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The use of Golden snail (*Pomacea canaliculata*) egg as source of carotenoid for improvement of Arabic Chicken egg quality

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Abstract. Consumers awareness of the usage of natural product is increasing but exploration of natural carotenoid originated from animal was conducted into a lesser extent. The objective of this study was to examine the use of Golden snail egg powder (GESP) for improvement of quality of Arabic chicken egg. Thirty six Arabic chicken (silver and golden chicken, 22 weeks of age, 1,123.9 $\pm$ 155.1 g initial body weight) were assigned in completely randomized design (CRD), consisting of 4 dietary treatments (R0 = control 0%; R1 = 4%; R2 = 8%; and R3 = 12% level of GESP) and 3 replications. Data were analysed using one way ANOVA with homogeneity and normality tested in advance. After one month of experiment, data showed that up to 12%, yolk carotenoid and yolk score significantly increased in line with dietary GESP level. However Haugh unit and yolk indices were not affected by GESP. This result indicates that Golden snail egg can be used as carotenoid for improvement of chicken egg quality.

1. Introduction

Chicken egg is widely consumed around the globe due to its essential nutrients content and inexpensive price[1]. Amongst poultry eggs that is available in Indonesia, kampong chicken is known to produce good quality egg compare to laying chicken. However, supply of kampong chicken egg is limited by its productivity, being below 100 eggs per annum[2]. Facing this situation, there was a shift of egg production, from kampong chicken to Arabic chicken[3].

Arabic chicken is local laying hen that has been introduced from Europe. It has prominent productivity compare to kampong chicken[3]. Despite its high productivity, egg quality of Arabic chicken is lower than kampong chicken. The main parameter of egg quality is the coloration of yolk[4] which has positive correlation with its carotenoid content. In this regards, yolk coloration of Arabic chicken egg tends to light or yellowish orange. Consumers, especially in Indonesia, prefer to choose strongly coloured yolk and the yolk colour of orange is associated with healthy egg[5].

Carotenoid is natural pigments that can be found in plants, algae as well as photosynthetic bacteria. Molecular structure of carotenoids were reported more than 1,100 forms[6] such as β-carotene, lutein and zeaxanthin. Alongside pigmentation, carotenoids play several functional roles in poultry, for example β-carotene is a pro-Vitamin A which is later converted to Vitamin A in intestinal mucosa. Furthermore, β-carotene and Vitamin E is able to stabilise oxidation of broiler meat[1; 7; 8].

Like other animals, birds cannot synthesis *de novo* carotenoid but it must present in integument as well as in egg. The existence of carotenoid in chicken egg is maternally carried over during egg formation[9; 10]. Thus, improvement of yolk colour in chicken can be done by incorporating dietary carotenoid. In commercial poultry farm, synthetic carotenoid, such as Carophyll® red[11], was elaborated *via* diet supplementation to achieve better pigmentation of yolk and meat. However, since consumers have concerned on the use synthetic product in food and feed, the interest of exploration of alternative source of natural carotenoid has been increasing. In compound feed, maize is a common ingredient which is formulated as energy source and it is also concomitant to carotenoid source. During previous decades, exploration of natural carotenoids were focused on vegetable source and it is well documented that carotenoid from tomato[12], carrot[13], marigold[14], red pepper[15], and microalgae: *Spirulina*[1; 16] successfully increased egg quality.

Golden snail (*Pamacea canaliculata*) egg is potential candidate of alternative natural carotenoid. Golden snail is invasive species that has high reproductive capacity. This oviparous animal rapidly occupy wet vegetation, spreading to tropical until sub-tropical region[17]. Golden snail has become a threat for ecosystem[18] and rice production due to their high ability in grazing young and emerging rice vegetations. Golden snail egg contains astaxanthin and total carotenoid as much as 40%[19] and 313.48 ppm[20] respectively. To our knowledge, the use of animal source carotenoid as diet in poultry is studied into a lesser extent. Hence the objectives of this study is to examine the use of Golden snail egg meal for improvement of quality of Arabic chicken egg.

