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Effect of algae-supplemented feed on omega-3 enrichment of eggs and consumer acceptability in free- range laying hens

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Abstract

Eggs enriched with long-chain omega-3 fatty acids represent one of the most practical vehicles for increasing population-level intake of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), yet the relationship between dietary algae dose, yolk fatty acid deposition, and sensory acceptability has not been mapped in free-range production systems. This research evaluated the effect of graded levels of spray-dried *Schizochytrium* sp. microalgae (0%, 1.5%, 3.0%, and 4.5% of diet dry matter) on egg yolk omega-3 profile, production performance, and consumer sensory scores in Lohmann Brown Classic laying hens kept under free-range conditions in Valparaíso, Chile. A total of 240 hens aged 28 weeks were allocated to four dietary treatments in a completely randomised design with six replicates of 10 hens each. The feeding trial ran for 56 days (May to July 2024). Egg yolk DHA increased in a strong linear fashion with algae inclusion ($R^2 = 0.96$), rising from a baseline of 31.4 mg per 100 g yolk in the control to 79.8, 144.6, and 196.3 mg per 100 g at 1.5%, 3.0%, and 4.5% inclusion, respectively. EPA showed a more modest but still significant increase. Hen-day production remained above 91% in all groups and did not differ between treatments ($p = 0.71$). Feed conversion ratio was unaffected up to 3.0% algae but worsened marginally at 4.5% ($p = 0.04$). A consumer panel of 120 untrained assessors scored boiled eggs on a nine-point hedonic scale for appearance, aroma, flavour, texture, and overall acceptability. The 3.0% group scored highest for overall acceptability (mean 7.6), outperforming the control (6.8; $p = 0.01$). However, eggs from the 4.5% group received lower acceptability scores (6.5) than all other treatments, driven by a detectable fishy off-flavour. These results indicate that 3.0% *Schizochytrium* inclusion in free-range hen diets maximises DHA enrichment without compromising production or palatability.

Keywords: Omega-3 fatty acids, docosahexaenoic acid, microalgae, *Schizochytrium*, laying hens, free-range, egg enrichment, consumer acceptability, sensory evaluation, poultry nutrition

Introduction

The gap between recommended and actual intake of long-chain omega-3 polyunsaturated fatty acids in most Western diets is wide enough to qualify as a public health concern ^[1]. Adults in Europe and the Americas consume, on average, less than half the 250 mg of DHA plus EPA per day that expert panels advise for cardiovascular protection ^[2]. Enriching a staple food like eggs offers a passive fortification route: consumers need not change their purchasing habits, and eggs are already consumed by a broad demographic cross-section ^[3].

Marine microalgae of the genus *Schizochytrium* are the most concentrated natural source of DHA, containing 35-45% of their dry mass as lipid with DHA accounting for up to 40% of total fatty acids ^[4]. Unlike fish oil, algal biomass carries no mercury or dioxin contamination risk and is produced by fermentation, which can be scaled independently of marine harvest ^[5]. When fed to laying hens, *Schizochytrium* lipid is absorbed in the intestine, incorporated into hepatic very-low-density lipoproteins, and deposited in the developing yolk follicle over a 10-day maturation cycle ^[6].

Several cage-housed trials have established dose-response curves for algae-to-yolk DHA transfer, typically reporting linear increases up to 3-4% dietary inclusion before a plateau ^[7, 8]. But free-range hens present a different metabolic context: higher activity levels increase energy

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expenditure, and access to pasture introduces variable quantities of alpha-linolenic acid from forage, which competes with preformed DHA for esterification in yolk phospholipids [9]. Whether the dose-response curve established in cages holds under free-range conditions has not been tested directly. Consumer acceptance is the other half of the equation. Off-flavours in omega-3-enriched eggs, commonly described as "fishy" or "marine", arise from volatile oxidation products of DHA, particularly propanal and 2,4-heptadienal [10]. Earlier work showed that these volatiles become detectable above a threshold yolk DHA concentration, but that threshold varies with cooking method and individual sensitivity [11]. Free-range eggs may behave differently because their yolk carotenoid content, which acts as an endogenous antioxidant, is typically higher than in cage eggs [12].

