Research Article

Identification and Effects of the Different Carotenoid on External and Egg Yolk Quality of Alabio Laying Ducks (*Anas plathyrhyncos Borneo*)

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Abstract | The purposed of this research was to identification effects in the different carotenoid on external and egg yolk quality of Alabio laying ducks (*Anas plathyrhyncos borneo*). To begin with, the carotenoid was identification using High Performance Liquid Chromatography (HPLC) for lutein content. Second, 200 day-old-Alabio laying ducks by age 4-5 months was used in this study. The basal diet was used in this study T0: basal diet, T1: basal diet + 10% lutein, T2: basal diet + 20% lutein, T3 basal diet + 30% lutein, T4 : basal diet + 40% lutein. A completely randomized design was adjusted with five treatments and six replications for each pen, consisting of ten Alabio laying ducks. A general linear model was used in this research to identification a significant effect. The results showed used different carotenoid give insignificant difference ($p < 0.05$) on the whole egg quality and yolk quality. In summary, even though present slightly different the carotenoid can be used as natural pigmentation on Alabio laying ducks.

Keywords | Alabio, Laying duck, Carotenoid, Egg yolk, Lutein

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INTRODUCTION

Nowadays the continuous demand for eggs are increased, while efficiency is required for produced egg. One policy that can improved egg production is optimizing the feed over laying periods (Adli et al., 2023). Waterfowl, in this case are Alabio ducks were still favorable kept non-intensive systems and growth well under low-quality feed ingredients (Maharani et al., 2019). As consequences of this condition, feed contributed to growth performances approximately 70-80% of total production costs. The used of feed in the Alabio laying ducks are mostly imported and relatively high cost (Sulaiman et al., 2022).

Moreover, Indonesia has abundant natural resource potential, both derived from animals and plants, which can be used as a source of food or medicine. One type of plant that can be used as feed ingredients. This type of legume is a type of plant that is rich in nutrients because it contains many vitamins, minerals, and anti-oxidants, respectively. Currently, international world, the cultivation of this type of legume is much encouraged because of the benefits contained in the leaves and stems. Almost all parts of several types of leguminose can be utilized, starting from leaves, fruit, seeds, flowers, bark, to roots (Suwignyo et al., 2022).

Leguminous plants (*Alfalfa, Moringa, Lamtoro*, and *Indigofera*) are easy-to-obtain feed ingredients, contain high crude protein, making them very profitable for poultry. High crude fiber is not good for poultry because it cannot digest the digestive tract of poultry very slowly (Suwignyo et al., 2022). Legumes contain balanced amino acids and are rich in vitamins, carotenoids and xanthophyll's which give yellow color to poultry carcasses and egg yolks (Tawalbeh et al., 2023). Legumes also contain 2-3% saponins which are efficacious as hypocholesterolemia (functions as a good cholesterol lowering of total cholesterol and triglycerides in the intestinal of waterfowl. At the end, their excreted through their excreta (Kowalska et al., 2020), anti-carcinogenic (preventing cancer), anti-inflammatory (reducing inflammation and pain), and antioxidants (Kuźniacka et al., 2020). determined. Therefore, the purposed of this research was to identification effects in the different carotenoid on external and egg yolk quality of Alabio Laying Ducks (*Anas plathyrhyncos Borneo*)

MATERIALS AND METHODS

Ethical approval

Ethical approval for the study was given by the Animal Care and Use Committee, University of Islam Kalimantan Muhammad Arsyad Al Banjary, No. 5/KEP/UNISKA/PPJ-2023.

