Agriculture and Food

AIMS Agriculture and Food, 10(2): 446–460.
DOI: 10.3934/agrfood.2025022
Received: 10 December 2024
Revised: 14 May 2025
Accepted: 29 May 2025
Published: 18 June 2025

https://www.aimspress.com/journal/agriculture

*Research article*

# Utilization of microalgae as a feed ingredient for laying hens: A meta-analysis

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**Abstract:** This meta-analysis aimed to evaluate the effects of supplementing varying levels and types of microalgae on the performance of laying hens. A total of 18 relevant studies, comprised of 71 data points, were analyzed using a mixed model approach. Microalgae supplementation was treated as a fixed effect, while the different studies were considered as random effects. The results showed that supplementation with any type of microalgae, up to 10%, did not negatively impact the laying hen performance, including egg production, feed conversion ratio, feed intake, body weight, and fatty acid composition. Increasing levels of microalgae supplementation resulted in a significant linear improvement ($P < 0.05$) in the egg quality parameters, including the eggshell strength, shell thickness, shell weight, haugh unit, and yolk color. Additionally, there were significant interaction effects ($P < 0.05$) between the supplementation levels and the types of microalgae on the albumen weight and the egg fatty acid composition (EPA, omega-6, and omega-3 fatty acids). Brown microalgae had a greater effect on increasing the n-3 fatty acid content of eggs compared to green microalgae. In conclusion, microalgae can be a promising source of n-3 fatty acids and bioactive compounds to improve the egg quality without negatively affecting the laying performance.

**Keywords:** laying hens; microalgae; supplementation; egg quality

## 1. Introduction

Eggs have gained recognition as a potential functional food due to their affordability, widespread availability, and associated health benefits. They are an excellent source of essential nutrients – including selenium, the B vitamin complex, provitamin A, amino acids, folic acid, and fatty acids – all of which contribute significantly to human health [1]. Enriched eggs are produced by incorporating raw materials, ingredients, or supplements into poultry diets to develop nutritional strategies [2]. The use of feed additives and supplements is a common practice to enhance the nutritional quality of eggs. This practice has become more important in light of growing consumer awareness and demand for healthier animal-based foods. At the same time, the growing global population is anticipated to significantly boost the demand for animal products, with estimates suggesting an increase of 50% to 70% from the present production levels. This trend is driving the expansion of the animal supplement market, as producers seek to enhance the feed efficiency, promote animal health, and optimize the production sustainability [3].

In recent years, there has been a notable rise in the utilization of microalgae as a supplement in both the food and feed [4]. There are around 25,000–50,000 species of algae, including macroalgae and seaweeds that are highly diverse and vary in sizes, forms, colors, and functional compounds. Microalgae are a source of bioactive metabolites that are unique and cannot be synthesized by terrestrial plants. Bioactive molecules, including carbohydrates, proteins, minerals, polyphenols, colors, mycosporine-like amino acids (MAAs), and polyunsaturated fatty acids (PUFAs), including omega-3 fatty acids, have been found to have various biological functionalities. They can serve as promising anti-microbial, anti-viral, anti-inflammatory, immunomodulatory, prebiotic, and cholesterol-lowering agents [5].

In laying hens, some studies have evaluated the effects of microalgae as a dietary supplement on the egg quality [6,7], productive performance [8,9], digestibility [10], and health [11]. Although numerous studies have been conducted, the results have been inconsistent and contradictory. High variability among studies in terms of age, breed/strain of laying hens, supplementation periods, doses, and the types of microalgae used could account for these inconsistencies. It has been reported that the use of meta-analytic methods can identify the source of such between-studies variability [12]. A meta-analysis is a statistical approach that employs rigorous procedures to combine and analyze datasets from multiple experiments. When conducted properly, a meta-analysis of studies is considered as decisive evidence because it occupies a top level in the hierarchy of evidence [13]. Therefore, this study aimed to evaluate the effects of the inclusion of microalgae (levels and their types) in laying hens' diet by integrating related studies using the meta-analysis method.

## 2. Materials and methods

### *2.1. Database development*

A search for potential articles was conducted in the Scopus database using keywords "microalgae" and "laying hens" to collect peer-reviewed articles that have investigated microalgae supplementation in laying hens' diets. A total of 49 potential articles were initially obtained, and after screening the abstracts, 26 articles were selected for a full-text review, which resulted in 18 articles meeting the criteria (Figure 1). Journals selected for database compilation had the following criteria: 1) the article was published in English; 2) the experiment was conducted on laying hens; 3) the type and dose of microalgae were reported; and 4) there was a control treatment (not added microalgae). The parameters integrated included the following: initial body weight (g), body weight gain (kg/weeks), final body weight (g), feed conversion ratio (FCR), feed intake (g), egg production (%), yolk percentage (%), shell thickness (mm), yolk weight (g), egg weight (g), shell weight (g), haugh unit, yolk color, albumen weight (g), myristoleic acid C14:1 (%), cholesterol (µg/dL), shell strength (kg/cm²), myristic acid C14 (%), saturated fatty acid (SFA) (%), palmitoleic acid C16:1n7 (%), oleic acid C18:1n9 (%), monounsaturated fatty acid (MUFA) (%), linoleic acid C18:2n6 (LA) (%), alpha-linolenic acid (ALA) C18:3n3 (%), arachidonic acid C20:4n6 (AA) (%), polyunsaturated fatty acid (PUFA) (%), palmitic acid C16 (%), docosahexaenoic acid (DHA) C22:6n3 (%), eicosapentaenoic acid (EPA) C20:5n3 (%), n-6 fatty acid (omega-6) (%), and n-3 fatty acid (omega-3) (%). The final study selection results showed that 18 journals with 76 data points could be included in the database, as shown in Table 1. The microalgae levels used in the studies ranged from 0 to 10%. When extracting the data into the database, the units of measurements of the variables were transformed into uniform units.

