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Original Article

Egg quality and cholesterol content of quail fed a combination of golden snails (*Pomacea canaliculata* L.) and *Azolla pinata*

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Abstract

Objective: This study aims to determine the effect of using golden snail flour (Pomacea canaliculata L.) and Azolla pinnata as sources of protein and carotene on the quality of quail eggs.
Methods: A total of 80 five-week-old laying quails were used in this study. Golden snail and Azolla pinnata were mixed into the feed ingredients in the form of flour. The experimental feed was formulated to be iso-protein and iso-energy (CP = 19% and ME = 2,900 kcal/kg). This study used a Completely Randomized Design (CRD) with 4 treatments and 5 replications, each replication consisting of 4 quails. The treatments were as follows: T0 = Control (without golden snail and Azolla pinnata); T1 = Azolla pinnata 10%; T2 = Golden snail 10%; T3 = Golden snail 10% and Azolla pinnata 10%.
Results: The results of the study showed that there was no significant effect on the quality and cholesterol content of quail eggs (P>0.05). However, the inclusion of 10% Azolla in the feed significantly improved the yolk color (P<0.01).
Conclusions: The inclusion of 10% Azolla pinnata in the feed improved the yolk color and tended to reduce the cholesterol content of quail egg.

Keywords: Golden snail; Azolla piñata; egg quality; cholesterol

INTRODUCTION

Quail is a small bird that feeds seeds and insects. The species *Coturnix coturnix japonica* is the most commonly farmed and studied species because it starts egg production rapidly., specifically 42 days after hatching [1]. In quail farming specifically and poultry farming in general, feed plays a crucial role as it accounts for 60-70% of the total production cost. Therefore, exploration is needed for finding cheap and high-quality poultry feed.

According to Retnani [2] feed is one of the primary commodities in the upstream agribusiness subsystem, or in other words, serving as a crucial resource for the livestock farming sector. The provision of high-quality feed has the potential to increase livestock populations, as well as meat production, and egg production as livestock products. Therefore, exploration and implementation of alternative feed materials that are cheap, high-quality, and easily accessible or produced are needed.

The golden snail (*Pomacea canaliculata* L.) is a pest species with a very high reproductive rate. This has the potential to cause a rapid pest outbreak in a short period of time. Its destructive power is highly dependent on its size; when the snail measures 31-40 mm, its destructive power reaches 97,38% [3]. Despite its pest status, Golden snails can serve as an alternative feed due to their high nutrient content, including 54% crude protein, 30% carbohydrates, 4-5% fat, and several minerals such as phosphorus, iron, calcium, magnesium and iodine and vitamin C. The inclusion of Golden snail flour at a level of 10% ration has been shown to enhance egg production in Tegal ducks [4].

Azolla is very common in still waters, such as lakes, ponds, swamps, and rice fields. *Azolla pinnata* is often considered an aquatic weed due to its ability to double its population in just three to four days. However, Azolla also has the potential as a feed ingredient because of its high nutritional content. According to Miranda et al. [5] that the dry matter (DM) content of sun dried Azolla meal is 89.73%. It contained 75.73% organic matter, 23.49% crude protein, 14.7% crude fiber, 3.7% ether extract, 24.26% total ash, 7.94% acid insoluble ash, 2.58% calcium and 0.26% phosphorus.

Golden snails and *Azolla pinnata* are both rich in, which contributes to the yellow coloration of egg yolks. According to Mahamuda and Islam [6] the cavities of Azolla leaves contain vitamin A, carotene, and beta-carotene, which enhance the color of egg yolks. Golden snails also contain carotene pigments that serve as natural coloring agents for egg yolks [7].

Carotene is part of to the pro-vitamin A group, which enhances the color of egg yolks, thereby increasing consumer acceptance and improving egg quality. Carotene enters the egg yolk and is transported along with lipids by the liver before being integrated into the egg yolk [8]. High-protein sources from golden snail and *Azolla pinnata* are used in this study to improve egg quality. The presence of animal carotene (Golden snails) and plants (Azolla) is expected to improve the quality and color of eggs. By combining both animal and plant protein feed ingredients, their amino acid profiles are likely to complement each other, thereby improving the overall quality of the feed. According to Donadelli et al. [9] that the essential amino acid content of animal protein is more complete than plant protein.

Quail eggs not only have high protein content, but also contain a high level of cholesterol (16-17%). Therefore, this study will also make efforts to produce feed that can reduce cholesterol levels in quail eggs. According to Akerina [10], the β-carotene compound is a carotenoid compound that functions as a provitamin A, gives yellow color to egg yolks and can reduce egg yolk cholesterol.

