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THE EFFECT OF CANDLENUT (*Aleurites moluccana* L Willd.) SEED FLOUR IN NATIVE CHICKEN FEEDING TOWARD THE INTERNAL EGG QUALITY AND CHOLESTEROL CONTENS

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ABSTRACT

The research aimed to examine effects of *Aleurites moluccana* (L.) Willd. (Candlenut) Seed Flour used in feed toward egg quality and cholesterol contents of egg-laying native chicken. The research method used was Completely Randomized Design and was continued with Duncan's test if significant different present. There were 5 treatments comprising of R0 = 100 % of Basal Diet (BD), R1 = 98.5% of Basal Diet (BD) + 1.5 % of Candlenut Seed Flour (CSF), R2 = 97 % of Basal Diet (BD) + 3% of CSF, R3 = 95.5% of Basal Diet (BD) + 4.5 % of CSF, R4 = 94% of Basal Diet (BD) + 6 % of CSF and replications, of which each replication consisted of 8 egg-laying native chickens. In addition, 50 eggs derived from egg-laying native chickens were taken weekly for quality analysis. Variables of this research were egg weight (g/egg), albumen (g/egg), yolk colour, yolk weight (g/egg), egg yolk cholesterol (mg/100g), blood cholesterol (ml/dl), blood LDL (ml/dl), blood HDL (ml/dl), and blood triglycerides (ml/dl), and egg shell's weight (g/egg) and thickness (mm). As a result, the effects of CSF up to 6 percent of feeding had significant result ($P<0.01$) to egg's weight, albumen, yolk colour, yolk weight, cholesterol, blood cholesterol, blood LDL, blood HDL and blood triglycerides, but not significant ($p>0.05$) on egg shell's weight and thickness. Therefore, the use of 6 % of Candlenut) Seed Flour in feed had positive effects to improve egg quality and cholesterol contents of egg-laying native chicken.

Keywords: Egg quality; candlenut seed flour; native chicken

INTRODUCTION

Native chickens are commonly raised by farmers in Indonesia. They could adapt tropical environment very well, more resistance to some diseases, and are usually kept in extensive system. However, currently the chickens have been selected and managed for breeding program in order to make higher egg productivity with higher egg quality (Leke *et al*., 2018). In fact, farmers have responded well toward production of more productive strain of native chickens. Moreover, native chicken farming has a significant role in supporting the local economy in rural communities. Due to being kept in scavenging system, it is believed that the chickens produce healthier eggs and meat. In addition, eggs are also recognized as functional food good for human health (Malik *et al.*, 2019). Recently, egg produced by native chicken has become the main choice for consumers having the risk of cholesterol and heart disease. However, the average egg production of native chicken is very low around 30–60 eggs/year. Beside genetic factors, the use of low nutritional quality of local feedstuffs contribute to low egg production

Aleurites moluccana (L.) Willd. (candlenut) is an importantly versatile plant in Indonesia and one of the local herbs from the Province of North Sulawesi. It is broken-white in colour and an elliptical shape with 4-5 cm of diameter. Biologically, candlenut is a plant having high oil content of 55 - 66% from its weight. The main component of candlenut oil is unsaturated fatty acids with few saturated fatty acids. The candlenut has been used for various purposes, such as ingredients for cooking and medical substances. Meanwhile, production of candlenut seed is intended for local and export consumption. Candlenut fruit is classified into indehiscent drupe fruit because of hard skin resembling a shell with a rough outer surface. The thickness of the seed shell is about 3-5 mm, and it has brown or blackish in colour. The firmness of candlenut is varied depending on its location produced. Generally, candlenut fruit is still traditionally managed and the removal of shell is mechanically cracked. This method is less effective and efficient because workers can only crack candlenuts for 9-10 kg of candlenuts/day, but easier and cheaper way to do. According to Leke *et al*., (2019), the addition of 4 % candlenut seeds in ration of native chickens can improve performance and production native chicken. It is recommended that further research use candlenut seed oil to reduce saponins in candlenut seeds. Xiaodong *et al*., (2018) said candlenut seeds have the highest antioxidant and phenolic compounds from other legume. Ketaren (2012) in his research using Candlenut Seed Flour (CSF) as a feed supplement (additional feed) showed higher gross energy and crude fat content than basal feed. Gross energy in CSF was 7690.57 Kcal/kg and basal feed was 4142.95 Kcal/kg. Crude fat content in candlenut flour was 64.92% while in basal feed was 6.37%. This CSF contains unsaturated fatty acids, namely Apha-Linolenic Acid (ALA) or Linoleic Acid (LA) or Oleic Acid (OC).