Experimental design

A total of 120-day old duck female Alabio duck with an average age 17 weeks were placed in colony-shaped pens, and each pens contained ten ducks. The Alabio duck was rearing from 17-21 weeks. The basal diet was used in this study T0: basal diet, T1: basal diet + 10% lutein, T2: basal diet + 20% lutein, T3 basal diet + 30% lutein, T4 : basal diet + 40% lutein. A completely randomized design was adjusted with five treatments and six replications for each pen, consisting of ten Alabio laying ducks. The diets were given every morning and evening according to the requirement of each maintenance, and were allowed *ad-libitum* access water through adjustable nipple drinkers. An Alabio laying duck was feed consisted yellow maize, bran, pollard, corn gluten meal, DDGS, soya bean meal, rapeseed meal, bone meal, crude palm oil, limestone, vitamin, mineral, and leguminous plant. About 500 gram of representative samples were taken to identified the metabolizable energy (Kcal/kg); crude protein (CP), crude fat (CF), crude fiber (%), calcium (%), and available phosphorus (C_{av}) by following (AOAC, 2000) method. Afterwards, the composition of formulated feed can be seen in Table 1.

Carotenoid preparation

First, the different of carotenoid was sun-dried for 7 days. A total 0.2 gram of sample powder was weighed and then 1 mL of 100% acetone was added. Then added $CaCO_3$ and sodium ascorbate to taste, then homogenized with a vortex for 1 minute. The pigment extract was separated from the residue by centrifuging at 6000 rpm at room temperature for 1 minute. Then the pigment extract is put into the vial. The residue was extracted in the same way by adding 1 mL of 100% acetone. Subsequently, the process was replicated until supernatant changes color become yellow pale. In the end, the samples were stored in the BioBase refrigerator at -30°C in the dark.

Identification of carotenoid using High Performance Liquid Chromatography (HPLC)

Dry carotenoids, the extract was dissolved in 1 mL of 100% acetone and filtered. Using a PTFE membrane syringe (0.22 μm, Shimadzu), then 20 μL of the extract was injected into the reverse phase (RP) HPLC. Pigment separation was carried out by RP-HPLC using a YMC C30 column (150 × 4.6 mm ID) (YMC, Wilmington, MA, USA), column oven (CTO 20A) at 30 °C, and photo-diode array detector (LC20A). The elution gradient program was set by a mixture of MeOH, MTBE, and HO (81:15:4, v/v/v) at 0 min to (6:90:4, v/v/v) at 70 min at 30 °C with flow rate of 1 mL/minute.

Egg quality assessment

At the end of the week, a total 10% of egg was individually weighed and collected for 9 weeks using scales 0.001 g. The yolk color was identified using Roche egg yolk color fan using 1-15 scales. The thickness of the eggshell is obtained by measuring the thickness of the shell with the egg membrane (mm). Measurement of shell thickness was carried out after the weight of the shell was weighed. The eggshell thickness was measured at the blunt end, the middle (equator), and the sharp end of the egg and then averaged.

Statistical analysis

A general linear model was used in this study using Proc. Mixed with general linear model (GLM) used online software of SAS studio. An error of standard mean was expressed (SEM). Subsequently, the probability test was subjected using Duncan Multiple Range Test by following method from (Adli et al., 2022; Sholikin et al., 2023).

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where Yij was parameters observed, μ was the overall mean, Ti the effect different the effect level of freshwater snail flesh incubated with bromelain enzyme, and eij the amount of error number. T0: basal diet, T1: basal diet + 10% lutein, T2: basal diet + 20% lutein, T3 basal diet + 30% lutein, T4 : basal diet + 40% lutein One-way ANOVA was used to compare the means of apparent digestibility protein, apparent metabolizable energy, feed intake, body weight gain, feed conversion and live weight and the significance threshold was established at $p < 0.05$ using the was used to compare the means. All analysis was carried out in six replications and significant of difference was defined as the 5% level (P<0.05). Duncan Multiple Range Test was subjected in order to determine the significance and probabilities values.