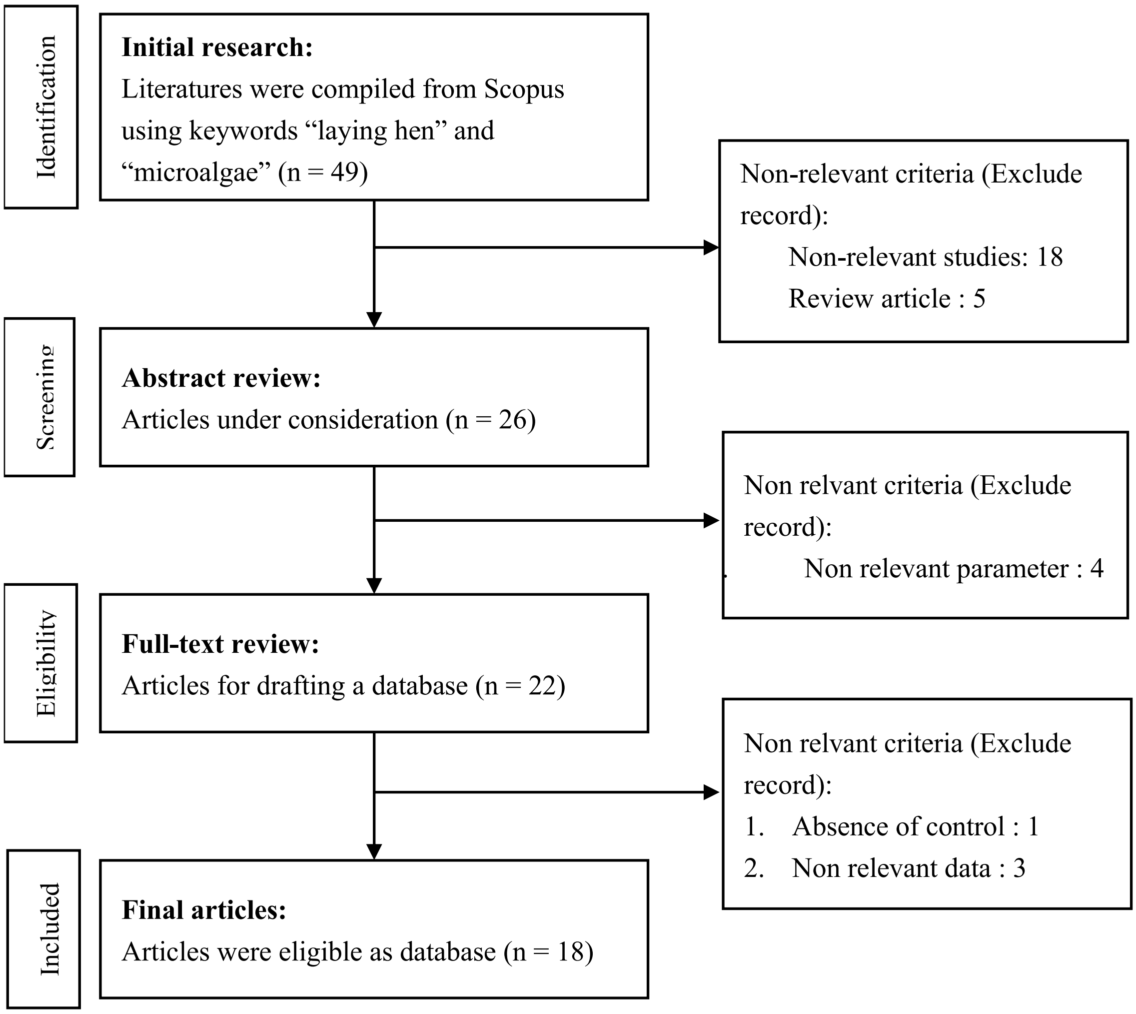


**Figure 1.** Study selection process in the meta-analysis.

**Table 1.** Articles used in the meta-analysis of microalgae supplementation in the diets of laying hens.

| No | Reference | Week | Strain | Algae Type | Species | Level (%) |
|---|---|---|---|---|---|---|
| 1 | Kim [6] | 26 weeks | Shaver Leghorn | Green | *Nannochloropsis sp.* | 0;3;5 |
| 2 | Lemahieu [7] | 29 weeks | Isa Brown | Green; Brown | *Phaeodactylum sp.; Nannochloropsis sp.; Isochrysis sp.; Chlorella sp.* | 0;0.125;0.25 |
| 3 | Panaite [8] | 38 weeks | Lohmann | Green | *Chlorella sp.; Spirulina sp.; Chlorella sp.* | 0;2 |
| 4 | Abbas [9] | 10–13 weeks | HY-Line W-36 | Green | *Spirulina sp.* | 0;3;6;9 |
| 5 | Mens [11] | 4 weeks | H&N Super Nick | Green | *Nannochloropsis sp.* | 0;1;2;3 |
| 6 | Ao [14] | 77 weeks | Hy-Line Brown | Brown | *Schizochytrium sp.* | 0;1;2;3 |
| 7 | Kim [15] | 21 weeks | Hy-Line Brown | Green | *Chlorella sp.; Tetradesmus sp.* | 0;0.5 |
| 8 | Park [16] | 46 weeks | Isa Brown | Brown | *Schizochytrium sp.* | 0;0.5;1 |
| 9 | Zhang [17] | 3 weeks | Isa brown | Brown | *Schizochytrium sp.* | 0;1.5 |
| 10 | Neijat [18] | 34 weeks | Lohmann | Green | *Marine Algae* | 0;0.2;0.4;0.6 |
| 11 | Kor [19] | 40 weeks | Lohmann | Green | *Chlorella sp.* | 0;0.01;0.02;0.03;0.04;0.05 |
| 12 | Wahyuni [20] | 81 weeks | Isa Brown | Green | *Spirulina sp.* | 0;0.3 |
| 13 | Lemahieu [21] | 33 weeks | Isa Brown | Brown | *Isochrysis sp.* | 0;0.03;0.06;0.09;1.2;1.5;2;3;4 |
| 14 | Manor [22] | 46 weeks | Shaver-White | Green | *Nannochloropsis sp.* | 0;2.85;5.75 |
| 15 | Rizzi [23] | 28 weeks | Isa Brown | Brown | *Schizochytrium sp.* | 0;1.67 |
| 16 | Wu [24] | 37 weeks | Lohmann | Green | *Nannochloropsis sp.* | 0;1;2;4 |
| 17 | Fredrikson [25] | 4 weeks | Hy-Line W-98 | Green | *Nannochloropsis sp.* | 0;10 |
| 18 | Bruneel [26] | 6 weeks | Isa Brown | Green | *Nannochloropsis sp.* | 0;5;10 |

*2.2. Data analysis*

This meta-analysis employed a mixed model methodology, as described by Sauvant et al. [12]. In the analysis, the studies were considered as random effects. Two statistical models were utilized [27], where the microalgae levels (continuous data) and types of microalgae (categorical) were considered as fixed effects:

$$Yij = A_0 + A_1Xij + A_2Xij^2 + Pi + aiXij + eij \quad (1)$$

where Yij is the response variable (dependent), A0 is the total intercept from all experiments, A1 is the linear regression coefficient Y on X, A2 is the quadratic regression coefficient Y on X, Xij is the value of the continuous forecaster variable (levels of microalgae), Pi is the random effect from the study i, ai is the random effect from the study i on the regression constant Y on X, and eij is the residual error. The study's statistical model relied on P-values. The data analysis of the effect of the types (color) of microalgae on the response variable used the following statistical model:

$$Yij = \boldsymbol{\mu} + Pi + \boldsymbol{\tau}j + eij \quad (2)$$

where Yij is the response variable, μ is the general mean, Pi is the random effect from the study i, τj is the fixed effect of the j-th level of factor τ, and eij is the residual error. The study's statistical model relied on P-values. The significance of the model was determined at a threshold of $P \leq 0.05$. When there was a statistically significant value among the categorical treatments (different types of microalgae), the Tukey's Honest Significant Difference (HSD) test was performed. The statistical analysis was performed using the PROC MIXED procedure of the SAS software, version 9.4.

## 3. Results and discussion

*3.1. Effect of microalgae supplementation on laying hens performance*

The results of meta-regression analysis demonstrated that the supplementation of microalgae up to 10% in the diet did not affect the laying hens' performance (Table 2). This finding suggests the suitability of microalgae as a feed ingredient for laying hens, regardless of the type. These results are consistent with Ao et al. [14], who reported no significant differences in the laying hens' performance, including egg production, between microalgae-supplemented feed and the control group. The absence of negative effects on the egg production further supports the conclusion that microalgae supplementation does not impair the laying capacity of hens. The type (color) of microalgae (green and brown) did not significantly ($P > 0.05$; Table 3) affect the performance parameters of the laying hens compared to the control diet. Therefore, supplementing any type of microalgae to the laying hens would not result in any detrimental effect on the laying performance when maintained at levels up to 10%. Moreover, it can also be inferred that microalgae may not have sufficiently increased the essential nutrient content necessary for body maintenance to enhance the laying hens' productivity. Generally, most studies that investigated microalgae supplementation in poultry feed, particularly in laying hens, did not observe significant changes in the productive performance [9]. This finding may indicate that the characteristics and composition of the microalgae do not contribute to improving the performance, particularly in laying hens. Some previous studies also suggest that while microalgae can influence the appearance and content of the laying hens' eggs, they did not enhance their overall performance [28]

