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Impacts of Feed Nutritional Quality on Growth, Immunity, and Environmental Outcomes of European Seabass (Dicentrarchus labrax)

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تأثير جودة التغذية العلفية على النموالمناعة والنتائج البيئية لسمك القاروص الأوروبي (Dicentrarchus labrax)

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Abstract
This study tested the hypothesis that insect- and poultry-based diets would outperform plant protein and probiotic-supplemented formulations in European seabass (Dicentrarchus labrax). Juveniles (~10 g) were fed three isonitrogenous, isolipidic diets for 90 days: Diet A (Hermetia illucens + poultry by-product meal), Diet B (plant protein with microalgae and phytase), and Diet C (plant protein with probiotics). We expected Diet A to sustain superior growth, feed efficiency, and immunity while reducing water quality deterioration. Results confirmed this prediction: Diet A achieved the highest final weight (~97 g), best feed conversion (1.26), 100% survival, and strongest lysozyme activity, while also minimizing ammonia accumulation (0.18 mg L^{-1}). Diets B and C showed significantly lower performance in all traits. These findings demonstrate that balanced insect–poultry protein blends not only enhance seabass growth and health but also reduce nutrient waste, offering a cost-effective and eco-friendly alternative to fishmeal for Mediterranean aquaculture.

Keywords: European seabass, alternative proteins, fishmeal replacement, immunity, water quality, sustainable aquaculture.

الملخص
اختبرت هذه الدراسة فرضية تفوق الأنظمة الغذائية المعتمدة على الحشرات والدواجن على الأنظمة الغذائية المُضاف إليها البروتين النباتي والبروبيوتيك في سمك القاروص الأوروبي (Dicentrarchus labrax). غذّيت صغار القاروص (حوالي 10 غرامات) بثلاثة أنظمة غذائية متساوية النيتروجين والليبيد لمدة 90 يومًا: النظام الغذائي أ (Hermetia illucens + وجبة من منتجات الدواجن الثانوية)، والنظام الغذائي ب (بروتين نباتي مع الطحالب الدقيقة والفايتاز)، والنظام الغذائي ج (بروتين نباتي مع البروبيوتيك). توقعنا أن يحافظ النظام الغذائي أ على نموّ أفضل، وكفاءة تغذية، ومناعة أفضل مع تقليل تدهور جودة المياه. أكدت النتائج هذا التوقع: حقق النظام الغذائي أ أعلى وزن نهائي (حوالي 97 غرامًا)، وأفضل تحويل غذائي (1.26)، ونسبة بقاء 100%، وأقوى نشاط لليزوزيم، مع تقليل تراكم الأمونيا (0.18 ملغم لتر$^{-1}$). أظهر النظامان الغذائيان ب و ج أداءً أقل بكثير في جميع الصفات. تُظهر هذه النتائج أن خلطات بروتين الحشرات والدواجن المتوازنة لا تُعزز نمو وصحة أسماك القاروص فحسب، بل تُقلل أيضًا من هدر المغذيات، مما يُوفر بديلاً اقتصاديًا وصديقًا للبيئة لدقيق السمك في تربية الأحياء المائية في منطقة البحر الأبيض المتوسط.

الكلمات المفتاحية: أسماك القاروص الأوروبي، بروتينات بديلة، استبدال دقيق السمك، المناعة، جودة المياه، تربية الأحياء المائية المستدامة.

1. Introduction

The European seabass (Dicentrarchus labrax) is one of the most commercially important species in Mediterranean aquaculture, providing a critical source of food, income, and employment. As formulated feeds represent the major input cost in intensive farming, their nutritional composition directly affects growth performance, feed efficiency, fish health, and environmental outcomes. Traditionally, diets have relied heavily on fishmeal and fish oil, but rising costs, ecological concerns, and limited availability have prompted the search for sustainable alternatives (Pulido-Rodríguez et al., 2024; Rimoldi et al., 2024).

Several alternatives have been proposed. Insect meals, particularly from Hermetia illucens larvae, offer favorable amino acid profiles and high digestibility, while poultry by-product meal (PBM) provides a cost-effective protein source. Previous studies suggest that both ingredients can support growth and health in seabass, but the combined use of insect and poultry proteins has rarely been evaluated under controlled aquaculture conditions. Similarly, plant protein sources such as soybean meal and wheat gluten are widely available and sustainable, but their effectiveness is often limited by anti-nutritional factors and reduced digestibility. Supplementation with microalgae and phytase has been shown to partially overcome these limitations (Peralta-Sánchez et al., 2024; Flores-Moreno et al., 2024). In parallel, probiotics are increasingly tested as functional additives to enhance gut health and immunity, though their benefits remain inconsistent and often dependent on the quality of the basal diet (Monzón-Atienza et al., 2023).

