

## FRESH-WATER GREEN ALGAE (*CHLOROPHYTA*) AS A NATURAL PIGMENT FOR MOJOSARI DUCKS

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### ABSTRAK

Penelitian dilakukan dengan rancangan acak lengkap untuk mengetahui penggunaan ganggang hijau air tawar segar sebagai pewarna alami kuning telur itik Mojosari selama periode bertelur terhadap performan produksi dan kualitas telur. Sebanyak 80 ekor itik petelur umur 36 minggu dibagi menjadi empat perlakuan dan empat ulangan terdiri dari 5 ekor. Pakan diformula menggunakan konsentrat komersial, dedak, dan jagung kuning (2:4:4) sesuai dengan standar pakan komersial sebagai kontrol, dan tiga perlakuan lain dengan menambahkan 2, 4, dan 8% ganggang hijau. Konsumsi pakan, produksi telur dan konversi pakan dipengaruhi oleh pemberian ganggang hijau ( $p < 0.05$ ). Produksi telur dan konversi pakan menunjukkan perbaikan pada level 2 dan 4%. Indek kuning telur, indeks albumen, Haugh Unit dan ketebalan kulit telur tidak dipengaruhi oleh pemberian ganggang hijau ( $P > 0.05$ ) kecuali warna kuning telur. Warna kuning telur mengalami perubahan lebih baik dalam waktu 7 hari setelah pemberian pakan yang mengandung ganggang hijau. Ganggang hijau dapat digunakan sebagai pewarna alami kuning telur dan pada level 8% dalam ransum meningkatkan pigmentasi kuning telur itik hingga mencapai skor tertinggi diukur Roche Yellow Color pada skala 15.

*Kata kunci: ganggang hijau, jagung, dedak, warna kuning telur, produksi telur.*

### ABSTRACT

An experiment in a completely randomized design was undertaken to study the use of fresh-water green algae as a yolk coloring agent in Mojosari ducks during a laying period on productive performance and egg quality from 36 to 44 wk of age. A total of 80 thirty-six wk-old laying ducks were divided into four dietary treatments and each of four replicates with 5 birds. Diets were formulated with a commercial concentrate, rice bran and yellow corn (2:4:4) according to a commercial standard diet as a control, and three other dietary treatments with 2, 4 or 8% of green algae were included. Fresh water green algae had a significant effect on the feed uptake, egg production, and feed conversion ratio (FCR) ( $p < 0.05$ ). Egg production and FCR improved at added 2 and 4% green algae. No differences were observed in egg yolk index, albumen index, Haugh Unit, and egg shell thickness ( $P > 0.05$ ) except egg yolk color. The yolk color increased within 7 days after feeding with the test diets. The present study indicated that fresh-water green algae could be used as a natural coloring agent in laying ducks and at 8% of green algae showed the highest score of (Roche Yellow Color-15).

*Keywords: green algae, corn, rice bran, egg production, yolk color*

### INTRODUCTION

The production of Ducks (*Anas platyrhynchos*) as a food source in Indonesia have

become of high importance in the last few years. Duck farming is regarded as the most reliable business in rural areas because it has a very large contribution to the small farmers in rural areas

both to increase their income and to support animal protein demand.

Mojosari ducks are one of the Indonesian superior laying ducks from areas of Mojokerto, East Java. In addition to producing high eggs, the duck meat is delicious and the demand of duck products tends to increase recently. Because ducks are more resistant to diseases and can easily be managed by small farmers, they become more popular and are of higher benefit compared with chicken. The value of selling duck eggs remains higher than chicken eggs because of the larger size of duck eggs and the price of individual eggs, not at the mass weight base. Duck eggs are also used as raw materials for salted eggs in food industries, which cannot be substituted by eggs from other layer birds of any types of poultry furthermore, egg yolk color is an important criterion for consumers.

Yellow corn is the main ingredient used in most poultry diets to supply xanthophylls for the yolk color, approximately 40 to 60% (Leeson and Caston, 2004) 30 to 50% (Cho *et al.*, 2013), 65% (Moeini *et al.*, 2013). However, xanthophylls for yolk color only relied on corn were not able to satisfy the consumer's preferences due to the fact that total carotenoid of corn was only 22 mg/kg (Kijparkorn *et al.*, 2010), which was lower than forage materials (Hammershøj *et al.*, 2010). Therefore the use of several natural materials to improve yolk color have been implemented in layers such as marigold and skin orange (Hasin *et al.*, 2006), marigold flower (MF), safflower petals (SP), red pepper meal (RPM) (Rowghani *et al.*, 2006), sano flower (Kijparkorn *et al.*, 2010), paprika (Lokaewmanee *et al.*, 2011), turmeric root (TR) and annatto seed (AS) (Laganá *et al.*, 2011) and duckweed in laying ducks (Indarsih and Tamsil, 2012). Indonesian small farmer cannot afford it because of limited commercial and natural availability as well as relatively expensive.