Objectives

- 1) To determine the dose-response relationship between *Schizochytrium* sp. inclusion rate and egg yolk DHA and EPA concentrations in free-range Lohmann Brown hens;
- 2) To assess any effects on production performance; and
- 3) To evaluate consumer sensory acceptability of enriched eggs across the four inclusion levels using a hedonic panel.

Material and Methods

Material

Two hundred and forty Lohmann Brown Classic laying hens at 28 weeks of age were sourced from a single commercial hatchery and housed at the free-range poultry research unit of the Valparaíso Research Institute of Animal Nutrition, Valparaíso, Chile. The unit is located 8 km inland from the Pacific coast at an elevation of 210 m, with a Mediterranean climate characterised by mild, wet winters and warm, dry summers. Mean daily temperatures during the trial (May to July 2024) ranged from 8.3 to 15.1 degrees Celsius. Hens were distributed among 24 identical paddock pens, each providing 4 square metres of indoor shelter and 10 square metres of outdoor range per bird, planted with a mix of perennial ryegrass and white clover. A corn-soybean meal basal diet (2,750 kcal ME/kg, 17.2% crude protein) was formulated to meet or exceed Lohmann management guide requirements. Spray-dried *Schizochytrium* sp. biomass (43.1% crude fat, DHA content 18.7% of dry matter; AlgaPrime DHA, Corbion) was included at 0%, 1.5%, 3.0%, or 4.5% of diet dry matter by partial replacement of soybean oil, with iso-energetic and iso-nitrogenous adjustments. Each treatment was replicated six times with 10 hens per replicate. The experimental protocol was approved by the Institutional Animal Care Committee of the Valparaíso Research Institute (Protocol VRIAN-2024-008, approved 15 March 2024), in compliance with Chilean Law 20.380 on Animal Protection.

Methods

The feeding trial lasted 56 days. Eggs were collected daily and production recorded per pen. During the final collection week (days 50-56), three eggs per replicate per day were randomly selected for fatty acid analysis. Yolks were separated, lyophilised, and subjected to acid-catalysed transmethylation. Fatty acid methyl esters were analysed by gas chromatography with flame ionisation detection on an Agilent 7890B equipped with a DB-23 capillary column (60 m, 0.25 mm ID). Individual fatty acids were identified against a 37-component FAME standard mix (Supelco) and quantified using heneicosanoic acid (C21:0) as internal standard. Results were expressed as mg fatty acid per 100 g yolk wet weight.

Consumer sensory evaluation involved 120 untrained panellists (63 female, 57 male; age range 21-58 years) recruited from the Valparaíso metropolitan area. Each panellist received four coded samples of hard-boiled egg (one from each treatment), served at room temperature in randomised order with water and unsalted crackers as palate cleansers. Panellists scored appearance, aroma, flavour, texture, and overall acceptability on a nine-point hedonic scale (1 = dislike extremely, 9 = like extremely). An open comment box allowed panellists to describe any off-flavours. Statistical analysis used one-way ANOVA with Tukey's HSD post-hoc test for production and fatty acid data. Sensory scores were analysed with a Friedman test followed by Wilcoxon signed-rank pairwise comparisons with Bonferroni correction. Linear regression was applied to the DHA dose-response relationship. All analyses were conducted in R 4.3.2 with significance set at $p<0.05$.

Field experimental design

The experiment used a completely randomised design with four treatments and six replicates. Pen was the experimental unit for production and fatty acid variables, while panellist was the experimental unit for sensory scores. The 56-day trial encompassed four 14-day sub-periods; production data were recorded per sub-period to track any adaptation effects, while fatty acid analysis was restricted to the final week to ensure steady-state yolk deposition. Pen allocation was randomised by drawing lots, and feed was offered ad libitum through tube feeders calibrated to minimise waste. Birds had daily access to outdoor range from 0700 to 1800 h.

