

THE SEAWEED (*Eucheuma cottonii*) AND EARTHWORM (*Lumbricus rubellus*) MEAL SUPPLEMENTATION: IMPACTS ON OMEGA-3 ENRICHMENT AND EGG QUALITY IN LAYING HENS

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ABSTRACT

High-quality feed with sufficient nutritional content plays an essential role in producing nutrient-dense eggs. This study aimed to evaluate the effectiveness of incorporating *Eucheuma cottonii* seaweed meal and *Lumbricus rubellus* earthworm meal into layer feed on omega-3 fatty acid concentration, yolk color, and egg pH in laying hens. The experiment was conducted at the Teaching Factory (TeFa) Layer Farm, Politeknik Pembangunan Pertanian (Polbangtan) Gowa, Bone Regency. A total of 16 laying hens were assigned to a Completely Randomized Design (CRD) consisting of four treatments and two replicates, with two hens per replicate: P0 (control), P1 (10% *L. rubellus* meal), P2 (10% *E. cottonii* meal), and P3 (10% *L. rubellus* + 10% *E. cottonii* meal). The results showed that the inclusion of 10% *E. cottonii* (P2) produced the highest omega-3 content in eggs. Yolk color differed significantly among treatments ($P < 0.05$), while egg pH did not show significant variation. The omega-3 content in P2 reached 58.8 mg/100 g, yolk color scores ranged from 9.50 to 12.50, and egg pH ranged from 7.12 to 7.97. Overall, the inclusion of 10% *E. cottonii* seaweed meal in layer feed effectively enhanced egg omega-3 levels and improved yolk pigmentation.

Keywords: Laying hens, Eggs, *Lumbricus rubellus*, *Eucheuma cottonii*, Omega-3.

ABSTRAK

Pakan berkualitas tinggi dengan kandungan nutrisi yang cukup berperan penting dalam menghasilkan telur yang kaya nutrisi. Penelitian ini bertujuan untuk mengevaluasi efektivitas penambahan tepung rumput laut *Eucheuma cottonii* dan tepung cacing tanah *Lumbricus rubellus* ke dalam pakan ayam petelur terhadap konsentrasi asam lemak omega-3, warna kuning telur, dan pH telur pada ayam petelur. Percobaan dilakukan di Peternakan Ayam Petelur Pabrik Pengajaran (TeFa), Politeknik Pembangunan Pertanian (Polbangtan) Gowa, Kabupaten Bone. Sebanyak 16 ekor ayam petelur dibagi ke dalam Rancangan Acak Lengkap (RAL) yang terdiri dari empat perlakuan dan dua ulangan, dengan dua ekor ayam per ulangan: P0 (kontrol), P1 (10% tepung *L. rubellus*), P2 (10% tepung *E. cottonii*), dan P3 (10% tepung *L. rubellus* + 10% tepung *E. cottonii*). Hasil penelitian menunjukkan bahwa penambahan 10% *E. cottonii* (P2) menghasilkan kandungan omega-3 tertinggi pada telur. Warna kuning telur berbeda secara signifikan antar perlakuan ($P < 0,05$), sedangkan pH telur tidak menunjukkan variasi yang signifikan. Kandungan omega-3 pada P2 mencapai 58,8 mg/100 g, skor warna kuning telur berkisar antara 9,50 hingga 12,50, dan pH telur berkisar antara 7,12 hingga 7,97. Secara keseluruhan, penambahan 10% tepung rumput laut *E. cottonii* dalam pakan ayam petelur secara efektif meningkatkan kadar omega-3 telur dan memperbaiki pigmentasi kuning telur.

Kata kunci: Ayam petelur, Telur, *Lumbricus rubellus*, *Eucheuma cottonii*, Omega-3.

INTRODUCTION

Chicken eggs are an integral component of the Indonesian diet and serve as an excellent source of animal protein. Containing approximately 12.8% protein and 11.8% fat, chicken eggs are not only rich in essential nutrients but are also widely recognized as a highly nutritious animal-derived food product (Kartikasari et al., 2018). The vitamin A and mineral content in chicken eggs also contributes to the nutritional value of this product in meeting the daily dietary needs of the population (Kralik et al., 2021). As consumer awareness regarding balanced nutrition increases, the demand for food products with enhanced functional properties continues to rise in Indonesia and globally. Eggs enriched with health-promoting components such as omega-3 fatty acids are increasingly viewed as strategic functional foods capable of contributing to public health improvement (Pongtambing et al., 2025).