Based on its bioactive substances, candlenut seed contains of non-synthesizable fatty acids included alpha-linolenic acid (ALA), linoleic acid (LA), and oleic acid (OC) (Ketaren, 2012). Essentially, candlenut seed also contains active

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substances, such as saponin and flavonoid. Flavonoid functions as an antioxidant that can stabilize free radicals; while, saponin acts as antibacterial.

Those bioactive substances have a significant role in human and animal health. Substantially, micro-nutrient substances derived from candlenut seed are protein, fat, and carbohydrate. The most dominant minerals are kalium, phosphor, magnesium, and calcium. In contrast, zinc, copper and selenium are present in small concentrations. Other important substances are vitamins, folate, and phytosterol which have ability to damage cholesterol-forming enzymes in liver, so it can retard cholesterol synthesis.

Protein in candlenut seed consists of amino acid, either essential or non-essential, of which the essential amino acid is essential for growth. The effects of Candlenut Seed Flour (CSF) in feed on production performance of *Coturnix coturnix japonica* (quail) had been reported to be no significantly affected the first day of egg-laying and laying interval. However, the treatments significantly affected feed consumption, Hen Day Egg Production, and gave highly significant effect on the total of egg's follicle cells. The use of CSF up to 3 % resulted the best laying performance of quail (Koen *et al.*, 2018). While the price of broken candlenut seed is considerably cheap, the attempts to use it as feed additive for egg-laying native chicken is challenging. Therefore, this research was necessary to be conducted with the purpose to evaluate effects of CSF in feed toward laying performance and egg quality of native chicken.

MATERIALS AND METHODS

Materials

The research location was native chicken farm located in the District of Minahasa. This research utilized 100 laying hens of native chickens having body weight of 1240 $\pm$ 8,30 g on average.

The CSF was made by easily squeezing the seed into flour. Preliminary research was initiated when the native chickens were of 4.5 months old, the beginning period of laying egg. The experimental basal feed used commercial concentrate for layer produced by PT Comfeed Indonesia Ltd. The nutritional contents were then analysed by proximate method in the Laboratory of of Biochemical and Nutrition, Faculty of Animal Science Universitas Gadjah Mada. Chemically, the composition of CSF was 5383 of metabolic energy (Kcal/kg), 24.62 % of protein, 38.81 % of fat, and 1.74 % of crude fibre. The results of laboratory analysis are presented in following Table 1.

Table 1. Chemical composition dietary treatments

Treatments	Crude protein (%)	Fat	Crude Fiber (%)	Calsium (%)	Phospor (%)	Metabolizable Energy/ME (Kcal/kg)
P0	16.38	6.77	4.49	1.98	0.47	2803
P1	16.41	7.25	4.45	1.44	0.59	2841
P2	16.62	7.73	4.39	1.44	0.59	2888
P3	16.74	8.22	4.36	1.44	0.59	2919
P4	16.87	8.69	4.32	1.44	0.59	2986

ME : 70 % x Gross Energy

Feed and water were provided *ad libitum*. The chickens were kept in individual battery system. Duration of research was 8 weeks. Eggs were collected for measuring quality and so blood sample for serum profiles. Data collection consisted of egg quality, weight, albumen weight, yolk weight, and egg shell weight and thickness. Blood serum collected were subjected to analysis of total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglyceride.