A natural source of xanthophylls, such as green algae from which its lutein is important for pigmentation in fish and poultry (Jin *et al.*, 2003) is a potential coloring agent. Green algae are different from the other plant divisions because they have a clear green color and contain the pigment chlorophyll: a, b and c, carotene and xanthophylls (Lewis and McCourt, 2004). Green algae (*unidentified chlorophyta*) growing in fresh water is probably one of alga groups which belongs to macroalgae and the largest group of algae (Chojnacka *et al.*, 2012) and a well-known water grass by duck growers. Feeding with 5%

macroalgae (*Ulva lactuca*) improved fish growth and as a protein source replacing common fish meal (Wassef, 2005). This potential macroalgae would be beneficial to reduce feed cost and improved performance when successfully applied. However, to the best of our knowledge, no previous studies have investigated the use of fresh water green algae (*chlorophyta*) for ducks. Therefore this study was conducted to evaluate fresh water green algae as a source of a natural pigment.

## MATERIALS AND METHODS

### Animals and management

A total of 80 thirty-six wk-old were obtained from the local breeder, then wing banded and weighed individually and reared in grouped-housed in an intensive barn from bamboo with average density of 4 birds/m<sup>2</sup> and zinc roof in an open-sided house with uncontrolled temperature. Floors were constructed from solid soil with rice hulls as the litter. The cages were equipped with two rounded plastic feeder with 20 cm in diameters for a feeder trough and for drinking water. Routine management practices such as health and disease control programs were applied with the ground for combating the recent disease outbreak on new castle diseases in Indonesia.

### Diets and Treatments

The birds were assigned to a completely randomized design. Eighty laying ducks were divided into 4 groups of dietary treatments with four replicates (20 birds/dietary treatment) and fed on a commercial layer concentrate containing 38 percent crude protein and mixed with yellow corn and rice bran in ratio of 2:4:4 respectively according to a commercial standard feed acting as the control group. This formula was commonly used and applied by the local growers. The three other dietary treatments were included 2, 4 and 8% of dry matter. Therefore the ratio of the basal diets was in a range to contain from 2,600 to 2,800 kcal of ME/kg until 8 weeks of the experimental period. Feeding method was restricted to 190 g/bird/day and provided twice a day (6.00 and 17.00) to avoid the over spilling feed. Birds had free access to drinking water during the experimental period. The diets were offered in a slurry form. Spillage of the feed was calculated by weighing and drying the remaining feed on the feeder. The fresh water green algae (*unidentified chlorophyta*) were harvested from

the local rivers near the duck farm, then washed and chopped into small size before feeding.

Prior to the study, all feed ingredients were investigated for the proximate composition (Table 1) according to the methods of the Association of Official Analytical Chemists (AOAC, 1995) and performed in the analytic laboratory of Mataram University.

### Measurements

The data on feed consumption, egg production and egg weight were recorded daily from 36 to 44 weeks (for the 8- wk of the experimental period). Egg quality characteristics viz. albumen index, egg yolk index, Haugh unit, shell thickness and yolk pigmentation were measured on wk 1 to 6 of the experimental production period. A total number of 192 eggs (8 eggs from each dietary treatment) per collection were taken and broken out on a clean Petri dish and then yolk color was measured against the Roche Yolk Color Fan scale (RYCF) as a tool for a standard color. This work was carried out at the Laboratory of Animal Product Processing Technology- Faculty of Animal Science, Mataram University.

### Data Analysis

The data collected for 8 wk of the experimental study were analyzed as a completely randomized design and subjected to the General Linear Model (GLM) procedure of SAS version 8 (SAS, 1999). To compare means, Duncan's multiple range test was applied at the level of less than 5%.