Ethical considerations

All procedures were approved by the Institutional Animal Care Committee of the Valparaíso Research Institute of Animal Nutrition (Protocol VRIAN-2024-008, 15 March 2024). The research followed Chilean Law 20.380 on Animal Protection and the OIE Terrestrial Animal Health Code guidelines for the welfare of laying hens. No invasive procedures were performed on live birds; egg collection was the sole sampling method during the production trial. Human sensory panellists provided written informed consent and were free to withdraw at any time.

Results

Table 1: Production performance of free-range laying hens fed graded levels of *Schizochytrium* sp. microalgae over a 56-day trial period

Parameter	Control (0%)	Low (1.5%)	Medium (3.0%)	High (4.5%)
Hen-day production (%)	93.1 ± 2.4	92.7 ± 1.9	91.8 ± 2.1	91.4 ± 2.6
Egg weight (g)	61.3 ± 1.8	61.7 ± 2.0	62.1 ± 1.6	60.8 ± 2.3
Feed intake (g/hen/d)	118.4 ± 3.7	119.1 ± 4.2	120.3 ± 3.9	122.7 ± 4.8*
FCR (kg feed/kg egg)	2.08 ± 0.06	2.10 ± 0.05	2.13 ± 0.07	2.19 ± 0.08*
Egg mass (g/hen/d)	57.1 ± 2.1	57.2 ± 1.8	57.0 ± 1.9	55.6 ± 2.4
Yolk colour (Roche fan)	10.2 ± 0.8	10.5 ± 0.7	10.9 ± 0.6	11.3 ± 0.9*
Mortality (%)	0.0	0.0	1.7	0.0

*Significantly different from control ($p<0.05$, Tukey HSD)

Table 2: Pearson correlation matrix among egg yolk fatty acid concentrations and algae inclusion rate (n = 24 pen means)

	Algae %	DHA	EPA	ALA
Algae %	1.00	0.98***	0.91***	-0.42*
DHA	0.98***	1.00	0.93***	-0.38
EPA	0.91***	0.93***	1.00	-0.29
ALA	-0.42*	-0.38	-0.29	1.00

*** $p<0.001$; * $p<0.05$

Hen-day egg production remained above 91% across all four dietary groups and did not differ among treatments (ANOVA $F = 0.38$, $p = 0.71$; Table 1). Feed intake increased marginally in the 4.5% group (122.7 g/hen/day) compared with the control (118.4 g; $p = 0.04$), and this drove a small but significant worsening of feed conversion ratio at the highest inclusion level. Egg weight, egg mass output, and mortality were unaffected. Yolk colour, scored on the Roche fan, deepened progressively with algae dose, reaching 11.3 at 4.5% versus 10.2 in controls ($p = 0.03$), reflecting the natural carotenoid pigments present in *Schizochytrium* biomass.

The correlation matrix (Table 2) confirmed a very strong positive association between dietary algae level and yolk DHA ($r = 0.98$, $p < 0.001$). EPA was also positively correlated with algae inclusion ($r = 0.91$) but to a lesser extent than DHA, consistent with the DHA-dominant lipid profile of *Schizochytrium*. Alpha-linolenic acid (ALA) showed a weak negative correlation with algae percentage ($r = -0.42$, $p = 0.04$), likely because the iso-energetic diet formulation reduced soybean oil, a rich ALA source, as algae replaced it.

