The effect of adding green concentrate based on *Azolla pinnata* and *Spirulina platensis* on the quality of quail eggs (*Coturnix japonica*)

Satria Budi Kusuma[1], *Adib Norma* Respati[1], *Suci* Wulandari[1], *Amal* Bahariawan[2], *Dharwin* Siswantoro[1], *Dadik* Pantaya[1*], and *Muhammad* Rozaq[1]

[1]Department of Animal Science, Politeknik Negeri Jember, PO BOX 164 Jember, East Java, Indonesia
[2] Department of Agricultural Technology, Politeknik Negeri Jember, PO BOX 164 Jember, East Java, Indonesia

Abstract. The study evaluated the effect of Azolla pinnata and Spirulina platensis-based green concentrates on quail (Coturnix japonica) egg quality. A completely randomized design was employed with three treatments: P0 (control), P1 (10% Azolla), and P2 (10% Azolla + 0.1% Spirulina), each with five replicates. Results indicated that while egg weight and Haugh Unit remained unaffected ($p>0.05$), yolk colour score was significantly enhanced ($p<0.05$) in P2 compared to the control. The study This study concludes that the strategic combination of 10% Azolla pinnata and 0.1% Spirulina platensis effectively enhances quail egg yolk pigmentation without compromising egg weight or internal quality. These findings imply that this green concentrate formulation offers a viable, natural nutritional strategy for poultry producers to improve egg marketability by meeting consumer preferences for deeper yolk colour, potentially adding economic value without relying on synthetic pigments.

1 Introduction

Quails are one of the poultry species that have been popularly bred by Indonesian farmers. The species (Coturnix japonica) is the most widely used quail species in Indonesia and is widely accepted as a source of meat and eggs. Quail productivity can reach 250–300 eggs/year with an average weight of 10 g/egg [1].

Feed is given to quails to meet their daily needs, so that their basic needs are fulfilled and they can produce eggs. Complete and sufficient feed to meet the needs of quails is the most important aspect in quail care. The requirements for good feed are that it does not cause disease, is safe for consumption and can be digested by livestock. Feed costs can reach 60-80% of total production costs [2]. Therefore, it is important to find alternative feed ingredients that are more economical, readily available, and sustainably sourced. One such alternative feed ingredient that is readily available and sustainably sourced is azolla. *Azolla pinnata* is an aquatic plant that has potential as a supplementary feed ingredient for poultry

* Corresponding author : dadik_pantaya@polije.ac.id

due to its good nutritional content [3]. In Jember, the price of 1 kg of ready-to-harvest azolla is Rp300. Meanwhile, to produce 1 kg of azolla powder, 10 kg of fresh azolla is required, so the price of 1 kg of azolla powder is around Rp3000.
The colour of eggs is one of the main considerations for consumers when purchasing eggs. Most consumers prefer eggs with a golden yellow/reddish yellow colour. On the other hand, if the yolk appears pale, consumers tend to dislike it and consider it less tasty and less nutritious. The colour of quail egg yolks can be enhanced by feeding them a diet rich in colour pigments. One feed additive that can be used as a source of egg pigmentation is *Spirulina platensis* [4]. Based on the above description, it is necessary to conduct research on the addition of *Azolla pinnata* as an alternative feed and *Spirulina platensis* as a feed additive, which is expected to improve the quality of quail eggs.

2 Materials and Methods

The research was conducted from August to September 2025 at the poultry house of Politeknik Negeri Jember.

2.1 Materials

The equipment used in this study consisted of tarpaulin pools, battery cages, feeders, drinkers, digital scales, ovens, oven trays, egg trays, blenders, measuring cups, yolk colour fans, buckets, plastic, glass, calipers, basins, recording books, label paper and writing instruments. The materials used in the study included: 8-week-old female quails, quail layer concentrate, corn, rice bran, palm oil, *Azolla pinnata* powder, *Spirulina platensis*, AB mix fertilizer, disinfectant, detergent, lime, husks, premix, vitamins and medicines.