Methods

The method was experiment using 5 treatment and 4 replications, 5 laying hens per unit. The treatment consisted of: R0 = 100 % of Basal Diet (BD), R1 = 98.5% of Basal Diet (BD) + 1.5 % of Candlenut Seed Flour (CSF), R2 = 97 % of Basal Diet (BD) + 3% of CSF, R3 = 95.5% of Basal Diet (BD) + 4.5 % of CSF, R4 = 94% of Basal Diet (BD) + 6 % of CSF.

The variables measured included egg weight, albumen weight, yolk weight, egg shell thickness, egg cholesterol, and blood serum profiles. The egg weight, albumen weight, and yolk weight were measured using a digital scale with 0.1 g of limit measurement.

Moreover, for the egg shell, it was cleaned from the rest of the albumen and, then weighed; yolk weight (g/egg) was measured by separating the yolk from albumen and subsequently weighed; and albumen weight (g/egg) was calculated by weighted egg minus by egg shell weight and yolk weigh. In this experiment, 50 eggs of native chicken were collected and given a specific code. Then, eggs were cracked down above glass surface. The measurement of egg yolk colour was by using Yolk Colour Fan, while egg thickness was assessed by using calliper with 0.05 mm limit measurement.

While serum and yolk cholesterol were analyzed using Liebermann Burchard (Kenny, 1952), and also being analyzed at Universitas Gadjah Mada, Yogyakarta. The procedure of analysis was firstly egg yolk was weighed to nearest 0.2 g, then added with 1 ml of alcohol KOH, and being stirred until sedimentation was formed. It was then idle in a water bath at a temperature of 39-40 °C for 1 hour. A total of 2 ml of ether petroleum by temperature of 40-60 °C was added, followed by 0.25 MI 1-120 shaken during 1 minute.

Using a standard pipet, each sample was injected 200 pl, added with boiling chips, and stored in a water bath at a temperature of 80 °C for 5 minutes. Later, it was dried in an oven at a temperature of 105-110 °C for 30 minutes. Thus, it was then cooled under room temperature, added with 4 ml solution of acetate anhydride sulphate acid, and shaken for 35 minutes. Subsequently, the result was read by using Spectrophotometer having wavelength of 630 nm and 0.5 nm of gap. Therefore, its calculation was as follows:

Cholesterol level = (YB) x engineering factor (mg/gr)/weight (gr) x 100

Where,
Y is a sample of absorbents, *a* is intercept, and *B* is slope

Blood cholesterol measurements of egg-laying native chicken consisted of total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglyceride. Initially, blood sample of egg-laying native chicken was taken for 2 ml, inserted into blood tube containing ethylene-diamine-tetra acetic-acid (EDTA) to avoid blood clothing, and stored in ice box for analysis process in the laboratory. The complete blood cholesterol standard procedure is by using photometer with cholesterol-oxidase-peroxidase-amino-antipyrine-phenol (CHOD-PAP) method having wave length of 546 and at temperature of 37°C. While measurement standard of leucocyte was by using haemocytometer method using Turk solution.

The blood sample was then inserted into a tube containing heparin and analysed in the Laboratory of Petra Manado, North Sulawesi.

Statistical Analysis

Statistically, data was analysed by using Completely Randomized Design analysis. Whereas the difference among treatments, was further tested by using Duncan’s Multiple Range Test.

RESULTS AND DISCUSSION

The Effect of Treatment on Egg Weight and Egg Albumen

The effect of treatment on egg weight, egg albumen showed at Table 2. The average egg weight was 39.92 - 44.95 grams/egg and egg albumen weight was 27.63 - 30.44 grams/egg. The results of statistical analysis showed that the adding of CSF had very significant effect on egg weight and egg white weight. The treatment of CSF up to 6% caused increasing egg weight and egg white weight. This could be due to the influence of feed consumption and the content of nutrients in feed and CSF.