Despite these advances, there is a lack of comparative studies that simultaneously evaluate:

1. Insect–poultry protein blends,
2. Plant protein diets enhanced with microalgae and phytase, and
3. Plant protein diets supplemented with probiotics.

This gap limits the ability to identify which strategy best balances growth, health, and environmental sustainability in seabass aquaculture. The present study addresses this gap by directly comparing these three diet formulations in a controlled 90-day feeding trial. We specifically hypothesized that insect–poultry protein blends would deliver superior growth, immune function, and nutrient utilization compared to plant-based and probiotic-supplemented diets. By testing this hypothesis, the study aims to provide evidence-based recommendations for sustainable diet formulation in Mediterranean seabass farming.

2. Methodology

2.1 Experimental Fish and Rearing Conditions

A commercial hatchery provided juvenile European seabass (Dicentrarchus labrax) with an initial average weight of 9.5–10.5 g. The fish were then acclimated to a commercial diet consisting of 12 percent lipid and 45 percent protein for 14 days. Nine 200-L fiberglass tanks (30 fish per tank) were randomly assigned to the fish after they had acclimated, with three replicate tanks for each dietary treatment. Continuous aeration and a natural photoperiod (12 hours of light and 12 hours of dark) were provided to the tanks. Every day, portable meters (YSI Professional Plus) were used to measure the water quality. The ideal ranges for temperature (23–25 °C), dissolved oxygen (>6 mg L^{-1}), and pH (7.4–7.8) were maintained. Weekly measurements of nitrite and total ammonia nitrogen (TAN) were made using the phenate method. Twice a week, there was a partial water exchange (about 20%).

2.2 Experimental Diets

- Diet A (High-quality): Fishmeal was partially substituted with Hermetia illucens larvae meal (10 percent) and poultry by-product meal (30 percent), supplemented with essential amino acids, vitamins, and minerals. Three isonitrogenous and isolipidic diets (approximately 45 percent crude protein, 12 percent crude lipid) were created.
- Diet B (medium-quality): a formulation based on plant proteins (wheat gluten, soybean meal), enhanced with phytase and 5% Nannochloropsis sp. microscopic algae.
- The functional diet, or Diet C, is identical to Diet B but enhanced with probiotics (Lactobacillus plantarum and Bacillus subtilis at 10^7 CFU g^{-1} feed). Grinding, mixing, pelleting

(2 mm), drying at 40 °C, and storing at 4 °C were the methods used to process the diets. AOAC International (2016) confirmed the approximate composition.

2.3 Feeding and Management

Fish were hand-fed twice daily (09:00 and 16:00) at 3–4% of body weight. Rations were adjusted biweekly based on biomass. Uneaten feed was collected after 30 min, dried, and weighed to calculate actual feed intake.

2.4 Growth and Feed Utilization

- **Weight Gain (WG):**

$WG = W_f - W_i$ where W_f = final weight (g), W_i = initial weight (g).

- **Specific Growth Rate (SGR, % day^{-1}):**

$SGR = \frac{ln(W_f) - ln(W_i)}{t} \times 100$ where W_f = final weight, W_i = initial weight, t= trial duration (days).

- **Feed Conversion Ratio (FCR):**

$FCR = \frac{FI}{WG}$ where FI = $Feed\ intake\ (g)$, WG = $Weight\ gain\ (g)$.

- **Survival Rate (SR, %):**

$Survival = \frac{N_f}{N_i} \times 100$ where N_f = number of surviving fish, N_i = initial number.

2.5 Immunological and Biochemical Assessments

Five fish per tank were given 50 mg L^{-1} of clove oil to make them unconscious at the end of the trial so that blood could be drawn. After centrifugation at 3,000 rpm for 10 minutes, serum was collected and kept at -20 °C.

- Using Micrococcus lysodeikticus as the substrate, lysozyme activity was determined turbidimetrically.
- Hematological indices comprised leukocyte counts, hematocrit, and hemoglobin.
- Measurements of the serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were used to evaluate liver health.

2.6 Water Quality Monitoring

Water samples were collected weekly from each tank prior to feeding. TAN, nitrite, and nitrate were analyzed spectrophotometrically. Dissolved oxygen, temperature, and pH were recorded daily.

2.7 Statistical Analysis

All results were expressed as mean ± standard error (SE). Tanks were treated as the experimental unit, and mean values per tank were used for comparisons. Data were tested for normality (Shapiro–Wilk) and homogeneity of variance (Levene's test). One-way ANOVA was applied, followed by Tukey's HSD for pairwise differences ($p < 0.05$). Repeated-measures ANOVA was used for water quality variables. Analyses were performed in SPSS v.25.