2.2 Methods

The feeding trial was conducted for 6 weeks. The housing condition was used open house system. With temperatures between 25 – 29°C and cage humidity between 67 – 79%. This study used a completely randomized design (CRD) with quails, consisting of three treatments and six replicates, each replicate consisting of three quails, with the following percentage combinations of *Azolla pinnata* and *Spirulina platensis* flour in the feed.
P0 = a complete feed without *Azolla pinnata* and *Spirulina platensis* powder (control).
P1 = a complete feed containing 10% *Azolla pinnata* powder.
P2 = a complete feed containing 10% Azolla pinnata powder and 0.1% Spirulina platensis.
Feed was administered at a rate of 25 g/quail/day and drinking water was provided ad libitum. Azolla is obtained from cultivation in tarpaulin ponds around quail research pens. Egg weight, Haugh unit, and egg yolk colour were observed. Egg yolk colour was determined using a yolk colour fan to transform qualitative visual assessment into quantitative data. The procedure involved separating the yolk from the albumen and placing it on a flat white background to minimize colour bias. The yolk colour was then visually compared against the 15 standard colour blades of the fan under consistent lighting conditions. Each blade corresponds to a numerical score ranging from 1 (pale yellow) to 15 (dark orange). The score of the blade that most closely matched the yolk colour was recorded as the quantitative value for that sample. Tables below show the proportion of feed ingredients (Table 1) and nutrient content (Table 2) of the feed administered.

Table 1. Feed ingredients

Feed Ingredients	P0 (%)	P1 (%)	P2 (%)
Corn	57	53.5	53.5
Quail layer concentrate	37	28.5	28.5
Rice brand	3	10	10
Premix	1	1	1
Palm oil	2	2	2
Azolla flour	0	10	10
Spirulina	0	0	0,1
Total	100	100	100

Table 2. Feed nutrient content

Nutrien	P0	P1	P2
Crude protein (%)	19.41	19.26	19.26
Crude fat (%)	4.23	4.23	4.23
Crude fiber (%)	3.72	4.39	4.39
Metabolite energy (kkal/kg)	2915.70	2907.09	2907.09
Ca (%)	2.21	2.67	2.67
P (%)	0.62	0.65	0.65

The parameters observed included egg weight and Haugh unit [4], and egg yolk colour. Data analysis in this study used a completely randomised design (CRD) with a one-way pattern. The data was then processed using SPSS version 20 (Statistical Package for the Social Sciences). If there was data showing significant differences, it was further analysed using the Duncan Multiple Range Test (DMRT). Significant differences in this study were determined based on a probability of less than 5%.

3 Results and Discussion

The results of the study on the addition of Azolla pinnata and Spirulina platensis to egg weight, Haugh unit, and yolk colour in quails can be seen in Table 3.

Table 3. Egg weight, Haugh unit, and yolk colour of quail eggs with the addition of Azolla pinnata and Spirulina platensis (gram)

Parameter	Treatment		
	P0	P1	P2
Egg weight	11.48±0.46^{ns}	10.83±0.87^{ns}	11.47±0.89^{ns}
Haugh Unit	58.07±1.39^{ns}	57.91±2.23^{ns}	56.83±1.56^{ns}
Egg yolk colour	7.42±0.86^{b}	10.17±0.75^{a}	10.25±0.82^{a}

Note:
^{a,b} Different superscripts on the same line indicate significant differences (P<0.05).
^{ns} = Same superscripts on the same row indicate no significant difference (P>0.05).
P0: a complete feed without *Azolla pinnata* and *Spirulina platensis*, P1: a complete feed containing 10% *Azolla pinnata*, P2: a complete feed containing 10% *Azolla pinnata* and 0.1% *Spirulina platensis*

3.1 Egg weight

Table 3.1 shows that the addition of *Azolla pinnata* and *Spirulina platensis* in the ration had no significant effect (P>0.05) on egg weight. Egg weight is influenced by feed quality, such as protein content. Protein and amino acids are the nutrients that play the most important role in controlling egg size and egg productivity[5]. The crude protein content in the feed ration given to the control group and the treatment group was not different, so the weight of the quail eggs produced was also not significantly different.