It is evidence that the use of 6 % of csf in feed resulted better egg quality. Since substitution of basal feed with CSF up to 6% did not change nutritional contents of feed, and the principle of feeding egg-laying chicken is by restricted feeding, then flavonoid and or saponin should play a role in the results of heavier egg weight and albumen weights.

Iskender *et al.,* (2017), reported that laying hens fed flavonoid containing feed did not showed any changes in egg quality. However, Pradikdo *et al.*,(2019), reported that betel leaf extract containing flavonoid did not change the intestinal development, but alter microflora of intestine in favour of development of non-pathogenic microflora. So, it might be possible act as antimicrobial agent. The mechanism by which egg weight increase in this research is due to increasing level of saponin in feed. Xiadong *et al*., (2018) reported that candlenut seed contains antioxidant and polyphenol. Wang *et al.* (2018) said that polyphenol significantly improved rate of production, egg weight and feed conversion ratio.

Saponin, polyphenol and might be also flavonoid, acts as antimicrobial, and alter the microorganism types and population, hence in favour of increasing non-pathogenic microbes (Widodo *et al.,* 2019) which enable to change the development of intestinal villi leading to an increase in nutrient absorption.

Table 2. Egg quality of egg-laying native chicken

Treatments	Egg weight (g/egg)	Egg albumen (g/egg)	Egg yolk color	Egg yolk weight (g/egg)	Egg shell weight (g/egg)	Egg shell thickness (mm)	Egg Cholesterol (mg/100g)
P0	39.92±0.51[a]	27.63±1.52[a]	13.58±1.05[a]	32.84±0.55[b]	5.98±0.10	0.36±0.01	212±2.52[a]
P1	44.69±1.00[b]	30.14±1.06[b]	14.15±1.04[ab]	32.23±0.51[ab]	5.99±0.06	0.37±0.02	195.4±3.06[b]
P2	43.40 ±1.00[b]	29.47±1.07[b]	14.21±1.10[ab]	32.89±0.54[ab]	6.09±0.06	0.36±0.00	195.9±3.07[b]
P3	44.16 ±1.03[b]	30.43±1.2[b]	14.17±1.15[ab]	31.75±0.42[a]	5.99±0.05	0.37±0.00	192.7±2.12[b]
P4	44.95 ±1.15[b]	30.44±1.04[b]	14.44±1.09[b]	31.76±0.45[a]	6.00±0.06	0.37±0.00	191.2±3.04[b]

*Mean values within followed by the different letters are significantly different at p < 0,05 according to Duncan's Multiple Range Test.

The Effect of Treatment on Egg Yolk Color and Egg Yolk Weight

The effect of treatment on egg weight and egg albumen wass shown in Table 2. The average color of yolk was 13.58 – 14.44 and egg yolk weight was 31.76 – 32.84 gram/egg. The quality of the yolk color was determined visually by comparing it with various standard colors from the Roche Yolk Color fan in the form of a standard color fan sheet with a score of 1-15 from pale to dark orange (dark orange). The more CSF, the stronger color of yolk, but the weight of the yolk decreased. The adding of CSF at 6% (P4) gave significantly difference effect on egg yolk color and egg yolk weight compare to P0.

The main natural dyes that affect egg yolk color are xanthophylls or carotenoids. The color of the yolk from pale to orange depends on the pigmentation of the yolk, and the type of carotenoids present in the feed. Factors affecting egg yolk color are quantity and quality of feed, xanthophyll concentration, chicken strain, genetic differences, disease, stress, antioxidants, lipid in feed, and nutrient content in feed. Barbosa *et al*., (2011), Li *et al.,* (2018), Conradie *et al*., (2018), Mortensen, (2006); Nys *et al*., (2000). Egg yolk is an indicator of consumer evaluation. Generally, consumers like yellow-orange color. The higher the egg yolk color score, the better the quality of the egg.