Previous studies have only used either a single source of animal-derived carotene, such as Golden snails, or solely plant-based carotene like Azolla [7]. Another study conducted by Tarigan and Manalu [11] focused solely on how Azolla pinnata affects poultry feed efficiency. Previous researchers generally have not compared the combination of the two ingredients (Golden apple snails and Azolla) which are sources of carotene and natural protein from animals and plants. This study aims to determine the effect of using golden snail flour (*Pomacea canaliculata* L.) and *Azolla pinnata* as sources of protein and carotene on the quality of quail eggs.

MATERIALS AND METHODS

Materials

The materials used in this study were 80 quail layers aged 5 weeks. Golden snails and *Azolla pinnata* that were already in flour form were mixed with other ingredients such as corn, laying hen concentrate, and premix to achieve iso protein and iso energy with a nutritional content of CP = 19% and ME = 2,900 kcal / kg (Table 1).

Research method

The method used in this study was experimental using a Completely Randomized Design (CRD) consisting of 4 treatments and 5 replications, each replication comprising 4 quails. The treatment feed was provided when the quails were 8 weeks old, following a one-week adaptation period. Drinking water was available ad libitum. The maintenance period lasted for 8 weeks.

The treatments given consisted of: T0 = Control (without Golden Snails and Azolla); T1= *Azolla pinnata* 10%; T2 = 10% Golden snails; T3 = 10% Golden snails and 10% *Azolla pinnata.* Egg collection for quality data began during the last two weeks of the maintenance period. The data were analyzed in duplicate. Cholesterol data from the egg yolk were collected from eggs harvested during the last week of the maintenance period. The observed parameters included: 1) Egg quality including egg weight, egg yolk index [12], egg white index [13], shell thickness [13], and egg yolk color [14]; and 2) Egg yolk cholesterol content [15].

Data Analysis

All data obtained from the study were analyzed using a Completely Randomized Design (CRD). Significant differences were further analyzed using Duncan's Multiple Range Test (DMRT) using SPSS version 16 software.

RESULTS

Egg quality

The observations of egg quality in this study included: egg weight, egg yolk index, egg white index, egg shell thickness, and egg yolk color. The average quality of the quail eggs is presented in Table 2. The results indicate that there were no significant differences between T0, T1, T2, and T3 in terms of egg weight, egg yolk index, egg white index, and egg shell ($P>0.05$), However, significant differences were observed in egg yolk color ($P < 0.05$) (Figure 1).

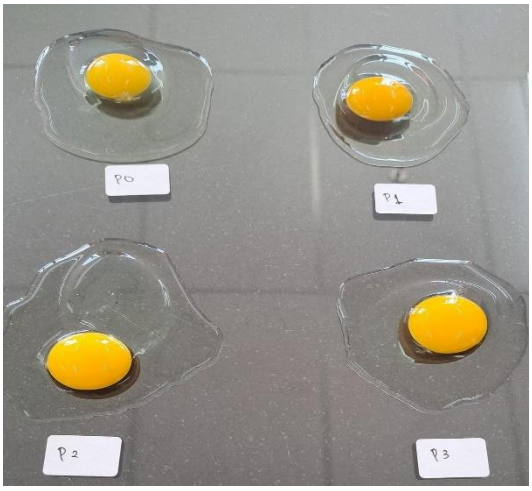

Figure 1. Egg yolk color for treatment T0 = Control (without Golden Snails and Azolla); T1 = Using *Azolla pinnata* 10%; T2 = Using 10% Golden snails; T3 = Using 10% Golden snails and 10% *Azolla pinnata*.

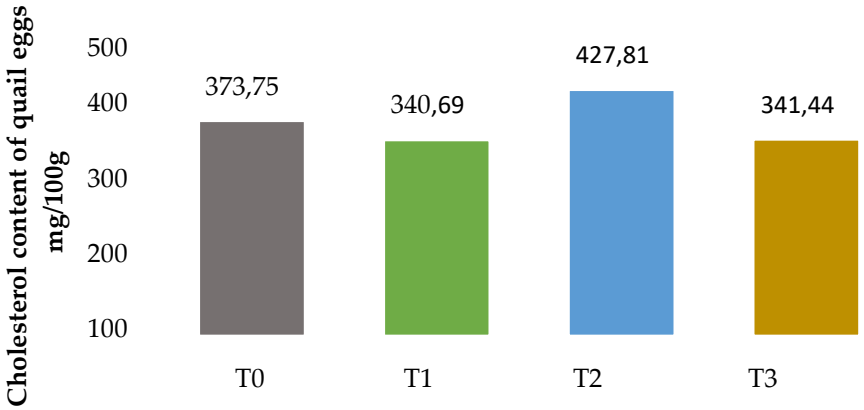

Figure 2. Cholesterol Content of Quail Eggs for treatment T0 = Control (without Golden Snails and Azolla); T1 = Using *Azolla pinnata* 10%; T2 = Using 10% Golden snails; T3 = Using 10% Golden snails and 10% *Azolla pinnata.*