2. Material and methods

2.1. Animal, experimental design, and diets

The research was conducted in State Polytechnic of Jember during 2 months. Chicken were rearing in an opened house using cage system. Prior to the experiment, birds were selected for its healthiness and uniformity. Thirty six Arabic chicken from Blitar region (silver and golden chicken, 22 weeks of age, 1,123.9 + 155.1 g initial body weight) were assigned in completely randomized design (CRD). Golden snail egg powder (GSEP) was made according to [21] and formulated as dietary treatments, namely R0 = control 0%; R1 = 4%; R2 = 8%; and R3 = 12% GSEP. Feed was *iso*-energetic and *iso*-nitrogenous. Feed composition is presented in Table 1. Treatment last for 1 month and each treatment was replicated 3 times. Feeding and drinking were given *ad libitum*.

2.2. Egg quality parameters

Yolk carotenoid (YC) was analysis using spectrophotometer[22]. Briefly, 1 g of yolk put in a flask that already added with 50 ml acetone. The solution was mixed then filtered (Whatman Nr. 4) then recovered acetone was diluted to 100 ml. Further it was measured in a spectrophotometer at 450 nm wavelength. Yolk colour score (YS) was measured using yolk Roche colour fan Haugh unit (HU) was calculated using the formula $HU = 100 \times \log (H - 1.7 \times W^{0.37} + 7.6)$, where H is the length of the albumen (mm) and W is the weight of the whole egg (g). Yolk indices (YI) was calculated by dividing yolk height (mm) with yolk width (mm).

2.3. Statistical analysis
Data of egg quality analysed using one way analysis of variance (ANOVA) after normality and homogeneity checked. Significant result of egg quality was further post hoc, using least significant different (LSD). Statistical analysis was conducted using SPSS 16.0.

Table 1. Formulation and composition of experimental feed.

Ingredient	Dietary treatment (%)			
	R0	R1	R2	R3
Maize	52	49	46	43
Soybean meal	29.3	28.5	24.6	23.4
Rice bran	17.2	17.5	20.4	20.6
GESP	0	4	8	12
Palm oil	0.5	1	1	1
Premix	1	1	1	1
Total	100	100	100	100
Analysed composition				
Crude protein	17.9	17.9	17.1	17.0
Crude fibre	1.5	1.6	1.8	1.9
Crude fat	4.9	5.2	5.5	5.4
Calculated composition				
Carotenoids[1]	17.16	28.69	40.22	51.75
Ca[2]	0.19	0.25	0.31	0.38
P[2]	0.27	0.26	0.25	0.24
Metabolizable energy[3] (kcal/kg)	2,874	2,849	2,800	2,735

[1] Based on carotenoid of maize and GESP
[2] Based on manufacturer (Medion – Indonesia)
[3] Laboratory of Feed Technology, State Polytechnic of Jember

3. Result and discussion
Egg quality of Arabic chicken fed various level of GESP is presented in Table 2. Yolk carotenoid (YC) of Arabic chicken fed diet containing GESP was in the range of 6.95 µg/g to 12.23 µg/g, meanwhile yolk score (YS) was in the range of 6.00 to 14.40. Both YC and YS are statistically affected by GESP ($P<0.05$). Data showed that up to 12 % of GESP level, YC and YS content increases in line with increasing dietary level of GESP and bird fed R3 exhibits the highest YC and YS. Haugh unit (HU) value is in the range of 87.36 until 99.90, meanwhile yolk indices (YI) is in the range of 0.43 until 0.46. GESP level did not affect ($P>0.05$).

The results of this research was in agreement with previous finding in[23, 24, 25], in which improvement of yolk colour happened in laying hen fed natural lutein and zeaxanthin from marigold flower in which 40 mg/kg of lutein increased the redness of yolk and tomato powder at 5 or 10 g/kg basal diet showed increasing lycopene content. Further, utilisation of xanthophyll from marigold can be deposited 13.4 % - 20.4 % in egg yolk. Data from this research showed that deposition of carotenoid in egg yolk decreased from 40.5 % - 23.63 % from total carotenoid consumed via GESP level. The efficiency of natural carotenoids depends on the types of carotenoid and the duration of carotenoids supplementation[26].