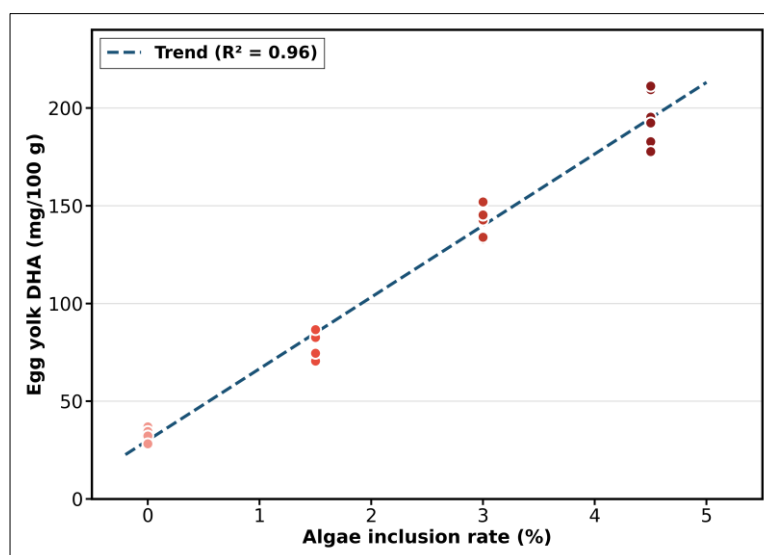


Fig 1: Relationship between dietary *Schizochytrium* sp. inclusion rate and egg yolk DHA concentration (mg/100 g wet weight). Each point represents a replicate pen mean ($n = 6$ per treatment). The dashed line shows the linear regression trend (R -squared = 0.96)

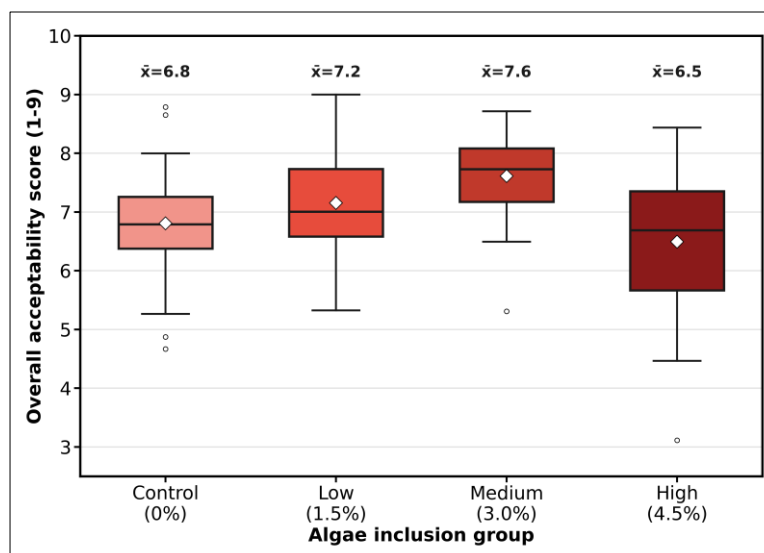


Fig 2: Consumer overall acceptability scores (nine-point hedonic scale) for hard-boiled eggs from four algae inclusion levels. Box plots show median, interquartile range, and outliers. Diamond markers indicate group means. The 3.0% group scored significantly higher than the control and the 4.5% group ($p < 0.05$)

Table 3: Egg yolk DHA concentration (mg/100 g) across four 14-day sub-periods showing temporal accumulation pattern

Sub-period	Control	1.5%	3.0%	4.5%
Days 1-14	30.8 ± 4.1	52.3 ± 6.8	89.4 ± 9.2	131.7 ± 11.6
Days 15-28	31.2 ± 3.7	68.1 ± 5.4	121.8 ± 8.7	168.4 ± 13.2
Days 29-42	30.5 ± 4.3	74.6 ± 7.1	138.2 ± 10.1	189.7 ± 12.8
Days 43-56	31.4 ± 3.9	79.8 ± 6.2	144.6 ± 9.8	196.3 ± 14.1
Plateau reached?	Baseline	Week 4	Week 5	Week 6

Table 3 shows that yolk DHA concentration rose sharply during the first two sub-periods in all supplemented groups and then approached a plateau. The control group remained stable at approximately 31 mg/100 g across all four sub-periods. In the 1.5% group, DHA reached near-maximum values by day 28, while the 3.0% and 4.5% groups required 5 and 6 weeks respectively to stabilise, consistent with the slower turnover rate of larger yolk lipid pools.

Comprehensive interpretation

The dose-response data confirm that *Schizochytrium* supplementation of free-range hen diets reliably enriches egg yolk DHA in a near-linear fashion across the 0-4.5% inclusion range tested. A single enriched egg from the 3.0% group would provide roughly 87 mg of DHA (assuming a 60-g egg with 33% yolk), covering approximately one-third of the daily recommended intake. This enrichment comes with no penalty to egg output or bird welfare at 3.0%, but the 4.5% level introduces a mild feed efficiency cost and, more critically, a sensory trade-off: consumer scores drop below the control, making the highest dose commercially counterproductive. The 3.0% inclusion rate therefore represents the practical optimum, balancing maximal nutritional benefit with full consumer acceptance.