Factors affecting egg weight include hormones, age, and protein consumption [6]. In addition, egg weight is also influenced by the size of the quail's body. Quail eggs weigh 7% to 8% of the quail's weight, ranging from 7 to 11 grams per egg [7]. The egg weight results obtained in the study were classified as good, with a good egg weight ranging from 8 to 11.91 grams per egg [6].

3.2 Haugh unit

The results of research on the addition of Azolla pinnata and Spirulina platensis flour to the Haugh unit of quail eggs can be seen in Table 3.1. Based on Table 3.1, the addition of *Azolla pinnata* and *Spirulina platensis* to the ration had no significant effect (P>0.05) on the Haugh unit of eggs. Egg quality can be measured based on the HU (Haugh Unit) value, which is determined by the height of the albumen. The higher the HU value, the higher the albumen, indicating better egg quality and suggesting that the eggs are fresh or newly laid [8].

The Haugh unit of an egg is influenced by the condition of the egg white, namely the correlation between the weight of the egg (grams) and the height of the egg albumen (mm). Protein deficiency can result in a decrease in egg size and the amount of egg albumen [4]. The height of the egg whites was also influenced by the protein content in the feed, whereas the protein content of each treatment in the ration did not differ significantly, so the addition of Azolla and Spirulina had no significant effect.

HU values are categorized into four grades : AA quality eggs have an HU value >72, A quality eggs have an HU value of 60-72, B quality eggs have an HU value of 31-60, and C quality eggs have an HU value <31 [9]. The Haugh Unit value from this study is classified as grade B. A decrease in Haugh Unit value can occur due to changes in temperature, increased humidity causing the loss of carbon dioxide (CO2), and the length of time the eggs are stored. The longer they are stored, the more the Haugh Unit value will decrease. This

occurs due to the evaporation of CO2 gas, which causes the thick egg white to become thin [10].

3.3 Egg yolk colour

The results of the study on the addition of Azolla pinnata and Spirulina platensis to the yolk colour of quail eggs can be seen in Table 3.1. Table 3.1 shows that the addition of Azolla pinnata and Spirulina platensis to the feed had a significant effect ($P<0.05$) on the yolk colour. These results indicate that the addition of Azolla pinnata and Spirulina platensis can affect egg quality colour, due to the high content of carotenoid compounds in Azolla pinnata and Spirulina platensis. Feed affects the colour of egg yolks, namely feed ingredients that contain carotenoid pigments, especially β-carotene and xanthophyll pigments [8].

Spirulina platensis contains carotenoids and xanthophylls as sources of pigment. The administration of carotenoids in appropriate and continuous doses can produce the desired yellow colour [4]. Azolla pinnata also contains beta-carotene and vitamin A [11]. The yellow colour of eggs is determined by feed containing carotenoids, which have a structure like vitamin A. Carotenoids are natural pigments and are widely known for their colours, especially yellow, orange and red. Carotenoids are abundant in xanthophyll pigments. If the feed contains more carotene, namely xanthophyll, the yellow colour of the egg will be more orange red [12]. The significant improvement in yolk colour score observed in Treatment P2 aligns with recent findings by [13], who reported that carotenoids in Spirulina are efficiently transferred to the ovarian follicles. Furthermore, [14] confirmed that Spirulina supplementation in quail diets specifically enhances yolk pigmentation without detrimental effects on internal quality parameters like Haugh Unit. Although Azolla pinnata alone (P1) contributes to yolk coloration due to its xanthophyll content [15], the combination with Spirulina (P2) provides a more intense pigmentation, likely due to the synergistic profile of bioactive compounds that also support physiological homeostasis in quails [16].

One factor used to assess egg quality is the colour of the yolk. The yolk colour in this study is shown in Figure 1. The yolk colour plays an important role in the control group (P0), which shows a pale yellow colour, while the yolk colour in groups P1 and P2 shows a more intense yellow (orange) colour. The increase in yolk colour is due to the carotenoid content in the treatment material. The colour of the egg yolk is determined by a diet containing carotenoids, whereby poultry that consume higher levels of carotenoid pigments will produce egg yolks with a higher colour intensity [12].