## RESULTS AND DISCUSSION

### Production Performance

The effects of the green algae inclusion in three different levels are presented in Table 2 and Tabel 3. There were significant differences observed all dietary treatments including the control diet groups for egg production, egg weight feed/kg eggs, and feed intake. Feeding green algae from 2 to 4% into the layer diets did not have any negative effect on egg production. However, by adding 8% a change in performance was observed which reduced egg production to 46.7%. This is inconsistent with previous studies (Rowghani *et al.*, 2006; Esfahani-Mashhour *et al.*, 2009; Kijparkorn *et al.*, 2010; Lokaewmanee *et al.*, 2011), who found no significant production performance due to additives. Our study indicates that green algae fed at lower levels (T-1 and T-2) improves feed conversion ratio (FCR), explaining the nutrient content of green algae particularly protein have contributed to the egg production. The T-3 groups, however, because of the lowest dietary energy and the highest dietary crude fibers increased the feed consumption at the expense of the lower production performance. In the work of Abudabos *et al.* (2013) where broilers were fed 0, 10 and 30 g/kg green seaweed (*Ulva lactuna*), the best results were noted in the groups with addition of 30 g/kg of green seaweed. Dressing percentage and breast meat yield performed higher in the birds fed 30 g/kg of green seaweed than those fed 10 g/kg. Similar trends were also observed for serum lipid, cholesterol and uric acid concentration. The reasons were claimed due to higher contents of limiting amino acids

Table 1: Chemical Composition of the Dietary Ingredients (%)

Chemical Composition	Ingredients			
	Commercial Protein Concentrate (CC)	Green Algae (GA)	Yellow Corn (YC)	Rice Bran (RB)
Moisture	11.00	89.25	11.75	9.46
Crude protein (CP)	38.01	21.74	8.53	6.80
Crude fibre (CF)	5.00	27.40	2.64	20.00
Ether extract	2.60	2.98	2.83	3.06
Ash	2.78	22.19	1.94	18.20
NFE*	40.62	25.69	84.06	51.94

\*Nitrogen-Free Extract (calculated by difference) = 100 – (protein + lipid + ash + fiber)

Table 2. Ingredient and Calculated Nutrient Contents of the Experimental Diets

Ingredient (%)	Dietary Treatment			
	T-0	T-1	T-2	T-3
Rice bran	40	40	40	40
Yellow corn	40	38	38	38
Commercial concentrates	20	20	18	14
Green algae	0	2	4	8
Calculated analysis				
Metabolizable energy (ME) (kcal/kg)	2,780	2,734	2,687	2,600
Crude protein (%)	13.77	14.07	13.22	13.09
Crude fibre (%)	10.06	10.20	10.65	11.55
Calcium (%)	2.70	2.82	3.27	2.40
Available phosphorus (%)	1.02	1.10	1.19	1.36

Ducks fed RB 40%, YC 40%, CC 20% , with no GA (T-0); ducks fed RB 40%, YC 38%, CC 20%, GA 2% (T-1); ducks fed RB 40%, YC 38%, CC 18%, GA 4% (T-2); ducks fed RB 40%, YC 38%, CC 14%, GA 8% (T-3)

Table 3. Effect of Different Levels of Green Algae on Production Performance of the Laying Ducks During The 8 -Wk Experimental Period (From Wk 36 to 44)

Performance	Dietary Treatment				
	T-0	T-1	T-2	T-3	SEM
Feed intake (g/bird)	154.0 <sup>b</sup>	161.6 <sup>ab</sup>	168.7 <sup>a</sup>	171.9 <sup>a</sup>	2.4
Egg production (%)	53.9 <sup>b</sup>	68.2 <sup>a</sup>	71.7 <sup>a</sup>	46.7 <sup>b</sup>	4.2
FCR (kg feed/kg eggs)	4.72 <sup>b</sup>	3.88 <sup>b</sup>	3.74 <sup>b</sup>	5.76 <sup>a</sup>	0.38
Mortality (%)	0	0	0	0	

<sup>a-b</sup> Means within a row with no common superscript differ significantly ( $p < 0.05$ ). Ducks fed RB 40%, YC 40%, CC 20% with no GA (T-0); ducks fed RB 40%, YC 38%, CC 20%, GA 2% (T-1); ducks fed RB 40%, YC 38%, CC 18%, GA 4% (T-2); ducks fed RB 40%, YC 38%, CC 14%, GA 8% (T-3) SEM: standard error of mean.

(methionine and threonine) of green seaweed. A study of Indarsih and Tamsil (2012) showed the much lower productive performance of laying ducks fed with 20% duckweed than this present study may explain the potential green algae as an additive or a feedstuff for ducks can be fed up 4% for the maximum egg production.