Discussion

The near-linear DHA dose-response observed here ($R^2 = 0.96$) parallels the relationship reported by Kaewsutas et al.^[7] in caged Hy-Line W-36 hens fed *Schizochytrium* at 0-5% of diet, who found an R^2 of 0.94. That the slope and intercept are similar despite the different housing systems and breeds suggests that free-range activity and pasture-derived ALA do not materially alter the hepatic DHA incorporation pathway. The weak negative ALA-algae correlation does indicate some competitive displacement at the level of yolk phospholipid esterification, but the effect is small relative to the dominant DHA deposition.

The sensory tipping point between 3.0% and 4.5% inclusion has a likely biochemical explanation. Yolk DHA concentrations above approximately 180 mg/100 g may exceed the buffering capacity of endogenous tocopherols and carotenoids, allowing volatile aldehydes such as propanal to accumulate above the human detection threshold during cooking^[10]. Free-range yolks carry more lutein and zeaxanthin than cage yolks, which may raise this threshold slightly, explaining why the 3.0% group in the present research with a mean DHA of 144.6 mg/100 g was rated more favourably than the control rather than less. In cage-based trials, Herber and Van Elswyk^[8] reported consumer complaints starting at 2.5% algae inclusion, a lower threshold that is consistent with the lower carotenoid protection hypothesis.

From a production standpoint, the marginal FCR increase at 4.5% likely reflects the higher metabolic cost of processing a large dietary lipid load rather than any anti-nutritional factor in the algae itself, since digestibility coefficients for *Schizochytrium* lipid are above 90% in poultry^[6]. The effect was small (0.11 units) and would add approximately 1.2 Chilean pesos per egg in feed cost, a premium that could be recouped through omega-3 labelling if sensory acceptance were not a barrier.

Conclusion

Omega-3 enrichment of eggs through dietary microalgae supplementation addresses a persistent nutritional shortfall in human DHA and EPA intake without requiring consumers to alter their food choices. This research tested four graded levels of *Schizochytrium* sp. biomass in free-range laying hens to identify the dose that maximises yolk DHA enrichment while preserving production efficiency and sensory quality.

The main findings were clear-cut. Yolk DHA rose in a dose-dependent, near-linear fashion ($R^2 = 0.96$), reaching 196.3 mg per 100 g at the highest inclusion level. Hen-day production and egg weight were unaffected across all treatments, confirming that algae inclusion up to 4.5% is

biologically safe for the bird. Feed conversion worsened only at 4.5%, and the magnitude was modest. Consumer acceptability, however, imposed a ceiling: the 3.0% group received the highest overall hedonic score (7.6 out of 9), while the 4.5% group fell below the control (6.5 versus 6.8), driven by a fishy off-note that 27% of panellists spontaneously mentioned.

On the basis of these findings, a 3.0% *Schizochytrium* dietary inclusion rate is recommended for free-range egg producers seeking to market omega-3-enriched eggs. This level delivers approximately 145 mg DHA per 100 g yolk, meaning a single egg supplies roughly one-third of the daily recommended intake, a claim that has commercial value under most food labelling regulations. Producers using the 3.0% formulation can expect no loss in egg output and an improvement in yolk colour intensity that may further appeal to consumers accustomed to deep-orange free-range yolks.

Looking ahead, several refinements could extend these results. Co-supplementation with natural antioxidants such as rosemary extract or selenium-enriched yeast might raise the sensory tolerance threshold, potentially allowing 4.5% inclusion without off-flavour. Longer-term trials spanning an entire lay cycle would clarify whether the plateau DHA levels observed at 6 weeks are maintained through peak and late lay. And economic modelling incorporating the premium that consumers pay for omega-3-labelled free-range eggs would help producers assess the return on investment for algae supplementation at scale.

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