.

Treatment with binahong leaf powder decreased the level of LDL in the quail egg yolk (Table-5). Afrose *et al*. [30] reported that saponins decrease the LDL content of quail egg. Likewise, Chaudhary *et al*. [33] stated that saponins potentially decrease the LDL content of meat. In addition, supplementation with saponin extract decreases LDL content in the blood, liver, kidneys, and heart tissues of white mice [31]. Flavonoids have been reported to decrease the LDL level in the blood of broiler [10,21,25]. Furthermore, Zhou *et al*. [25] stated that flavonoid supplementation also decreases the LDL content of the breast meat of broiler. Binahong leaf extract contains saponins, which bind bile acid and form large mixed micelles. Cholesterol in the micelles cannot be absorbed by microvilli on the surface of intestinal epithelial cells, which causes a decrease in total and LDL cholesterol levels [34]. Saponins inhibit fat metabolism through

Table-5: Influence of the addition of binahong leaf powder on cholesterol, triglyceride, HDL, LDL, and protein of quail egg yolk.

Parameters	Addition of binahong leaf powder				p-value
	T0 (0%)	T1 (2%)	T2 (4%)	T3 (6%)	
Yolk cholesterol (mg/g)	56.15±5.04[a]	23.77±3.75[b]	51.97±4.63[a]	51.35±7.55[a]	<0.01
Yolk triglyceride (mg/g)	903.22±58.21[a]	608.62±22.46[c]	778.34±53.59[b]	811.63±104.01[b]	<0.01
Yolk HDL (mg/g)	36.86±1.32[b]	34.8b±1.58[b]	48.62±1.67[a]	47.52±1.58[a]	<0.01
Yolk LDL (mg/g)	29.18 ±1.59[a]	16.06b±0.33[b]	17.09±0.37[b]	16.28±0.36[b]	<0.01
Protein of yolk (%)	30.16±2.78	30.37±.82	29.15±0.81	30.19±2.55	0.00

Mean within a row for each parameter with different superscripts are significantly different (p<0.01)

the inhibition of lipase secretion and decrease cholesterol and LDL [35], but increase HDL [36], contents in the blood. Flavonoids are active compounds that reduce the activity of fatty acid synthase and reduce the level of LDL in animal tissues [10].

The addition of 2-6% binahong leaf powder did not significantly influence the protein content of egg yolk. This contrasted with a previous study. Iskender *et al*. [16] reported that supplementation with flavonoids (hesperidin, naringin, and quercetin) increases egg protein. Ahmad *et al*. [37] documented that bioactive compounds from *Moringa oleifera* leaf extracts increase the protein content in yolk.

In general, the protein content in an egg is derived from the feed. In this study, the inclusion of binahong leaf powder was accompanied by the decreased crude protein in the ration. Hence, the protein-increasing effect by binahong leaf powder seemed to be inhibited by lower protein content in the quail ration due to the binahong leaf inclusion.

Conclusion

The addition of 2% binahong leaf powder is best for lowering cholesterol, triglyceride, and LDL levels in quail egg.

Authors' Contributions

SK conducted the research, data collection, and drafting of the article. HIW developed the feeding concept and supervised the research, RM advised the experimental design, DS conducted data analysis, and SS performed laboratory analysis. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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Authors' Contributions

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"chickens" should be "quails".

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Addition of binahong (*Anredera cordifolia*) leaf powder to diets to produce eggs with low cholesterol

Sri Kismiati, Hanny Indrat Wahyuni, Rina Muryani, Dwi Sunarti and Sri Sumarsih

has been published online at http://www.veterinaryworld.org/Vol.13/March-2020/31.pdf on 31-03-2020.

Citation: Kismiati S, Wahyuni HI, Muryani R, Sunarti D, Sumarsih S (2020) Addition of binahong (Anredera cordifolia) leaf powder to diets to produce eggs with low cholesterol, Veterinary World, 13(3): 604-608.
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Corresponding author: Sri Kismiati, Department of Animal Science, Faculty of Animal and Agricultural Science, Diponegoro University, Tembalang Campus, Semarang, Central of Java, Indonesia.
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