**Table 2**. Effects of dietary microalgae supplementation on production performance of laying hens.

| Parameter | Unit | N | Model | Intercept | SE Intercept | Slope | SE Slope | P-value | L × S |
|---|---|---|---|---|---|---|---|---|---|
| **Performance** | | | | | | | | | |
| Initial body weight | g | 11 | L | 1430 | 1263 | −0.056 | 1.62 | 0.974 | NA |
| Body weight gain | kg/weeks | 10 | L | 1.58 | 0.13 | 0.008 | 0.009 | 0.440 | 0.441 |
| Final body weight | g | 8 | L | 1539 | 57.8 | 0.370 | 1.45 | 0.808 | NA |
| FCR | | 22 | L | 2.08 | 0.116 | −0.007 | 0.007 | 0.350 | 0.851 |
| Feed intake | g/d | 41 | L | 109.5 | 2.94 | −0.427 | 0.234 | 0.079 | 0.544 |
| Egg production | % | 49 | L | 91.7 | 1.84 | 0.091 | 0.176 | 0.610 | 0.833 |

Note: P ≤ 0.01: very significant, P ≤ 0.05: significant, P >0.05: not significant, FCR: feed conversion ratio, M: model, L: linear, Intercept: average value of the response parameter when the microalgae level is equal to zero, SE Intercept: standard error of intercept, Slope: value of the slope of the line, SE Slope: standard error of slope, L × S: level × source.

**Table 3.** Influence of various types of microalgae on production performance of laying hens.

| Parameter | Unit | Control | Green | Brown | P-value |
|---|---|---|---|---|---|
| **Performance** | | | | | |
| Initial body weight | g | 1428 ± 138 | 1431 ± 138 | NA | 0.833 |
| Body weight gain | kg/weeks | 1.54 ± 0.159 | 1.64 ± 0.154 | 1.54 ± 0.159 | 0.342 |
| Final body weight | g | 1555 ± 60.8 | 1538 ± 60.3 | NA | 0.124 |
| FCR | | 2.06 ± 0.116 | 2.06 ± 0.114 | 1.99 ± 0.131 | 0.621 |
| Feed intake | g/d | 108.6 ± 3.16 | 108.9 ± 3.10 | 107.68 ± 3.66 | 0.884 |
| Egg production | % | 90.7 ± 1.93 | 91.9 ± 1.92 | 93.1 ± 2.14 | 0.102 |

Note: P ≤ 0.01: very significant, P ≤ 0.05: significant, P > 0.05: not significant, FCR: feed conversion ratio, NA: not available.

### *3.2. Effect of microalgae supplementation on egg quality*

Microalgae are recognized as a valuable source of both macronutrients and micronutrients for animal nutrition, thereby providing proteins and lipids as macronutrients, along with a rich array of micronutrients, including vitamins, minerals, and various bioactive molecules such as polyphenols, polysaccharides, peptides, and pigments [29]. Historically, the nutrition of laying hens has focused on sustaining and improving egg production along with other traits related to the egg's quality and the animal's health. Dietary microalgae supplementation significantly influenced several egg quality parameters in the laying hens. The results suggest that microalgae can be included in the diet of laying hens, up to 10%, to enhance the eggshell, haugh unit, and yolk color (Table 4). Notably, it improved the eggshell quality (P < 0.05), as evidenced by the significant increase in the shell thickness and strength. Additionally, the shell weight was significantly affected (P < 0.05), thus indicating a possible increase in the shell material deposition. The observed increase in the eggshell quality can be attributed to an improved mineral absorption in the digestive tract [30]. These findings align with previous studies that demonstrated that microalgae contained bioavailable minerals such as calcium and phosphorus, which contributed to the eggshell formation and strength [31,32]. Calcium is present in all microalgae species, with concentrations reaching up to 8.2%, which is considerably higher than those found in conventional poultry feed ingredients such as soybean meal (0.2%–0.31%) [33]. This high calcium content is particularly relevant in poultry nutrition, as calcium is essential for egg production. During the laying period, hens require approximately 4% dietary calcium to support the formation of eggshells, thus highlighting the potential of microalgae as a valuable calcium source in the layer diets [34]. Additionally, microalgae are rich in bioactive compounds that enhance the eggshell's mineralization and structure, as reported by Park et al. [35]. The eggshell quality parameters, such as the shell strength, shell weight, and shell thickness, significantly impact the profitability of the egg industry [36]. However, parameters such as the yolk weight, egg weight, and albumen weight were not significantly ($P > 0.05$) affected, thus indicating that microalgae supplementation did not alter these aspects of the egg composition. The egg quality parameters are crucial for consumer acceptance, with the Haugh unit being particularly important. As an indicator of egg freshness [37], the Haugh unit showed a positive effect ($P < 0.05$). Haugh units with values greater than 70 indicate fresh eggs with low protein and lipid oxidation rates [38]. Including antioxidants in poultry diets can delay the egg component oxidation. Many molecules found in microalgae are known for their antioxidant properties [35]. In this study, increasing levels of microalgae supplementation were associated with enhanced Haugh units, thus indicating fresher and higher quality eggs.