One of the most widely explored methods for improving egg quality is dietary manipulation. Specifically, the incorporation of natural feed ingredients rich in polyunsaturated fatty acids (PUFAs) has shown substantial potential for increasing the omega-3 content of eggs. Among the available feed resources, *Eucheuma cottonii* (seaweed) and *Lumbricus rubellus* (earthworm) were selected because they are abundantly available in Indonesia, relatively inexpensive, environmentally sustainable, and remain underutilized as feed ingredients in commercial laying hen production. Their utilization also supports the use of locally available resources to reduce dependence on imported feed ingredients while promoting sustainable livestock production. *Eucheuma cottonii* is notably recognized for its high concentrations of omega-3 fatty acids;

studies indicate it contains approximately 45.72% omega-3 fatty acids, outperforming other species of seaweeds (Matanjan et al., 2008; Silva et al., 2024). The nutritional profile of *Eucheuma cottonii* is characterized by high levels of eicosapentaenoic acid (EPA) and other polyunsaturated fatty acids (PUFA), which can contribute to improved health outcomes for consumers of the eggs produced (Silva et al., 2024). Incorporating such a fatty acid-rich source into laying hens' diets can positively impact the fatty acid composition of the eggs, enhancing their nutritional value (Sefer et al., 2011; Berenjian et al., 2021). The use of *Eucheuma cottonii* is also advantageous from a sustainability perspective, as it is abundantly available, cost-effective, and environmentally friendly compared to conventional marine-based omega-3 sources. For instance, the use of marine-derived oils or microalgae has been shown to significantly boost the n-3 PUFA content in eggs, correlating with findings for *Eucheuma cottonii* (Fraeye et al., 2012). Inclusion of earthworm meal from *Lumbricus rubellus* in the diets of laying hens has shown potential benefits in improving egg production and certain hematological parameters, such as hemoglobin and hematocrit levels. Research indicates that supplementation with earthworm meal can positively affect egg quality and the overall health of hens, particularly when included at levels up to 20% in combination with other supplements like seaweed (Arshad et al., 2023). Earthworm meal is also considered a sustainable protein source due to its rapid biomass production, low environmental footprint, and ability to utilize organic waste as a growth substrate. This combination may enhance omega-3 fatty acid content in eggs and support better physiological health.

Based on the regional potential assessment, Bone Regency exhibits strong prospects for the development of omega-3–enriched eggs in laying hens. Awangpone District, in particular, supports this potential, as it hosts several layer farms with a total population of approximately 27,872 birds. However, the use of *Lumbricus rubellus* earthworm and *Eucheuma cottonii* seaweed as alternative

feed ingredients with the potential to enhance the nutritional value of eggs remains largely unfamiliar to local farmers. This condition provides the rationale for conducting the present study, which aims to evaluate the effectiveness of *Eucheuma cottonii* algae meal and *Lumbricus rubellus* earthworm meal in increasing the omega-3 content of eggs produced by laying hens.

MATERIALS AND METHODS

The research was conducted at the Teaching Factory (TeFa) for Laying Hens, Campus II of the Agricultural Development Polytechnic (Polbangtan) Gowa, located in Bakunge Hamlet, Mappesangka Village, Ponre Subdistrict, Bone Regency. The analysis of omega-3 fatty acid content in the eggs was carried out at the Saraswanti Indo Genetech (SIG) Laboratory, West Java. The study was conducted from March to May 2025.

Design, Animals, Diets, and Management:

The experiment is a single-factor experiment arranged as a completely randomized design with 4 treatment groups and 2 replications. A total of 16 ISA Brown laying hens aged 81 weeks were used, with

each experimental unit containing two hens. The hens were randomly allocated into eight experimental units according to the design.