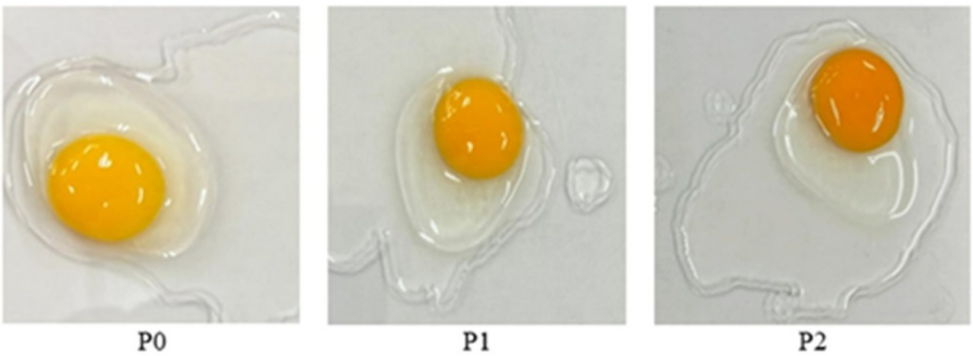

Fig. 1. Egg yolk colour

Note : P0: a complete feed without *Azolla pinnata* dan *Spirulina platensis.*
P1: a complete feed containing 10% *Azolla pinnata*
P2: a complete feed containing 10% *Azolla pinnata* and 0,1% *Spirulina platensis.*

The finding that adding 10% Azolla pinnata and 0.1% Spirulina platensis significantly improves egg yolk colour scores has important practical implications for quail farmers. Egg yolk colour is a primary consideration for consumers when purchasing eggs, with most consumers preferring a golden yellow or reddish yellow colour. The increase in egg yolk colour score from treatments P1 and P2 compared to the control (P0) indicates that these green concentrates successfully meet market preferences.

4 Conclusion

This study concludes that the strategic combination of 10% Azolla pinnata and 0.1% Spirulina platensis effectively enhances quail egg yolk pigmentation without compromising egg weight or internal quality. These findings imply that this green concentrate formulation offers a viable, natural nutritional strategy for poultry producers to improve egg marketability by meeting consumer preferences for deeper yolk colour, potentially adding economic value without relying on synthetic pigments.

The authors would like to express their gratitude to P3M Politeknik Negeri Jember for funding this research through the 2025 PNBP fund with contract number 0918/PL17.4/PG/2025.

References

[1] A.E. Setyawan, E. Sudjarwo, E. Widodo, H. Prayogi, Pengaruh penambahan limbah teh dalam pakan terhadap penampilan produksi telur burung puyuh, Jurnal Ilmu-Ilmu Peternakan 23 (2013) 7–10. http://jiip.ub.ac.id/.

[2] J.W. Utomo, E. Sudjarwo, A.A. Hamiyanti, F. Peternakan, U. Brawijaya, J. Veteran, M.-J. Timur, Pengaruh penambahan tepung darah pada pakan terhadap konsumsi pakan, pertambahan bobot badan, konversi pakan serta umur pertama kali bertelur burung puyuh, Jurnal Ilmu-Ilmu Peternakan 24 (n.d.) 41–48. http://jiip.ub.ac.id/.

[3] M. Sutri Diani, L. Malianti, R. Zurina, N. Definiati, Pengaruh penggunaan tepung azolla microphylla dalam ransum terhadap performan burung puyuh, Jurnal Embrio 1 (2023) 46–56. https://doi.org/https://doi.org/10.31317/embrio.v15i1.890.

[4] H. Tistiana, dan Lutfiah Puti Utami, F. Peternakan, U. Brawijaya, J. Veteran, K. Lowokwaru, K. Malang, J. Timur, The Effect of Spirulina platensis on the Performance and Quality of Quail Eggs, Juni 2023 OPEN ACCES Freely Available Online J. Ternak Tropika 24 (2023) 20–28. https://doi.org/10.21776/ub.jtapro.2023.024.01.4.