**Table 4**. Effects of dietary microalgae supplementation on egg quality of laying hens.

| Parameter | Unit | N | Model | Intercept | SE Intercept | Slope | SE Slope | P-Value | L × S |
|---|---|---|---|---|---|---|---|---|---|
| Yolk percentage | % | 12 | L | 26.4 | 1.46 | 0.018 | 0.097 | 0.858 | 0.364 |
| Shell thickness | mm | 28 | L | 0.410 | 0.030 | 0.011 | 0.002 | < 0.001 | 0.671 |
| Yolk weight | g | 46 | L | 17.5 | 1.31 | −0.004 | 0.046 | 0.926 | 0.279 |
| Egg weight | g | 59 | L | 60.8 | 0.749 | −0.267 | 0.158 | 0.093 | 0.396 |
| Shell weight | g | 33 | L | 7.73 | 0.416 | 0.074 | 0.035 | 0.044 | 0.286 |
| Haugh unit | | 25 | L | 89.73 | 3.81 | 0.647 | 0.176 | 0.002 | 0.612 |
| Yolk color | | 23 | L | 9.09 | 0.620 | 0.429 | 0.119 | 0.003 | 0.975 |
| Albumen weight | g | 29 | L | 35.45 | 1.24 | −0.016 | 0.235 | 0.944 | < 0.001 |
| Shell strength | $kg/cm^2$ | 10 | L | 4.47 | 0.500 | 0.100 | 0.016 | < 0.001 | 0.674 |

Note: $P \leq 0.01$: very significant, $P \leq 0.05$: significant, $P > 0.05$: not significant, FCR: feed conversion ratio, M: model, L: linear, Intercept: average value of the response parameter when the microalgae level is equal to zero, SE Intercept: standard error of intercept, Slope: value of the slope of the line, SE Slope: standard error of slope, L × S: level × source.

The yolk color is a critical parameter of egg quality. In this study, a significant change in the yolk color was observed with the inclusion of microalgae, as detailed in Table 4. This was related to nutrition, as algae contain high levels of carotenoids, especially β-carotene, which are converted into retinol and deposited into the yolks. This indicated that microalgae have the potential to replace synthetic pigments in the laying hens' diets without detrimental effects on the performance [39]. The nutritional profiles of microalgae significantly vary among different species. Table 5 demonstrates that different types of microalgae had distinct effects on the yolk color compared to the control. Both green and brown microalgae contain carotenoids, although their specific pigment compositions differ. Green microalgae, such as *Chlorella* and *Spirulina*, are rich in chlorophyll, lutein, and zeaxanthin, all of which contribute to the yolk pigmentation upon ingestion. On the other hand, brown microalgae are abundant in fucoxanthin, a particular carotenoid of this group [15]. In the present study, supplementation with brown microalgae resulted in the most intense yolk coloration, followed by green microalgae, while the control group without microalgae showed the lowest pigmentation (Table 5). The egg yolk color is primarily influenced by the hens' diet, as they cannot synthesize pigments but can accumulate them from their feed [40]. Herber-Mcneill and Van Elswyk [41] noted that dietary microalgae induced shifts in the yolk color due to the inclusion of carotenoids such as canthaxanthin and β-carotene. Notably, the inclusion of brown microalgae significantly increased the yolk color, which corresponded to a higher total carotenoid content in these treatments [42]. A greater deposition of carotenoids in the yolk enhances its pigmentation [43]. Since carotenoids influence the food coloration, this attribute is among the first noticed by consumers, which directly impacts their choices [44].

**Table 5.** Influence of various types of microalgae on egg quality of laying hens.

| Parameter | Unit | Control | Green | Brown | p-value |
|---|---|---|---|---|---|
| Yolk percentage | % | 26.32 ± 1.44 | 26.34 ± 1.44 | 26.5 ± 1.48 | 0.937 |
| Shell thickness | mm | 0.414 ± 0.027 | 0.425 ± 0.026 | 0.410 ± 0.027 | 0.517 |
| Yolk weight | g | 17.4 ± 1.31 | 17.5 ± 1.31 | 17.4 ± 1.32 | 0.589 |
| Egg weight | g | 60.4 ± 0.798 | 60.1 ± 0.786 | 61.1 ± 0.89 | 0.545 |
| Shell weight | g | 7.64 ± 0.411 | 7.96 ± 0.401 | 7.87 ± 0.434 | 0.113 |
| Haugh unit | | 89.8 ± 3.76 | 91.4 ± 3.77 | 89.4 ± 3.86 | 0.355 |
| Yolk color | | $8.58^{b}$ ± 0.823 | $9.07^{ab}$ ± 1.31 | $11.2^{a}$ ± 0.72 | 0.038 |
| Albumen weight | g | $34.6^{a}$ ± 1.28 | $35.7^{ab}$ ± 1.23 | $36.2^{b}$ ± 1.43 | 0.197 |
| Shell strength | $kg/cm^2$ | 4.73 ± 0.489 | 4.60 ± 0.467 | 4.62 ± 0.551 | 0.916 |

Note: P ≤ 0.01: very significant, P ≤ 0.05: significant, P > 0.05: not significant.