Table 1: Experimental diet composition

Proximate composition of composed feed	T0	T1	T2	T3	T4
Dry matter (%)	89.7	89.69	89.69	89.68	89.68
Crude protein (%)	18	18.19	18.38	18.56	18.75
Crude fibre (%)	6	6.10	6.21	6.31	6.42
Fat (%)	4	4.06	4.13	4.19	4.26
Metabolizable energy (kcal/kg)	2879.45	2885.01	2890.77	2895.23	2892.22
Calcium (%)	3	3.04	3.07	3.11	3.14
Available phosporus (%)	0.6	0.60	0.61	0.61	0.61

RESULTS AND DISCUSSION

The concentration of lutein after identification using HPLC

The following Table 2 accounts for the concentration of lutein after identification using HPLC. In *Moringa oleifera* presented very large majority reached until 237.94 µg/g bb while, Indigofera accounted a very small number at 0.96 µg/g body weight. Afterwards, *alfa-alfa* leaves presented approximately 60 µg/g bb. Yet along with this the boost of the river tamarind reached 95.84 µg/g bb. However, exposing the xanthophyll commonly found in the leguminous, which presented as energy and photosynthesis processed (Schlatterer and Breithaupt, 2006). Xanthophyll can be found in the cell of the animals including humans as well as animal products, which derivate from the plant as source of pigmentation. Specifically, the color of egg yolk, fat, and others was come from digested xanthophyll's especially lutein, which is added to chicken feed. Xanthophyll is the main contributor to determine the color of egg yolk (Mamatha et al., 2011). Consumers in South Kalimantan prefer the color of yolk which is reddish yellow rather than pale yellow. Xanthophyll is absorbed from the intestinal tract of chickens and deposited in egg yolk and fatty tissue in the same form as consumed (Perry et al., 2009). Xanthophyll not only affects the color of the yolk but also the skin color of the chicken. Therefore, this pigment is not deposited on the white skin of chickens and eggs that have not been released by any birds (Ortiz et al., 2021) Xanthophyll are carotenoids containing hydroxyl groups (Miao et al., 2023). Xanthophyll are usually monohydroxycarotene (e.g. lutein, rubixanthin), hydroxycarotene (zeaxanthin), or dihydro or dihydroxyepoxyepoxycarotene (violaxanthin) (Perry et al., 2009). Xanthophyll Both are insoluble in water, but soluble in alcohol, petroleum ether, acetone and many other organic solvents (Chowdhury et al., 2008).

Effects of The Different of the Carotenoid On External and Egg Yolk Quality of Alabio Laying Ducks (*Anas plathyrhyncos Borneo*)

The following Table 3 accounts for the differences of the carotenoid on external and egg yolk quality of Alabio Laying Ducks (*Anas plathyrhyncos Borneo*) (Table 2). In brief it can be clearly seen that across all the tables in egg quality, the carotenoid effect on external quality present insignificant different ($p > 0.05$) (Table 2) (Figure 1). However, the pace of the egg weight begins to rise at the T1 (73.83) then slightly dropped down until rest of the treatments (Table 2) (Figure 2). Yet along with this both of yolk color and yolk height remain same at the moment (Figure 3). Afterwards, shell thickness present at very small number across the dataset. To do so, the ducks that are reared intensively require food completely from feeding by breeders. If ducks have good genetics and provide quality feed and according to standard requirements with good management, it will provide benefits. Duck productivity is determined by two main factors, namely genetics and the environment (Chowdhury et al., 2008). Genetic factors are determined by the arrangement of genes and chromosomes possessed by an individual and are hereditary as long as no mutations occur in the genes that compose them, while environmental factors do not always change and cannot be passed on to their offspring (Titcomb, 2019). Therefore, improving the quality of seeds, feed and maintenance procedures will increase the productivity of these ducks. Rostini et al. (2021) stated that the average egg production of Alabio ducks in the battery cage system was higher and more stable than the litter cage system.