The diets used in the experiment consisted of a basal diet and mixed diets containing *Lumbricus rubellus* earthworm meal and *Eucheuma cottonii* seaweed meal. The equipment used included battery cages, feeders, drinkers, basic processing tools, an oven, a blender, a scale, a Roche Yolk Colour Fan, and a pH meter. Accordingly, the four dietary treatments were defined as follows:

P0= 100% Basal Feed (Control)

P1 = 90% Basal Feed + 10% Earthworm Meal

P2 = 90% Basal Feed + 10% Seaweed Meal

P3 = 80% Basal Feed + 10% Earthworm Meal + 10% Seaweed Meal

Table 1. Nutrient composition of feed

Ingredient	PK(%)	LK(%)	SK(%)
Corn	8.84	3.0	1.01
Concentrate	33	4	8
Earthworm Meal	60.42	16.61	5.74
Seaweed Meal	2.60	0.40	0.90

Notes = Crude Protein (CP), Crude Fat (CF), Crude Fiber (CFb)

Table 2. Nutrient Composition of the Treatment Diets

Ingredient (%)	P0	P1	P2	P3
Corn	50	50	50	50
Concentrate	50	40	40	30
Earthworm Meal	0	10	0	10
Seaweed Meal	0	0	10	10
Total	100	100	100	100
PK	20.92	23.66	17.88	20.62
LK	3.50	4.76	3.14	4.40
SK	4.51	4.28	3.80	3.57

Notes: Analyzed primary data, 2025

The results of the nutritional content analysis including crude protein, crude fat, and crude fiber of each feed ingredient are presented in Table 1. Meanwhile, the total nutritional composition of each treatment diet used in this study is shown in Table 2.

The maintenance period was carried out using the dietary treatments for four weeks. During the first week, feed acclimatization was conducted to allow the chickens to adjust to the changes in feed composition. The feeding treatments from weeks 2 to 4, after the acclimatization period, were recorded as observation days. The chickens were fed twice daily, at 06:00 WITA in the morning and 15:00 WITA in the afternoon. Drinking water was provided *ad libitum*, meaning it was supplied continuously without restriction.

This study was conducted by incorporating seaweed meal and earthworm meal into the feed formulation for laying hens. The samples were selected as representative portions of the population, with the omega-3 analysis performed using 4 eggs collected from each dietary treatment. Egg samples for further analysis were obtained during the fourth week of the rearing period, and all research data were compiled at that stage.

Producing Seaweed Meal and Earthworm Meal:

The procedural steps for producing *E. cottonii* seaweed meal begin with sorting the seaweed to remove impurities, followed by washing it thoroughly with clean water. The seaweed is then soaked in clean water for 12 hours to reduce odor and soften the thallus. After soaking, the seaweed is coarsely chopped to accelerate the drying process. The chopped seaweed is then dried either under direct sunlight for 2–3 days or in a hot-air oven at 60°C for approximately 24 hours until the moisture content reaches approximately 10%. Once dried, the seaweed is ground using a blender to obtain a fine powder. The final step involves sieving the seaweed

meal to produce a more uniform and fine texture (Gultom et al., 2015).

The procedure for producing *Lumbricus rubellus* earthworm meal starts with washing the earthworms thoroughly with clean water to remove any remaining soil particles. The next step involves boiling the earthworms for 10–15 minutes to eliminate mucus. After boiling, the earthworms are chopped into approximately 3 cm pieces to facilitate the removal of gut contents and ensure a cleaner final product. The chopped earthworms are then dried either under direct sunlight for 2–3 days or in a hot-air oven at 60°C for approximately 24 hours until the moisture content reaches approximately 10%. Proper drying is indicated by a brittle texture, characterized by the worms crumbling easily when pressed. Once fully dried, the earthworms are ground into a fine powder. The final step is sieving the earthworm meal to obtain a more refined and uniform texture.

Omega-3 Fatty Acid Content:

The omega-3 fatty acid content of egg yolk was determined using gas chromatography coupled with a flame ionization detector (GC-FID). A 100 mg egg yolk sample was weighed into a 20-mL screw-cap vial, and lipids were extracted using methyl tert-butyl ether (MTBE) according to the method described by Matyash et al. (2008). The extracted lipids were then converted into fatty acid methyl esters (FAMES) through transesterification following the procedure of Ichihara and Fukubayashi (2010). After the addition of hexane and a neutralization solution, the mixture was centrifuged to separate the organic phase. The resulting organic layer containing the FAMES was transferred into a 2-mL vial and injected into the GC-FID system. Fatty acid components were identified by comparing their retention times with those of authenticated FAME standards, and the omega-3 fatty acid content was quantified

accordingly (Matyash et al. (2008); Ichihara & Fukubayashi, 2010).