### *3.3. Effect of microalgae supplementation on egg`s fatty acid profile*

Our results indicated that the effects of microalgae on the n-3 and n-6 fatty acid profiles of eggs depended on the type of microalgae used (Tables 6 and 7). However, the effects were insignificant for some other saturated and unsaturated fatty acids. Brown microalgae appeared to be more effective in increasing the n-3 levels in eggs, while green microalgae did not significantly change the n-3 content of the eggs (Table 7). The primary mechanism that explains this difference lies in the omega-3 fatty acid synthesis pathway in brown microalgae, particularly Schizochytrium sp. This microalga utilizes the polyketide synthase (PKS) pathway, which is more efficient than conventional pathways because it requires less NADPH as a reducing power. This efficiency enables Schizochytrium sp. to produce high levels of docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA) with minimal energy expenditure [45]. Therefore, this microalga serves as a superior DHA source compared to other n-3 sources such as perilla and flaxseed [17]

In comparison, green microalgae such as Chlorella, Isochrysis, and Nannochloropsis only contain about 2.75% of their total fatty acids in the form of n-3, which is significantly lower than brown microalgae such as Schizochytrium, which can contain up to 37.6% of total fatty acids as DHA [16]. The high n-3 concentration in brown microalgae is directly associated with an increased n-3 intake, which has been previously reported to have a strong linear relationship with n-3 transfer into the eggs of laying hens [46]. When animals consume microalgae rich in DHA, the omega-3 fatty acids are incorporated into their egg lipid profiles, thus contributing to the nutritional quality of the eggs, especially in terms of the omega-3 content.

**Table 6.** Effects of microalgae supplementation on eggs' fatty acid profiles of laying hens.

| Parameter | Unit | N | Model | Intercept | SE Intercept | Slope | SE Slope | P-value | L × S |
|---|---|---|---|---|---|---|---|---|---|
| Cholesterol | ug/dL | 12 | L | 141 | 15.7 | 3.18 | 4.44 | 0.494 | 0.941 |
| Myristioleic acid C14:1 | % | 8 | L | 0.230 | 0.100 | −0.0011 | 0.0087 | 0.907 | NA |
| Myristic acid C14 | % | 9 | L | 0.340 | 0.030 | 0.0006 | 0.0026 | 0.822 | NA |
| SFA | % | 13 | L | 28.5 | 4.93 | −0.176 | 0.392 | 0.666 | 0.297 |
| Palmitoleic acid C16:1n7 | % | 17 | L | 4.13 | 1.82 | −0.034 | 0.109 | 0.761 | 0.654 |
| Oleic acid C18:1n-9 | % | 20 | L | 33.4 | 6.61 | −0.036 | 0.092 | 0.705 | 0.732 |
| MUFA | % | 13 | L | 35.1 | 6.45 | −0.289 | 0.377 | 0.465 | NA |
| Linoleic acid C18:2n-6 | % | 20 | L | 15.4 | 2.40 | −0.258 | 0.158 | 0.127 | 0.759 |
| Linoleic alfa acid C18:3n3 | % | 15 | L | 4.43 | 3.38 | 0.069 | 0.109 | 0.538 | 0.671 |
| Arachidonic acid C20:4 n-6 | % | 14 | L | 0.990 | 0.620 | −0.017 | 0.016 | 0.302 | 0.463 |
| PUFA | % | 15 | L | 16.8 | 2.78 | −0.254 | 0.178 | 0.189 | NA |
| Palmitic acid C16 | % | 9 | L | 18.4 | 5.78 | 0.0046 | 0.115 | 0.969 | NA |
| DHA C22:6n3 | % | 17 | L | 0.990 | 0.350 | 0.068 | 0.057 | 0.257 | 0.284 |
| EPA C20:5n3 | % | 13 | L | 0.150 | 0.110 | 0.0042 | 0.017 | 0.810 | 0.023 |
| n-6 fatty acid | % | 14 | L | 14.2 | 2.31 | −0.108 | 0.198 | 0.602 | < 0.001 |
| n-3 fatty acid | % | 14 | L | 1.41 | 0.370 | 0.096 | 0.066 | 0.183 | 0.022 |

Note: P ≤ 0.01: very significant, P ≤ 0.05: significant, P >0.05: not significant, NA: not available, SFA: saturated fatty acid, MUFA: monounsaturated fatty acid, PUFA: polyunsaturated fatty acid, DHA: docosahexaenoic acid, EPA: eicosapentanenoic acid, M: model, L: linear, Intercept: average value of the response parameter when the microalgae level is equal to zero, SE Intercept: standard error of intercept, Slope: value of the slope of the line, SE Slope: standard error of slope, L × S: level × source.