Table 2: Concentration of Lutein after identification using HPLC

Kind of Leguminose	Konsentrasi (µg/g bb)
	Lutein
Alfalfa (*Medicago sativa L.*)	56.61
Moringa (*Moringa oleifera*)	237.94
Indigofera (*Indigofera zollingeriana*)	0.96
River Tamarind (*Leucena leucocephala*)	95.84

The quantity and quality of the feed given determines the production and quality of eggs both physically/externally and chemically/internally. The egg production would have achieved at peak production depend on the feed, management, and economical value (Zarghi et al., 2023). Egg pro

Table 3: The effect of the different carotenoid on the egg quality of the Alabio Laying ducks

Parameters	T0	T1	T2	T3	T4	SEM	R^2
Egg Length (cm)	5.67	5.65	5.61	5.48	5.61	0.03	0.177
Egg width (cm)	4.61	4.83	4.36	4.34	4.36	0.01	0.80
Egg Weight (g)	71.90^{ab}	73.83^{b}	67.11^{a}	67.09^{a}	67.06^{a}	3.00	0.55
Diameter of Albumin (cm)	14.55	15.13	15.13	14.98	15.14	0.36	0.34
Diameter of Yolk (cm)	4.80	4.43	4.40	4.50	4.17	0.27	0.41
Shell Thickness (mm)	0.36^{b}	0.41^{b}	0.20^{a}	0.23^{a}	0.15^{a}	0.004	0.75
Albumin height (cm)	0.46	0.54	0.35	0.50	0.46	0.001	0.28
Yolk Color (cm)	16.00	17.00	16.25	16.00	15.75	0.31	0.71
Yolk Height (cm)	1.58	1.72	1.77	1.63	1.75	0.17	0.18

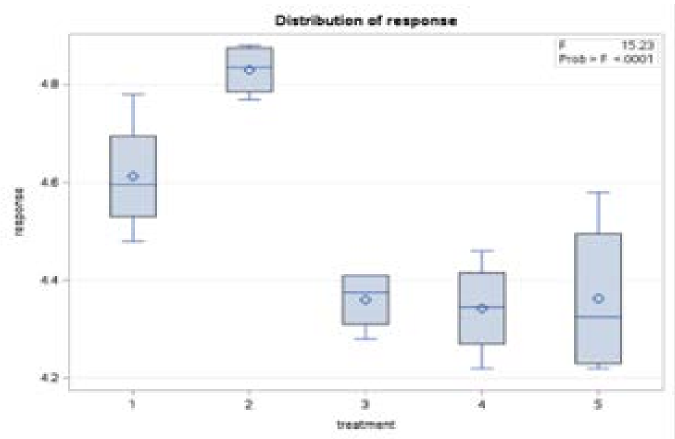

Figure 1: Distribution response of egg width (cm)

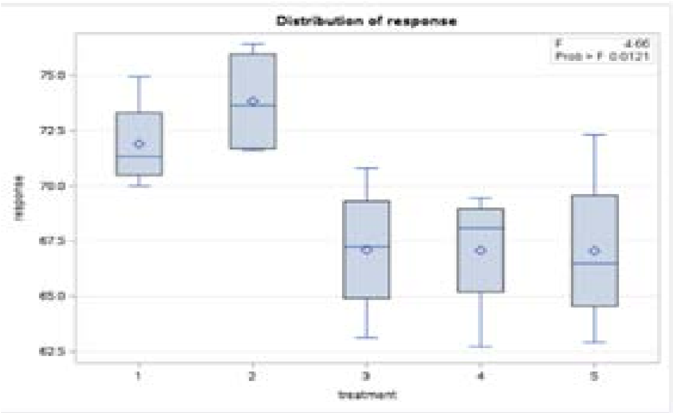

Figure 2: Distribution response of egg weight

duction is one of the important quantitative characteristics that have high economic value from the performance of laying hens. Egg production in various regions of South Kalimantan is relatively different. The differences of the price, quality may depend on the various factors.