Egg Yolk:

The egg samples were broken, then measured using a Roche Yolk Color Fan with a score of 1-15, from pale to dark yellow (concentrated), to measure the color of the egg yolk produced. The egg yolk color score was obtained based on color measurements that were close to those on the Egg Yolk Color Fan (Mustakim et al., 2023).

Acidity Level Analysis (pH):

The egg samples were broken, and the egg whites and yolks were mixed until homogeneous; then, the pH was measured

using a pH meter. Before use, the pH meter was stabilized by dipping it in distilled water, then dipped into the homogeneous sample to obtain the pH value. The pH scale ranges from 1 to 14, with values from 1 to 7 classified as acidic, 7 as neutral, and 7 to 14 as alkaline (Ramadani et al., 2021).

Statistical Analysis

Data were analyzed using a statistical package SYSTAT vs 13.2 (Wilkinson, 2009), based on one-way Analysis of Variance of a randomized completely design. Significant differences between mean values are stated at a level of 5% maximum.

RESULT & DISCUSSION

Omega-3 Fatty Acid Content

Omega-3 fatty acids are a group of unsaturated fatty acids that are very important for human health. They cannot be synthesised by the body and must be obtained from food sources. There are three main types of omega-3 fatty acids that play an important role in physiological functions, namely alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). ALA is

commonly found in plant-based sources such as flaxseed oil, chia seeds, and nuts; while EPA and DHA can be obtained from fish, particularly fatty varieties such as salmon, tuna, and sardines (Nava et al., 2023; Gerevengood et al., 2021). The results of laboratory tests on omega-3 levels in chicken eggs in this study can be seen in Table 3 below.

Table 3. Omega-3 Fatty Acid Content

No	Treatment	Omega-3 (mg/100g)
1	P0	43.6 ^{ab}
2	P1	54.9 ^{bc}
3	P2	58.8 ^c
4	P3	29.2 ^a

Notes:

- P0 (100% Basal Feed (Control), P1 (90% Basal Feed + 10% Earthworm Meal), P2 (90% Basal Feed + 10% Seaweed Meal), P3 (80% Basal Feed + 10% Earthworm Meal + 10% Seaweed Meal).
- ^{a,b} Mean values in rows followed by different superscript letters are significantly different (P<0.05).

In laying hens, the omega-3 fatty acid content of eggs is primarily influenced by dietary intake. After omega-3-rich feed ingredients are consumed, fatty acids are

digested and absorbed in the small intestine, incorporated into chylomicrons, and transported via the bloodstream to the liver. In the liver, omega-3 fatty acids are

incorporated into very-low-density lipoproteins (VLDL) and vitellogenin, which are subsequently transported to the developing ovarian follicles and deposited into the egg yolk during yolk formation. Therefore, increasing the concentration of omega-3 fatty acids in the diet can enhance their deposition into egg yolks. Feed ingredients naturally rich in omega-3 fatty acids, such as marine algae, seaweed, flaxseed, fish oil, and other marine resources, have been widely reported to increase the omega-3 content of eggs (Calder, 2020; Givens, 2022; Silva et al., 2024).

The relationship between dietary composition and omega-3 deposition in eggs is particularly relevant to the present study. Differences in the type and proportion of feed ingredients may affect the availability of fatty acids for deposition into the egg yolk. In this study, the experimental diets differed in the inclusion of *Eucheuma cottonii* seaweed meal and *Lumbricus rubellus* earthworm meal, which may have contributed to differences in egg omega-3 content among treatments. However, because the fatty acid profile of the complete experimental diets was not analyzed, a direct quantitative relationship between the omega-3 content of the feed and the omega-3 content of the eggs cannot be established. Therefore, the differences in egg omega-3 content observed among treatments are discussed as being potentially associated with differences in dietary composition rather than as evidence of a direct causal relationship.

The results of this study indicate that the feed formulation with the addition of 10% *Eucheuma cottonii* seaweed meal (P2) resulted in the highest omega-3 content in eggs (58.8 mg/100 g) compared with the other treatments. This result is consistent with the mechanism of omega-3 deposition in egg yolks, whereby increasing the dietary supply of omega-3-rich feed ingredients can enhance the transfer of these fatty acids into the yolk. This finding

suggests that the inclusion of *E. cottonii* seaweed meal may contribute to omega-3 enrichment in eggs. Seaweed is also a source of minerals, vitamins, sulfated polysaccharides, and other bioactive compounds that may support nutrient metabolism in livestock. Several studies have shown that seaweed contains minerals such as iodine, magnesium, and calcium, which are involved in various physiological processes (Aakre et al., 2021).