The different microalgae color types influenced the eggs fatty acid composition, with a reduction in the omega-6 content and an increase in the omega-3 content, as shown in Table 7. Furthermore, there were significant interactions ($P < 0.05$) between the microalgae supplementation levels and the types for some fatty acid contents in eggs (i.e., omega 6 (Figure 2), omega 3 (Figure 3), and EPA C20:5n3 (Figure 4)). Generally, eggs have a higher omega-6 content than omega-3. Increasing the omega-3 content in eggs reduces the omega-6/3 ratio. The high omega-3 content may result from microalgae containing sufficient DHA [18]. Adding microalgae to the laying hens' feed up to 2% significantly increased the DHA content in eggs [47]. Additional research [17] indicated that microalgae supplementation can elevate the omega content in eggs and help reduce the omega-6/3 fatty acid ratio. An excessive intake of omega-6 compared to omega-3 fatty acids can lead to negative health impacts associated with cardiovascular diseases. Therefore, balancing the omega-6 and omega-3 fatty acids is crucial to maintain human health [48]. Lower levels of microalgae supplementation may yield better fatty acid profiles than higher levels [35]. Therefore, increasing the level of microalgae supplementation in the feed may not necessarily enhance its fatty acid content. The results indicate that green and brown microalgae exhibit better performances than the control in balancing the omega-6/3 ratio. The brown microalgae were more effective in reducing the omega-6 fatty acid content at lower supplementation levels (< 3%). However, at higher supplementation levels (> 3%), the green microalgae demonstrated a greater effectiveness in reducing the omega-6 fatty acids.

**Table 7.** Influence of various microalgae color sources on egg's fatty acids parameters of laying hens.

| Parameter | Unit | Control | Green | Brown | *p*-value |
|---|---|---|---|---|---|
| Cholesterol | ug/dL | 150.8 ± 14.3 | 149.1 ± 13.2 | 125 ± 19.1 | 0.451 |
| Myristioleic acid C14:1 | % | 0.209 ± 0.083 | 0.212 ± 0.083 | 0.305 ± 0.088 | 0.187 |
| Myristic acid C14 | % | 0.324 ± 0.030 | 0.345 ± 0.031 | 0.354 ± 0.031 | 0.133 |
| SFA | % | 27.6 ± 4.93 | 28.2 ± 4.92 | 29.4 ± 5.31 | 0.700 |
| Palmitoleic acid C16:1n7 | % | 3.89 ± 1.81 | 4.28 ± 1.86 | 4.05 ± 1.86 | 0.851 |
| Oleic acid C18:1n-9 | % | 33.1 ± 6.62 | 33.2 ± 6.63 | 33.8 ± 6.64 | 0.561 |
| MUFA | % | 34.2 ± 6.49 | 34.6 ± 6.43 | Na | 0.805 |
| Linoleic acid C18:2n-6 | % | 15.9$^{a}$ ± 2.24 | 14.7$^{ab}$ ± 2.29 | 13.5$^{b}$ ± 2.33 | 0.041 |
| Linoleic alfa acid C18:3n3 | % | 4.07$^{c}$ ± 3.15 | 4.17$^{b}$ ± 3.15 | 6.95$^{a}$ ± 3.15 | < 0.001 |
| Arachidonic acid C20:4 n-6 | % | 1.03 ± 0.627 | 0.931 ± 0.629 | 0.882 ± 0.632 | 0.281 |
| PUFA | % | 16.9 ± 2.97 | 16.5 ± 2.96 | 13.4 ± 3.18 | 0.069 |
| Palmitic acid C16 | % | 18.2 ± 5.82 | 18.0 ± 5.92 | 18.7 ± 5.83 | 0.855 |
| DHA C22:6n3 | % | 0.735 ± 0.361 | 1.33 ± 0.404 | 1.44 ± 0.398 | 0.052 |
| EPA C20:5n3 | % | 0.092 ± 0.099 | 0.092 ± 0.107 | 0.364 ± 0.125 | 0.120 |
| n-6 fatty acid | % | 15.0$^{a}$ ± 3.09 | 14.6$^{a}$ ± 2.37 | 11.4$^{b}$ ± 3.53 | 0.015 |
| n-3 fatty acid | % | 1.23$^{b}$ ± 0.418 | 1.59$^{ab}$ ± 0.437 | 2.39$^{a}$ ± 0.492 | 0.038 |

Note: P ≤ 0.01: very significant, P ≤ 0.05: significant, P > 0.05: not significant, NA: not available, SFA: saturated fatty acid, MUFA: monounsaturated fatty acid, PUFA: polyunsaturated fatty acid, DHA: docosahexaenoic acid, EPA: eicosapentanenoic acid.