Age at first laying indicates that the duck is sexually mature, although ovulation may have occurred (Liu et al., 2023). Mass production as a very important variable is related to the selling price of duck eggs at the market level. The quality of Alabio duck were evaluated based on the (1)

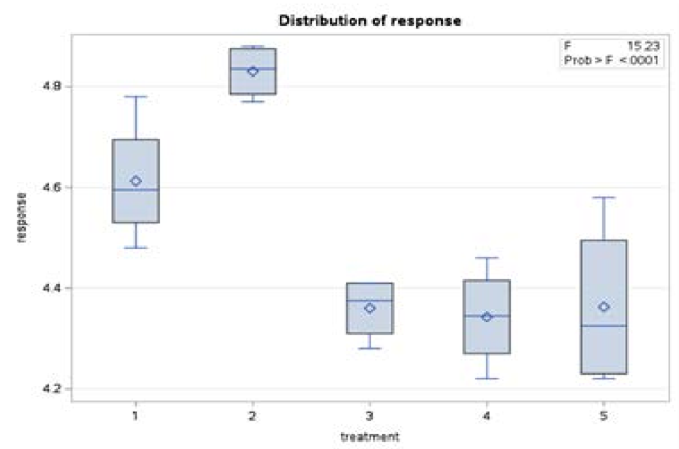

Figure 3: Distribution response of egg yolk diameter (cm)

egg size; (2) egg weight; (3) and egg yolk color. This means that not only is the level of production being sought to increase, but the size or size of the eggs is sought to be large enough to be sold at an optimal selling price (Siddiqui et al., 2022). Thus the profit from selling eggs will be higher when these two parameters are taken into account in the Alabio duck farming business, while the average egg mass of Alabio duck eggs is 39.50 ± 2.60 g/head/day.

The yolk color score (yolk) was obtained by comparing the yolk color with the yellow color on the yolk color fan which is done every two weeks during the study. The yolk index is the ratio between the height of the yolk and the diameter of the yolk. Fresh egg yolk index ranged from 0.33-0.50. According to Yang et al. (2023) that the more *Moringa* leaf powder in the ration, the better the intensity of the egg yolk color. Riley et al. (2023) stated that feed affects the color of egg yolks, feed ingredients containing carotenoid pigments, especially beta-carotene and xanthophyll pigments. Karwanti et al. (2023) states that the egg yolk index is an index of freshness quality as measured by the height and diameter of the yolk. Furthermore, Chen et al. (2023) stated that the yolk index value is used to determine egg freshness. The yolk index indicates a progressive decline in the function of the vitelline membrane in eggs, the smaller the yolk index, the lower the egg quality. Cholesterol is a product of animal metabolism and is therefore present in foods of animal origin such as meat, liver, brains and egg yolks (Yenilmez and Atay, 2023).

According to Ma et al. (2023) that there was a decrease in egg yolk cholesterol levels in laying hens given Cellulolytic penicillin of *Bacillus Anthracis* and *Ochrobactrum* Anthropic (YZ-1). Patel et al. (2023) stated that this probiotic can suppress the activity of the enzyme 3-hydroxy-3-methylglutaryl Co-A which functions for cholesterol synthesis in the liver and cellulolytic bacteria are able to grow and assimilate cholesterol in the small intestine. Ouyang et al. (2023) which stated that giving probiotics could increase growth and reduce serum cholesterol in ducks. The same thing was reported by Yao et al. (2023) who said that probiotic supplementation in rations can significantly reduce blood serum cholesterol levels.

CONCLUSIONS

In summary, using different carotenoid source can be used as pigmentation on Alabio laying ducks without any adverse effects. It's envisaged the different carotenoid on the egg quality help to increasing the egg weight of Alabio laying duck.

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CONFLICT OF INTEREST

The remain article does not report any conflict of interest.

NOVELTY STATEMENT

The novelty of this research is the use of different source of carotenoid to enhance the egg yolk quality. There is limited information related to the used of the carotenoid on the local waterfowl in this case was Alabio laying ducks.

AUTHOR'S CONTRIBUTION

MSD contributed to data collection, in-vivo and in-vitro, software and writing original manuscript. HH, OS, ID conducted the conceptualization, supervision, validation, and writing revised draft. All authors read and approved the final version of the manuscript in the present journal.

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