The increase in egg yolk color was due to the presence of carotenoid pigments in the CSF which was able to affect efficiently absorbed and utilized by laying hens where this carotenoid pigment was able to increase egg yolk color and egg yolk weight. In addition, the increase in egg yolk color occurred due to the presence of -carotene in the treatment ration which served as an enhancer of egg yolk color.

The β -carotene acts as a precursor for vitamin A which can be used as a pigment in egg yolks. In addition, CSF contains flavonoid compounds and other phenolic compounds that act as natural antioxidants. The use of CSF in the ration of laying hens significantly increased the yellow color of the skin (Purnayasa *et al*., 2018).

The Effect of Treatment on Egg Shell Weight and Egg Shell Thickness

The effect of treatment on egg shell weight and egg shell thickness showed in Table 2. The average egg shell weight was 5.98 g/egg and egg thickness was 0.36 – 0.37 mm. The higher the level of CSF, the more egg shell weight and shell thickness. Statistically showed that the addition of CSF up to 6% gave no significant effect on egg shell weight and egg shell thickness. The results showed that the CSF did not affect the weight and thickness of the eggshell.

The egg shell is the outermost part that encloses the contents of the egg and functions to reduce physical damage and biological damage. The shell is equipped with shell pores that are useful for gas exchange from inside and outside the egg shell. Shell strength is related to the supply of calcium obtained during shell formation. The weight of the shell was influenced by the nutrient content of the ration, health, maintenance management and environmental conditions.

The eggshell is the main defence for eggs against damage during transport and storage. Eggs have thick shells that slow down evaporation of CO_2 and H_2O through the egg pores during storage so that the decline in egg interior quality takes longer and eggs still have good quality (Pasaribu *et al*., 2019)

The Effect of Treatment on Egg Cholesterol

The data can be seen in Table 1. The average egg cholesterol is 191.2 – 212 mg/100 g. It was numerically indicated that total egg cholesterol decreased with increasing levels of CSF up to 6%. The CSF used in laying hens feed contains essential fatty acids, namely linoleic acid, linolenic acid, and oleic acid which can stimulate the reproductive organs to secrete LH and FSH hormones in the formation of egg follicles. Linoleic acid will control the proteins and lipids needed for follicular development and directly control the size of the egg so that the shape of the egg becomes larger. Egg production is also affected by linoleic acid and methionine. Linoleic fatty acid affects egg production because it is needed as a constituent of lipoprotein complexes then synthesized in the liver, stimulated by estrogen hormone and transferred to follicle formation and directly controls the weight of the eggs to be produced (Koen *et al*., 2018).

Essential fatty acids are one of the important food substances for poultry because these fatty acids are associated with the integrity of the mitochondrial membrane structure and are present in high concentrations in the reproductive organs. Increased production and reproduction of local chickens is very dependent on the feed given, especially essential fatty acids, high double bond fatty acids are responsible for permeability and membrane activation to bind enzymes and regulate cell proliferation, components of bile acids and constituents of steroid hormones including reproductive hormones. Omega-3 fatty acids in oil can stimulate the production of hormones that function as anti-aggregators, namely antiblood clots in arteries, and these hormones are needed for follicular development (Koen *et al*., 2018)

The Effect of Treatment on Blood Cholesterol

The effect of treatment on blood cholesterol up to level of 6% showed in Table 3. The average blood cholesterol was 121 – 124 mg/dl, blood LDL was 16.28 – 22.66 mg/dl, blood HDL was 109-20.3 mg/dl and blood triglycerides was 44.60 – 52.04 mg/dl.