Quail Egg Cholesterol

The cholesterol content in quail eggs is presented in Figure 2. The observation indicate that there were no significant difference between treatments T0, T1, T2, and T3 on the cholesterol content of quail eggs (P>0.05).

DISCUSSION

Egg quality

Egg weight was measured daily using a digital scale and expressed in grams per egg (g/egg) over a 4-week observation period. The average weight of quail eggs during the study were 12.26±1.02 g/egg (T0); 11.26±1.02 g/egg (T1); 10.92±0.74 g/egg (T2), and 11.48±0.65 g/egg (T3) (Table 2). The results indicate that the addition of Azolla flour and Golden snail flour did not significantly affect egg weight. This lack of effect is likely due to the similar nutrient content in the feed, which had iso-protein and iso-metabolic energy levels. Similar findings were reported in the study by Alamsyah, Kismiati, and Yunianto [16], where the weight of the eggs produced was not significantly different due to the same protein and metabolic energy content between treatments. The results of this study can be considered quite good compared to the findings of Gubali, Zainudin, and Dako [17] which reported egg weights ranging from 9.36 to 9.80 g/egg, the research by Rondonuwu et al. (Rondonuwu et al., 2018) which ranged between 7.98 - 8.96 g/egg, and the research by Satria, Harahap, and Adelina [18] which ranged between 8.08 - 8.96 g/egg.

The egg yolk index reflects the quality of the viscosity of the egg yolk, measured based on its height and diameter [14]. The results indicate that the average egg yolk index of the quail eggs produced was not significantly affected by the inclusion of Golden snail and *Azolla pinnata* flour. Throughout the study, the average egg yolk index ranged from 0.33 to 0.36, which aligns with the Indonesian National Standard (SNI 3926:2008), stating that the index should be between 0.33 and 0.52.

This condition is possible because the nutritional content of the feed given from all treatments is sufficient for the needs of quail livestock, one of which is indicated by the observation variable of the egg yolk index. consistent with the views of Mustakim, Munir, and Irmayani who stated that the protein in the feed affects the viscosity of the egg, which reflects its quality, specifically the egg yolk index. Satria, Harahap, and Adelina [18] noted that egg yolk is composed of fat and protein, forming lipoproteins synthesized by the liver under the influence of estrogen. Therefore, the egg yolk index is influenced by the protein, fat and essential amino acids contained in the ration.

The effect of giving Golden snail flour and *Azolla pinnata* to the feed also did not affect the egg white index. This condition is similar to the egg yolk index, where the protein and energy content in the feed for all treatments are the same. Alamsyah, Basuki, Prarudiyanto, dan Cicilia [19] added that the egg white index is also influenced by egg weight. Since the weight of the eggs produced is not significantly different, so the egg white index is also not significantly different. The same situation was observed for shell thickness, which was unaffected by the provision of Golden snail flour and Azolla flour. This was due to the nutrient content in the ration given to quail has a relatively similar Ca and P content, namely in the range of Ca 2.2% - 2.7% and P 0.5% - 0.7% (Table 1). This also happened in the study conducted by Paryanta, Sudrajat, and Anggraeni [20], where calcium content of 3.2% - 4.6% and a phosphorus content of 0.6% - 0.9% did not significantly affect eggshell thickness.