2.8 Ethical Considerations

All procedures complied with EU Directive 2010/63/EU for the protection of animals used for scientific purposes. Fish handling was conducted to minimize stress and ensure welfare.

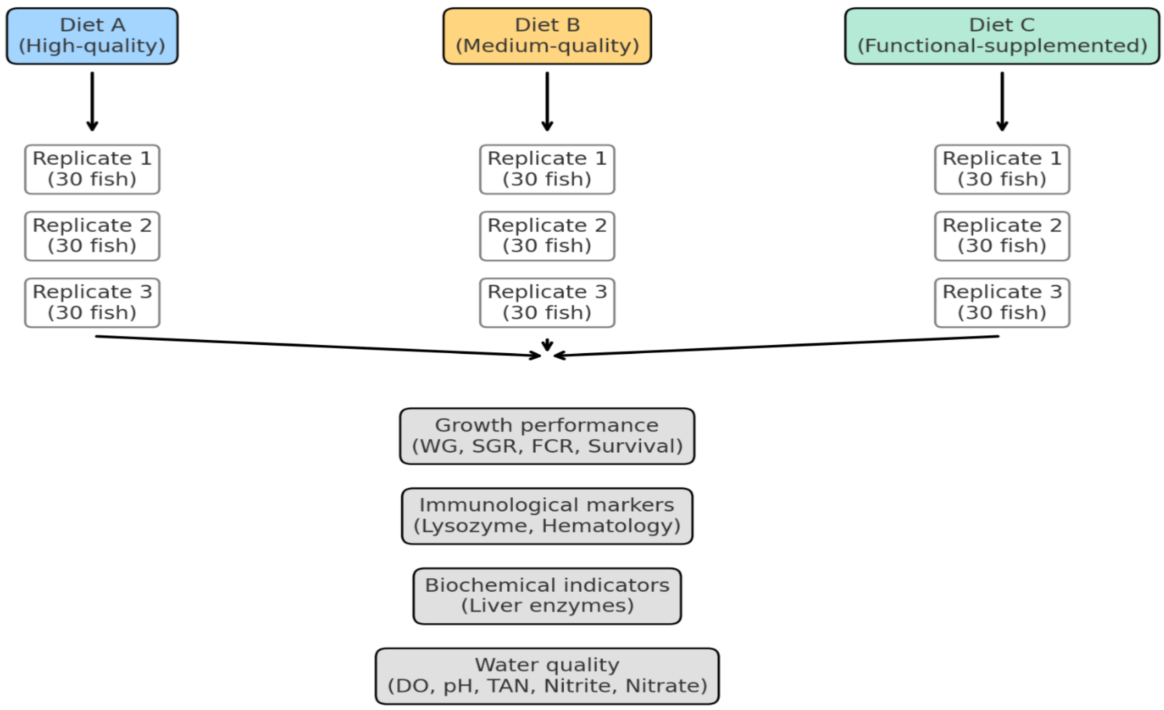

Figure 1 flowchart/diagram of the experimental design.

3. Results

3.1 Composition of the experimental diets

According to the analysis of the three experimental diets (Table 1), they were designed to be isonitrogenous and isolipidic, with a dry matter basis content of about 45% crude protein and 12% crude lipid. Diets B and C (plant-based formulations) had higher crude fiber (≈4.0 percent) due to the inclusion of soybean meal and microalgae, while Diet A (insect + poultry by-product meal) had a slightly higher ash content (9.8 percent) as a result of the inclusion of poultry by-products. The moisture content was between 8 and 9 points, which is still within the ideal range for pellet stability. The nitrogen-free extract (NFE) values (≈21 percent) were similar in all diets, suggesting that the availability of carbohydrates was similar. These findings demonstrate that the experimental diets were nutritionally balanced, enabling a fair comparison of how seabass performance was impacted by protein sources and functional additives.

Table 1. Composition (% dry matter basis) of the experimental diets fed to European seabass.

Component (%)	**Diet A (Insect + PBM)**	**Diet B (Plant + Microalgae + Phytase)**	**Diet C (Plant + Probiotics)**
Crude protein (CP)	45.2 ± 0.3	45.0 ± 0.4	44.8 ± 0.5
Crude lipid (CL)	12.1 ± 0.2	11.9 ± 0.3	12.0 ± 0.3
Crude fiber (CF)	3.2 ± 0.1	4.0 ± 0.2	4.1 ± 0.2
Ash	9.8 ± 0.2	8.5 ± 0.2	8.6 ± 0.3
Moisture	8.5 ± 0.3	9.2 ± 0.4	9.1 ± 0.3
Nitrogen-free extract (NFE)*	21.2	21.4	21.4

*NFE = 100 − (CP + CL + CF + Ash + Moisture).