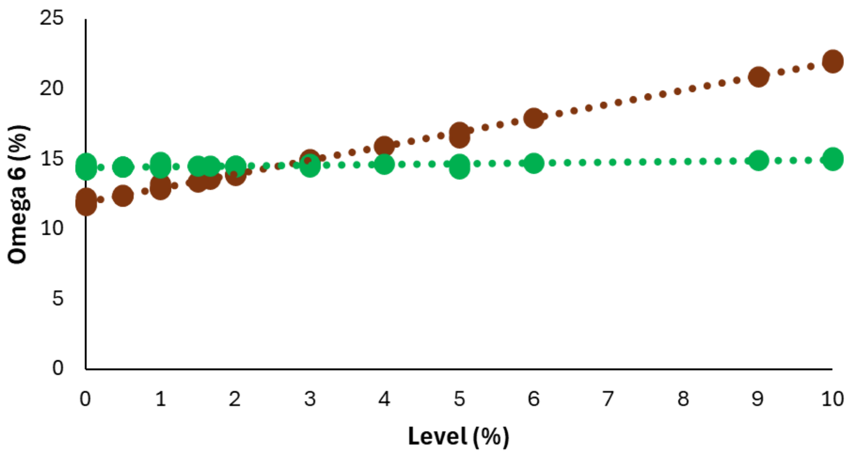


**Figure 2.** Interaction between level and type (green and brown) of dietary microalgae supplementation on omega 6 egg fatty acid content.

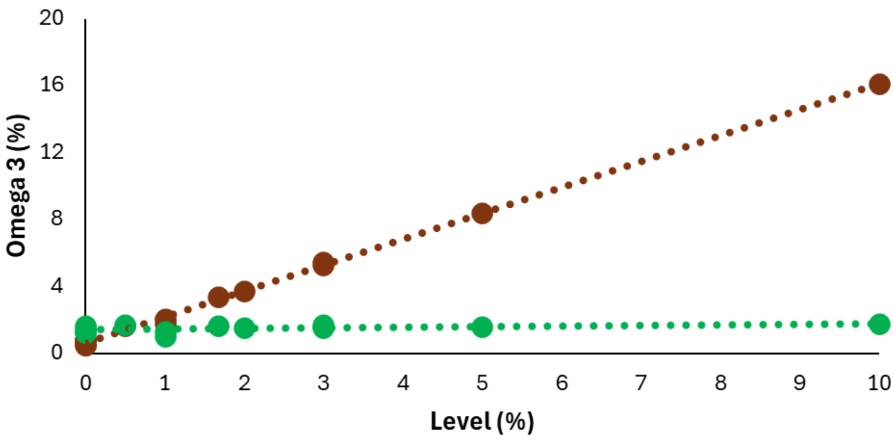


**Figure 3.** Interaction between level and type (green and brown) of dietary microalgae supplementation on omega 3 egg fatty acid content.

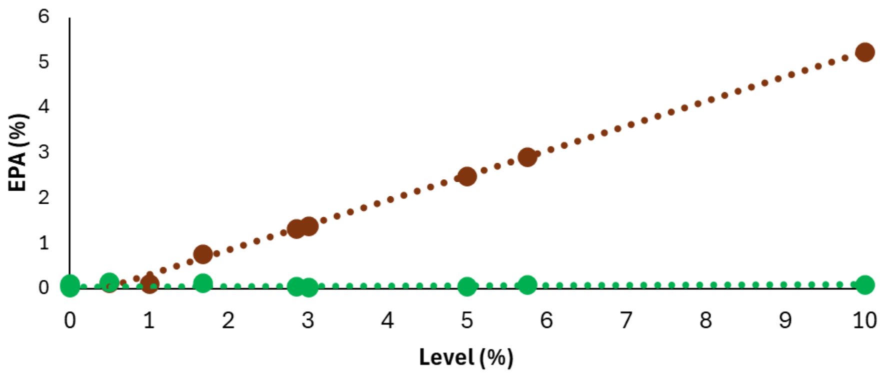


**Figure 4.** Interaction between level and type (green and brown) of dietary microalgae supplementation on EPA C20:5n3 egg fatty acid content.

## 4. Conclusions

The meta-analysis on supplementing microalgae at various levels and types in laying hens concluded that neither the level nor the type of microalgae significantly affected the overall laying hens' performance metrics. However, higher levels of microalgae supplementation notably enhanced the egg quality, such as the eggshell quality, haugh unit, and yolk color. Moreover, the inclusion of different microalgae types (green and brown) significantly improved both the egg quality and the fatty acid composition of the eggs. Additionally, a significant interaction between the level and color of microalgae was observed, which influences parameters such as the egg quality (albumen weight) and fatty acid composition (EPA C20:5n3, omega-6, and omega-3) in the eggs.

## Author contributions

Raihani Indah Kusuma: Writing – original draft, Formal analysis; Giovani Giovani: Writing – original draft, Formal analysis; M Sulaiman Daulai: Writing – original draft, Formal analysis; Arif

Darmawan: Data curation, Validation; Hasliza Abu Hassim: Conceptualization, Resources; Nor Dini Rusli: Conceptualization, Resources; Yuan-Yu Lin: Conceptualization, Resources; Agung Irawan: Conceptualization, Resources; Anuraga Jayanegara: Supervision, Writing – review & editing, Data curation. All authors have read and agreed to the published version of the manuscript.

## Use of AI tools declaration

The authors declare that they have not used artificial intelligence tools in the creation of this article.

## Acknowledgments

This research was funded by the Directorate of Research and Innovation, IPB University, Indonesia, through the International Collaborative Research Scheme (RI-KOIN), year 2023-2024, contract number 562/IT3.D10/PT.01.03/P/B/2023.

## Conflict of interest

The authors declare no conflict of interest related to this study or its publication.

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