The difference observed was in the color of the egg yolk, with the highest value observed in quail eggs from the T1 treatment (using 10% Azolla) (10.10 ± 0.24), followed by T3 (using 10% Azolla and 10% Golden snails) (9.80 ± 0.25), T2 (using 10% Golden snails) (7.10 ± 0.36) and the lowest was in the T0 treatment (control feed with factory concentrate) (6.70 ± 0.25) (Figure 1). This indicates that the carotene compound in the Azolla plant (with a dose of 10%) can affect the color of the egg yolk. According to Rondonuwu et al. [7] that the carotene compounds in the Azolla plant can affect the color of the egg yolk. However, this effect was not observed with the T2 feed (containing 10% Golden snails) where the effect of carotene in Golden snails at a 10% dosage did not

significantly enhance the color of the quail egg yolk. The inclusion of protein sources with a combination of Azolla and golden apple snails (T3) has reduced the consumption (palatability) of Azolla. This is because, although Azolla was finely ground, it could still be selectively eaten by the quails due to its very light weight, making it easy to separate from other ingredients, particularly from the golden snail flour. As stated by El-Ghany [21] reduced consumption of Azolla in poultry may occur due to palatability issues and an increase in the bulkiness of Azolla. The decrease in Azolla consumption as a source of carotene resulted in a lower yolk color score for T3 (combination of 10% golden snail and 10% Azolla pinnata) compared to T1 (10% Azolla pinnata) (Table 2).

Table 1. Proportion of Feedstuff and Nutritional Content of The Experimental Diet

Feed Ingredients/ Nutrients	Treatments (%)			
	T0	T1	T2	T3
Jagung	57.0	50.5	56.0	53.0
Konsentrat	37.0	28.0	30.4	23.0
Keong mas	0.0	0	10.0	10.0
Azolla pinnata	0.0	10.0	0	10.0
Premix	1.0	1.0	1.0	1.0
Bekatul	3.0	10.5	0	0.4
Minyak Sawit	2.0	2.0	2.6	2.6
Nutrient Composition				
Dry Matter (DM)	89.3	92.5	89.6	87.7
Crude Protein (CP)	19.4	19.4	19.2	19.3
Crude Fiber (CF)	3.7	4.9	3.8	4.2
Extract Ether (EE)	4.2	4.8	1.7	3.5
Metabolic Energy (ME)	2,915.7	2,931.5	2,900.7	2,904.3
Calcium (Ca)	2.2	2.6	2.8	2.5
Phospor (P)	0.6	0.7	0.6	0.6

Table 2. Quail Egg Quality

Parameters	Treatments			
	T0	T1	T2	T3
Egg Weight (g) ^{ns}	12.26±1.02	11.26±1.02	10.92±0.74	11.48±0.65
Egg Yolk Index ^{ns}	0.35±0.07	0.35±0.01	0.36±0,02	0.33±0.01
Egg White Index ^{ns}	0.08±0.02	0.06±0.01	0.08±0.00	0.07±0.01
Egg Shell Thickness (mm) ^{ns}	0.26±0.01	0.33±0.03	0.30±0.03	0.31±0.03
Egg Yolk Color	6.70±0,25 ^{b}	10.10±0.24 ^{a}	7.10±0.36 ^{b}	9.80±0.25 ^{b}

$^{a, b}$ = Different lowercase letters in the same row indicate significant differences in each treatment ($P<0.05$).

^{ns} = non significant

Cholesterol content of quail eggs

The provision of Golden snail flour and *Azolla pinnata* flour in the feed aims to reduce the cholesterol content of quail eggs. Quail eggs that with lower cholesterol content are expected to be popular with the public, thus increasing the value of livestock products. While the cholesterol content in quail eggs did not show significant differences, there was a tendency for the treatments using *Azolla pinnata* (T1 and T3) has a lower cholesterol content compared to the control group which was the feed containing Golden snail animal protein. This is because *Azolla pinnata* not only contains carotene that can lower cholesterol but also contains crude fiber that binds fats and eliminate them from the body through excretion. According to Alfauzi et al. [22] that fiber can reduce fat absorption, so that body fat deposition is reduced. Nurfianti and Tribudi [23] further explained that crude fiber

enhances the excretion of fat, including cholesterol, through feces.

The combination of Azolla with Golden snails resulted ini egg quality that was not significantly different from other treatments. According to Donadelli, Jones, and Beyer [9] that the essential amino acid content in animal protein is more complete compared to that in plant protein. Furthermore, daily egg production is influenced by the amino acid content in the protein in the feed [24]. The rate of egg production causes variation in yolk color when egg production increases. This is because carotene and xanthophylls in the feed are distributed to many egg yolks, causing the yolk color to decrease, and vice versa[7].

CONCLUSION

The inclusion of 10% *Azolla pinnata* in the feed improved the yolk color score and tended to reduce the cholesterol content of quail eggs. Further research is needed in the future to explore the use of plant and animal protein and carotene sources that can effectively reduce cholesterol and improve other egg quality parameters, such as egg weight, yolk index, egg white index, and shell thickness.

CONFLICT OF INTEREST

The authors declare no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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