Table 2. Egg quality of Arabic chicken hen fed dietary treatment containing GESP

Parameters	R0 (0% GESP)	R1 (4% GESP)	R2 (8% GESP)	R3 (12% GESP)	p-value
YC (µg/g)	6.95[a]	9.7[b]	11.01[bc]	12.23[c]	0.01
YS	6.00[a]	12.51[b]	13.91[bc]	14,40[c]	0.01
HU	90.65	88.88	87.37	99.90	0.71
YI	0.46	0.43	0.46	0.45	0.25

Means value in a row with unlike letters were significantly different (P<0.05) assigned using LSD

The quantification of yolk carotenoid of this research was different from [23] and [24] due to analytical methods that has variation in its detection limits. Although those of investigators used different analytical protocol, it can be inferred that birds have ability to utilised natural carotenoid that has been incorporated in diet. Carotenoid is essential pigment for improvement of egg quality. In the present research, GESP represents the source of carotenoid originated from animal. Our data suggested that dietary GESP can be utilised by Arabic chicken and eventually yolk coloration increased toward intensely orange colour in line with GESP level (Figure 1).

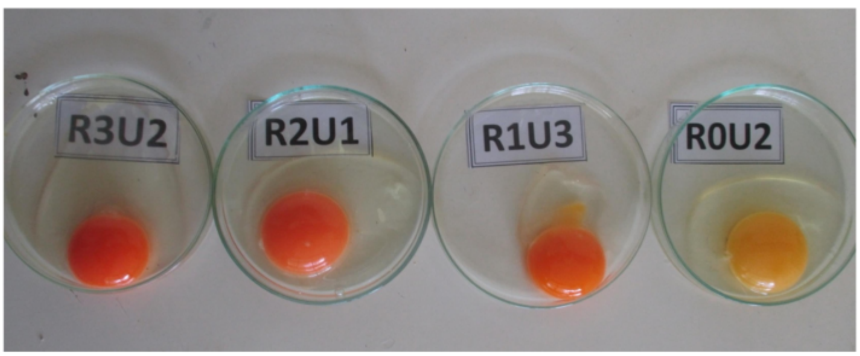

Figure 1. Yolk colour of Arabic chicken fed various level of GESP (right to left R3, R2, R1 and R0 accordingly).

It is notably that total carotenoid consumed by Arabic chicken at present experiment was not merely on GESP contribution. Maize was elaborated as ingredient in this experimental feed. Therefore, yolk colour was derived from both GESP and maize. Since there was decreasing level of maize and increasing level of GESP in (R0 to R3), it can be indicated that GESP is possible to replace maize although degree of replacement was low.

HU and YI are intrinsic parameter of egg quality in which the higher value of HU and YI is considered as high quality. The value of HU in Arabic chicken is higher than egg of 35 weeks old laying hen at room temperature, reported 67.608 of HU [27]. Other investigators reported that HU of 50 weeks old laying hen was 64,84 (fresh egg) and YI was 44.09%[28] and 43.7% - 44.8%[16] .

According to several researchers[27; 29], HU and YI depend on some factors such as dietary protein, storage, and hen strain. These factors were maintained to be identical in this experiment, with the exception of total carotenoid content. As a result, HU and YI values is similar.

4. Conclusion

To sum up, GESP significantly improved quality of Arabic chicken egg with regard of yolk carotenoid and yolk colour score ($P<0.01$). However Haugh unit and yolk indices were not affected by GESP ($P>0.05$). Golden snail (*Pamacea canaliculata*) egg is a potential candidate of alternative natural carotenoid for improvement of Arabic chicken. Further study on maximum level of GESP is recommended to provide comprehensive information for its efficiency as dietary supplement.

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