The mineral content in seaweed can contribute to improving the quality of animal feed. For example, research by Ribeiro et al. shows that the use of *Laminaria digitata* in pig feed contributes to an increase in the nutritional value of meat, thanks to its high mineral content (Ribeiro et al., 2022). In addition, Aakre et al. reported that individuals who consume seaweed have better iodine status, which can meet vital nutritional needs in regulating thyroid function, although there are potential risks associated with excessive iodine intake. Therefore, the high omega-3 content observed in treatment P2 may be associated with the inclusion of *E. cottonii* seaweed meal in the diet, particularly through its contribution to the fatty acid composition of the feed. However, because the fatty acid profile of the complete experimental diets was not analyzed, the specific contribution of seaweed to the omega-3 content of the eggs cannot be confirmed directly.

Treatment P1, which involved the addition of 10% *Lumbricus rubellus* earthworm meal, produced an omega-3 content of 54.9 mg/100 g, which was lower than P2 but higher than the control treatment. Earthworm meal is a source of animal protein and contains essential amino acids and various micronutrients. The lower omega-3 content in P1 compared with P2 may indicate that the inclusion of *E. cottonii* seaweed meal contributed more strongly to omega-3 enrichment under the conditions of this study (Hartono et al., 2021).

Different results were observed in the P3 treatment, which showed a substantial decrease in omega-3 content to 29.2 mg/100 g, the lowest value among all treatments. This difference may be related to the different proportion and combination of feed ingredients used in P3. Although P2 containing 10% *E. cottonii* seaweed meal showed the highest omega-3 content, the combination of 10% *L. rubellus* earthworm meal and 10% *E. cottonii* seaweed meal in P3 did not produce a similar response. Differences in the type and proportion of dietary ingredients may affect the availability and deposition of fatty acids into the egg yolk. However, because the

fatty acid profile, particularly omega-3 fatty acids, of the complete experimental diets was not analyzed, the specific mechanism responsible for the lower omega-3 content in P3 cannot be confirmed. Therefore, the lower omega-3 content observed in P3 may indicate that the proportion and combination of feed ingredients influence omega-3 deposition in eggs, rather than demonstrating a direct causal relationship (Hartono et al., 2021); Jumsurizal et al., 2021).

Egg Yolk

The yellow egg colour results produced in this study can be seen in Table 4, as follows:

Table 4. Duncan Test Results for Egg Yolk Colour

No	Treatment	Egg Yolk Colour Score
1	P0	10.00 ^a
2	P1	10.50 ^a
3	P2	12.50 ^b
4	P3	9.50 ^a

Notes:

- P0 (100% Basal Feed (Control), P1 (90% Basal Feed + 10% Earthworm Meal), P2 (90% Basal Feed + 10% Seaweed Meal), P3 (80% Basal Feed + 10% Earthworm Meal + 10% Seaweed Meal).
- ^{a,b} Mean values in rows followed by different superscript letters are significantly different ($P < 0.05$).

The results also showed that the P2 treatment had a significant effect on egg yolk color. In Table 4, P2 showed the highest color score of 12.50, which was significantly different from the other treatments. The carotenoid pigment content in *Eucheuma cottonii* seaweed plays an important role in increasing the intensity of egg yolk color, which can be seen in its use as a feed supplement for laying hens. Natural pigments such as carotenoids are known to increase egg yolk color scores, and this is an important factor in consumer assessment of egg quality (Kljak et al., 2021). Treatment P1, although there was an increase in yolk color score, the result did not show a significant difference compared to the control. Treatment P3 showed the lowest yolk color score (9.50), but this value was not significantly different from P0 and P1. The lower numerical value observed in P3 indicates that the

combination of earthworm meal and seaweed meal did not produce the same pigmentation response as the 10% seaweed meal treatment (P2). (Zhang et al., 2024).

Previous studies have shown that an increase in carotenoid levels in chicken feed is directly related to the quality of the yellow color of the eggs produced. According to (Zhang et al., 2024), adding mulberry leaf extract to chicken feed can significantly improve egg yolk color thanks to its carotenoid content. Carotenoids from natural sources such as seaweed not only provide a more appealing color but also offer additional benefits for livestock health and the quality of the products produced.