#### Egg quality

The dietary treatments had no significant

effect on egg yolk index, egg weight, Haugh unit and egg shell thickness (Table 4). This is consistent with earlier studies (Kijparkorn *et al.*, 2010; Abiodun *et al.*, 2014). However, the yolk color was significantly changed ( $p < 0.05$ ) according to the added levels of green algae into the diet. The differences in yolk color between the experimental diets showed that an increase in pigmentation of egg yolk color was also dependent on the differences feed intake from

which algae intake was also different. This is similar to previous studies (Rowghani *et al.*, 2006; Laganá *et al.*, 2011; Abiodun *et al.*, 2014). In the present study, improvement in yolk colors was observed 7 days after feeding the test diets, and yolk color hanged rapidly in a response to feeding green algae, showing the average values were higher than the control depending on the treatments, then it remained constant until observation of day 42. The highest level showed the best pigmentation (15.0) (Table 5). A study of Rowghani *et al.* (2006) showed that the yolk color improvement by adding marigold flower, safflower petals, and red pepper meal was longer than this present study. These authors claimed that increased yolk color by feeding basal diets (wheat

and yellow corn based diet) with those additive sources can be observed 10-13 days after feeding. The work of Laganá *et al.* (2011) showed the linier improvement in yolk color for birds fed 2% annato seed from day 0 to 28 with maximum score was 11.8. It means that green algae used in our study contained high xanthophylls and were fast deposited into yolk although the content of xanthophylls of green algae was not analyzed. It is interesting to note that 4% (40 g/kg) green algae in the present study produced RYC values higher than 4% using marigold or skin orange (13.33 versus 8.2 or 3.3) as observed by Hasin *et al.* (2006) which marigold and skin orange contained 156.32 mg/kg and 83.02 mg/kg DM xanthophylls respectively. In other studies, egg

Table 4. Egg Quality From Laying Ducks Fed a Diet Containing Different Levels of Green Algae During the 8- Wk Experimental Period (From Wk 36 to 44)

Variable	Dietary Treatment				SEM
	T-0	T-1	T-2	T-3	
Egg yolk index	0.466	0.443	0.432	0.374	0.005
Albumen index	0.127	0.130	0.136	0.135	0.007
Egg weight (g)	62.97	65.71	66.22	67.59	1.29
Haugh unit	90.87	94.94	86.03	99.03	6.14
Egg shell thickness (mm)	0.47	0.45	0.46	0.43	0.01
Egg yolk color (RYC)	9.95 <sup>c</sup>	3.90 <sup>b</sup>	14.28 <sup>b</sup>	15.00 <sup>a</sup>	0.17

<sup>a-c</sup> Means within columns with no common superscript differ significantly ( $p < 0.05$ ). Ducks fed RB 40%, YC 40%, CC 20% (T-0); ducks fed RB 40%, YC 38%, CC 20%, GA 2% (T-1); ducks fed RB 40%, YC 38%, CC 18%, GA 4% (T-2); ducks fed RB 40%, YC 38%, CC 14%, GA 8% (T-3).

SEM: standard error of mean

Table 5. Yolk Color of Duck Eggs Fed Diets Containing Different Inclusion Levels of Green Algae During the 8- Wk Experimental Period (From Wk 36 to 44)

Treatment	Yolk Color Values on Different Observation Days					
	7	14	21	28	35	42
T-0	9.01	9.73	10.71	9.73	9.31	10.71
T-1	13.73	13.71	14.00	13.70	14.00	14.22
T-2	14.03	14.32	14.31	14.31	14.32	14.34
T-3	15.00	15.00	15.00	15.00	15.00	15.00

Ducks fed RB 40%, YC 40%, CC 20% with no GA (T-0); ducks fed RB 40%, YC 38%, CC 20%, GA 2% (T-1); ducks fed RB 40%, YC 38%, CC 18%, GA 4% (T-2); ducks fed RB 40%, YC 38%, CC 14%, GA 8% (T-3)

yolk color reached to  $13.70 \pm 1.4$  RYC with 20% added duckweed (Indarsih and Tamsil, 2012). In a study where layers were fed with 2% annato seed, pigmentation showed intensively after 28 days, reached to 11.8 (Laganá *et al.*, 2011). The highest yolk color index (5.5) was observed by feeding 1% of costmary (Nobakhtand Moghaddam, 2013). Feeding with 2% red algae in laying hens, yolk color was lower ( $12.51 \pm 0.87$ ) than the control diet ( $13.21 \pm 0.69$ ) as the company's formula (Ozaki *et al.*, 2013). Observed by Moeini *et al.* (2013), incorporating of 3% red pepper in 65% corn diet, resulted in 13.3 RYC. This clearly suggests that green algae can be used as a natural potential coloring agent for producing desirable egg-yolk color at low levels. A possible explanation for this is again that the level of b-carotene and xanthophylls in fresh green algae was quite high. Therefore further studies are needed to clarify more details concerning the xanthophylls content and to identify the species of this *chlorophyta*.

## CONCLUSIONS

The results of the present study indicated that fresh-water green algae could be used as a natural coloring agent in laying ducks. To the best yolk color values (15.0) was observed in a diet containing 8% of green algae added into a conventional ration for laying ducks without any negative effect on productive performance.

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