On the aspects of serum and egg chemical profiles, the treatments gave significant effect (P<0.01) to all variables. Cholesterol and triglyceride decreased with increasing level of CSF in egg-laying native chickens, and it seems that 6% CSF addition could produce healthier egg products. Brown egg contains 13.76 mg cholesterol/g yolk or 200.2 mg cholesterol/g egg. In the study of the effect of phytobiotic supplementation and magnetized drinking water on the production performance and quality of chicken eggs, the averages of egg weight were 61.13 – 64.12 (g) and yolk weight were 16.38 – 17.00, while for yolk cholesterol were 218.12 – 225.34 (mg/100g) (Marwi *et al*., 2021).

Table 3. Blood cholesterol of egg-laying native chicken

Treatments	Blood Cholesterol(mg/dl)	Blood LDL (mg/dl)	Blood HDL (mg/dl)	Blood Triglyceride (mg/dl)
P0	124.06±4.91[a]	22.66±3.95[a]	109.1±2.1[a]	52.04±3.5[c]
P1	124.4±4.74[a]	21.64±2.67[b]	111.1±3.20[b]	55.78±2.10[d]
P2	124.69±34.77[b]	20.80±2.2[b]	112.0±2.2[b]	48.24±2.2[b]
P3	120.647±4.24[b]	21.16±1.11[a]	120.3±2.11[d]	45.73±1.26[a]
P4	121.04±5.88[b]	16.28±2.13[b]	117.1± 4.13[c]	44.60±2.32[b]

*Mean values within followed by the different letters are significantly different at p < 0,05 according to Duncan's Multiple Range Test.

Laying hens'endogenous cholesterol is synthesized in the liver and transported to the extrahepatic tissues by blood LDL, while blood HDL was mainly responsible for transporting the cholesterol from the peripheral tissues to the liver and excreting it through bile pathway. The shift of cholesterol distribution from plasma into the tissue was due to increasing of LDL catabolism acceleration, resulted from addition of total of LDL receptors by single non-saturated fatty acid (Murray, *et al.*, 2012). Lipid and cholesterol deposition in eggs were closely related to plasma TG, TC and LDL-C levels (Qiu *et al.*, 2018). Wang *et al.*, (2021) reported that decrease in yolk and serum cholesterol related to the act of tea polyphenol in lipid metabolism.

The adding CSF up to 6% in laying hens feed can reduce blood cholesterol levels, blood LDL levels and blood triglyceride levels but increase blood HDL. The effect of CSF to blood cholesterol, blood LDL, blood HDL and blood triglycerides indicate that active ingredients contained in CSF have an important role in reducing cholesterol levels. The CSF contains phenolic compounds, polyphenolic compounds and various types of antioxidants which when added to feed can be processed, absorbed, and have an important role in lowering cholesterol (Sunarno and Djaelani, 2018).

The phenolic compounds or various kinds of polyphenol antioxidants can increase the oxidation of fatty acids in the body to be used as energy and maintenance of the body. Feed with the addition of CSF has an important role in inhibiting the formation of triacylglycerol. Phenolic compounds, tannins, flavonoids, and saponins in CSF has function as anti-hypercholesterolemia. Phenolic compounds will be distributed to all body systems and used in supporting cholesterol metabolism. Polyphenol compounds (flavonoids), in

CSF, has function as antioxidants that has important function in lowering cholesterol levels. In addition to polyphenolic compounds, CSF contains other chemical compounds, namely niacin/B3, pyridoxine/B6 and niacin (vitamin B3) which play in fat metabolism in lowering LDL and triglycerides, as well as increasing HDL levels so that CSF can reduce blood vessel and coronary heart disease (Sunarno *et al.*, 2019).

CONCLUSION

The effects of 6% of *Aleurites moluccana* (L.) Willd. (Candlenut) Seed Flour (CSF) in feed had improved egg weight, albumen, yolk colour, yolk weight, egg cholesterol, blood cholesterol, blood LDL, blood HDL and blood triglycerides, but no significant impact on egg shell weight and thickness. Therefore, the use of 6 % of CSFin feed was suggested to improve egg quality and serum cholesterol of egg-laying native chicken.

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