The improvement in egg yolk color observed in treatment P2 may also be associated with the increased omega-3 content, although the relationship is indirect. Egg yolk color is primarily determined by the deposition of carotenoid pigments, particularly lutein and

zeaxanthin, whereas omega-3 enrichment results from the deposition of dietary polyunsaturated fatty acids into the yolk. These two nutritional components are deposited through different metabolic pathways; however, feed ingredients such as *Eucheuma cottonii* contain carotenoids, antioxidants, and bioactive compounds that can simultaneously enhance yolk pigmentation and improve the nutritional quality of eggs. Consequently, the higher egg yolk color score observed in treatment P2 was accompanied by higher omega-3 content because the seaweed provided

multiple beneficial nutrients rather than because yolk color directly reflects omega-3 concentration. Therefore, egg yolk color should not be considered a direct indicator of omega-3 concentration, although both characteristics may improve simultaneously when laying hens are fed nutrient-rich ingredients containing carotenoids and omega-3 fatty acids (Cherry et al., 2019)

Acidity Level Analysis (pH)

The results of the egg pH measurements can be seen in Table 5, as follows:

Table 5. Average Degree of Acidity (pH)

No	Treatment	Average Degree of Acidity (pH)
1	P0	7.97 ± 0.08 ^a
2	P1	7.48 ± 0.08 ^a
3	P2	7.12 ± 0.06 ^a
4	P3	7.20 ± 0.37 ^a

Notes:

- P0 (100% Basal Feed (Control), P1 (90% Basal Feed + 10% Earthworm Meal), P2 (90% Basal Feed + 10% Seaweed Meal), P3 (80% Basal Feed + 10% Earthworm Meal + 10% Seaweed Meal).

The results of the egg albumen pH are presented in Table 5. The mean albumen pH ranged from 7.12 to 7.97. The highest pH was observed in P0 (7.97 ± 0.08), followed by P1 (7.48 ± 0.08), P3 (7.20 ± 0.37), and P2 (7.12 ± 0.06). However, all treatments were marked with the same superscript letter (a), indicating that the dietary treatments did not significantly affect albumen pH ($P > 0.05$).

Fresh chicken egg albumen generally has a pH ranging from approximately 7.5 to 8.5, while albumen pH tends to increase during storage as carbon dioxide (CO₂) escapes through the pores of the eggshell. Based on this reference range, the albumen pH of P0 (7.97) was within the typical range for fresh eggs, whereas P1 (7.48), P2 (7.12), and P3 (7.20) were slightly below this range (Obianwuna et al., 2022). Therefore, the observed values should not be interpreted solely as an indication of improved or reduced egg quality based on pH. Moreover, SNI 3926:2023, which is the current standard

for chicken eggs for consumption, does not specify a numerical requirement for albumen pH. Instead, egg quality is assessed based on physical characteristics, including albumen cleanliness, consistency, and albumen index, as well as other quality parameters.

The lower numerical albumen pH observed in the seaweed-containing treatments may be related to the nutritional components of *Eucheuma cottonii*. Seaweeds contain minerals and various bioactive compounds that can contribute to the nutritional and physiological status of laying hens. However, the mechanism by which seaweed supplementation may influence albumen pH cannot be confirmed from the present study because the specific buffering capacity, carbon dioxide dynamics, or biochemical composition of the albumen was not measured. Seaweed supplementation should therefore be considered a possible nutritional factor affecting egg internal characteristics rather than a direct pH-stabilizing agent. The

relatively small numerical differences among treatments and the absence of a significant statistical effect further indicate that seaweed and earthworm meal

supplementation did not substantially alter albumen pH under the conditions of this experiment.

CONCLUSION

The addition of seaweed flour and earthworm flour in the formulation of layer feed, especially in treatment P2 (10% *E. cottonii* seaweed), was proven to significantly increase omega-3 levels by 58.8 mg/100 g. This treatment also produced the highest egg yolk color score of 12.50. Meanwhile, egg pH ranged from

7.12 to 7.97, and the differences among treatments were not statistically significant ($P>0.05$). These results indicate that the inclusion of *E. cottonii* seaweed meal and *L. rubellus* earthworm meal did not significantly affect egg pH under the conditions of this study.

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