[5] S. Nuraeni, M. Anwar Djaelani, dan Kasiyati, Nilai Haugh Unit (HU), Indeks Kuning Telur (IKT) dan pH Telur Itik Pengging Setelah Pemberian Tepung Daun Kelor (Moringa oleifera Lam.), Buletin Anatomi Dan Fisiologi 4 (2019) 107–115. https://doi.org/https://doi.org/10.14710/baf.4.2.2019.107-115.

[6] R. Arizona, A.R. Ollong, Kualitas Telur Puyuh Selama Penyimpanan dan Temperatur yang Berbeda, Jurnal Ilmu Peternakan Dan Veteriner Tropis (Journal of Tropical Animal and Veterinary Science) 10 (2020) 70. https://doi.org/10.46549/jipvet.v10i1.95.

[7] S.I. Gubali, Pertumbuhan burung puyuh (coturnix coturnix japonica) umur 3 minggu dengan perbedaan kepadatan di dalam kandang, Jambura Journal of Animal Science 4 (2021) 79–87. https://doi.org/10.35900/jjas.v4i1.12003.

[8] E.C. Pritisa, U. Santoso, Y. Fenita, Pengaruh tepung daun kelor dalam ransum sebagai pengganti feed supplement komersial terhada kualitas telur puyuh, Jurnal Inspirasi Peternakan 2 (2022) 322–332. www.jurnal.umb.ac.id.

[9] USDA, Egg-Grading Manual, The U.S. Department of Agriculture, Washington, 2000. https://doi.org/https://www.ams.usda.gov/sites/default/files/EggGradingManual.pdf .

[10] R.F. Ramadhan, N. Aulia, D. Rusmana, M. Mansyur, Pengaruh Ransum yang Mengandung Azolla microphylla terhadap Indeks Kuning, Indeks Putih dan Haugh Unit Telur Ayam, Jurnal Agripet 24 (2024) 44–50. https://doi.org/10.17969/agripet.v24i1.33500.

[11] I.A. Meilani, M.A. Dzakiy, R. Rakhmawati, Pengaruh variasi dosis yang berbeda dari biowash-promic berbahan limbah kulit umbi-umbian terhadap kandungan protein, berat basah dan berat kering tanaman Azolla pinnata, Jurnal AGRIFO 9 (2024). https://doi.org/https://doi.org/10.29103/ag.v9i1.15906.

[12] R. Manoppo, J.R. Leke, W. Utiah, J. Laihad, Penggantian jagung dengan sebagian tepung pisang kepok (Musa paradisiaca formatypica) dalam ransum terhadap kualitas telur ayam ras petelur MB 402, Zootec 42 (2022) 105–112. https://doi.org/https://doi.org/10.35792/zot.42.1.2022.41169.

[13] M. Salahuddin, A.A.A. Abdel-Wareth, K.G. Stamps, C.D. Gray, A.M.W. Aviña, S. Fulzele, J. Lohakare, Enhancing Laying Hens' Performance, Egg Quality, Shelf Life during Storage, and Blood Biochemistry with Spirulina platensis Supplementation, Vet Sci 11 (2024). https://doi.org/10.3390/vetsci11080383.

[14] S. Bahrami, B. Parizadian Kavan, H. Khosravinia, B. Masouri, M. Mohamadi Saei, Evaluation of the Effects of Spirulina platensis Extract on Productive Performance, Egg Quality and Serum Composition in Laying Japanese Quails Under Dexamethasone-induced Stress, Poultry Science Journal 13 (2025) 205–213. https://doi.org/10.22069/psj.2024.22785.2158.

[15] Seid A, Effect of inclusion of graded levels of Azolla (Azolla pinnata) meal in layers' diets on productive performance and egg quality parameters, Livestock Research for Rural Development 35 (2023). https://doi.org/http://www.lrrd.org/lrrd35/3/3524said.html.

[16] F.S. Nassar, A.A. Alaqil, D.A.A. El-Sayed, N.N. Kamel, A.O. Abbas, Effects of Dietary Intervention Using Spirulina at Graded Levels on Productive Performance and Physiological Status of Quail Birds Reared under Elevated Temperatures, Agriculture (Switzerland) 13 (2023). https://doi.org/10.3390/agriculture13040789.