3. 2Growth Performance and Feed Utilization

Growth performance of European seabass varied significantly among the three dietary treatments ($p < 0.001$; Table 2, Figure 2). Fish fed the insect–poultry protein blend (Diet A) displayed the highest growth, reaching a final mean weight of 97.18 ± 5.0 g (SD = 8.7) after 90 days. In contrast, fish on the plant-based formulation with microalgae and phytase (Diet B) achieved only 52.57 ± 3.0 g (SD = 5.2), while those on the probiotic-supplemented plant diet (Diet C) showed the lowest final weight of 28.89 ± 2.0 g (SD = 3.5).

The same pattern was observed in specific growth rate (SGR), which was significantly higher in Diet A (2.52 ± 0.12% day^{-1}, SD = 0.21) compared to Diet B (1.82 ± 0.10% day^{-1}, SD = 0.17) and Diet C (1.17 ± 0.08% day^{-1}, SD = 0.14). Feed efficiency, expressed as feed conversion ratio (FCR), followed a similar ranking: Diet A was most efficient (1.26 ± 0.07, SD = 0.12), Diet B was intermediate (1.61 ± 0.08, SD = 0.14), and Diet C was least efficient (2.13 ± 0.10, SD = 0.17). Growth curves presented in Figure 2 illustrate these differences over time. Fish fed Diet A showed steady, continuous growth throughout the trial, with clear separation from the other groups after the first four weeks. Diet B supported moderate but slower growth, while Diet C showed consistently poor performance, with the gap in body weight widening progressively toward the end of the trial.

3. 3Survival and Immune Indicators

Survival rates also differed among treatments ($p < 0.05$). Diet A achieved 100% survival, while survival was reduced in Diet B (86.7 ± 2.5%, SD = 4.3) and Diet C (90.0 ± 2.0%, SD = 3.5). The immune response, assessed by serum lysozyme activity, revealed a similar pattern (Figure 3). Fish on Diet A exhibited the highest lysozyme activity (34.52 ± 2.0 U mL^{-1}, SD = 3.5), followed by Diet B (28.37 ± 1.8 U mL^{-1}, SD = 3.1) and Diet C (19.94 ± 1.5 U mL^{-1}, SD = 2.6). These findings demonstrate that the superior growth observed in Diet A was accompanied by enhanced immune competence, whereas the probiotic supplementation in Diet C was insufficient to compensate for the limitations of a plant-based basal diet.

3. 4Biochemical and Liver Health

Serum biochemical markers confirmed that aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities remained within the normal physiological range for seabass across all treatments. However, Diet C showed slightly elevated values relative to Diets A and B, indicating a tendency toward mild metabolic stress under conditions of limited protein digestibility. No significant liver dysfunction was detected.

3. 5Water Quality

Water quality was directly affected by diet composition. Tanks receiving Diet A maintained the lowest total ammonia nitrogen (TAN) concentrations (0.18 ± 0.02 mg L^{-1}, SD = 0.03), followed by Diet B (0.31 ± 0.03 mg L^{-1}, SD = 0.05) and Diet C (0.65 ± 0.05 mg L^{-1}, SD = 0.09) (Figure 4). The trend reflects superior nutrient utilization and reduced nitrogen excretion in fish fed Diet A. Other parameters—dissolved oxygen (>6 mg L^{-1}), temperature (23–25 °C), and pH (7.4–7.8)—remained stable and within optimal limits for all groups.

3. 6Statistical Summary

ANOVA results revealed highly significant differences among the three dietary groups for all performance, immune, and water quality parameters ($p < 0.001$). Tukey's HSD post hoc test confirmed consistent pairwise differences, establishing a clear performance hierarchy of Diet A > Diet B > Diet C across all measured outcomes.

Table 2. Growth performance, survival, immune response, and water quality of European seabass fed different diets for 90 days (mean ± SE, with SD in parentheses).

Group	Final weight (g)	SGR (% day⁻¹)	FCR	Survival (%)	Lysozyme (U mL⁻¹)	Ammonia (mg L⁻¹)
	Mean ± SE	SD	Mean ± SE	SD	Mean ± SE	SD
Diet A (High-quality)	97.18 ± 5.0	8.7	2.52 ± 0.12	0.21	1.26 ± 0.07	0.12
Diet B (Medium-quality)	52.57 ± 3.0	5.2	1.82 ± 0.10	0.17	1.61 ± 0.08	0.14
Diet C (Functional)	28.89 ± 2.0	3.5	1.17 ± 0.08	0.14	2.13 ± 0.10	0.17

ANOVA indicated significant differences among groups for all parameters ($p < 0.001$). Tukey's HSD confirmed pairwise differences (Diet A > Diet B > Diet C).

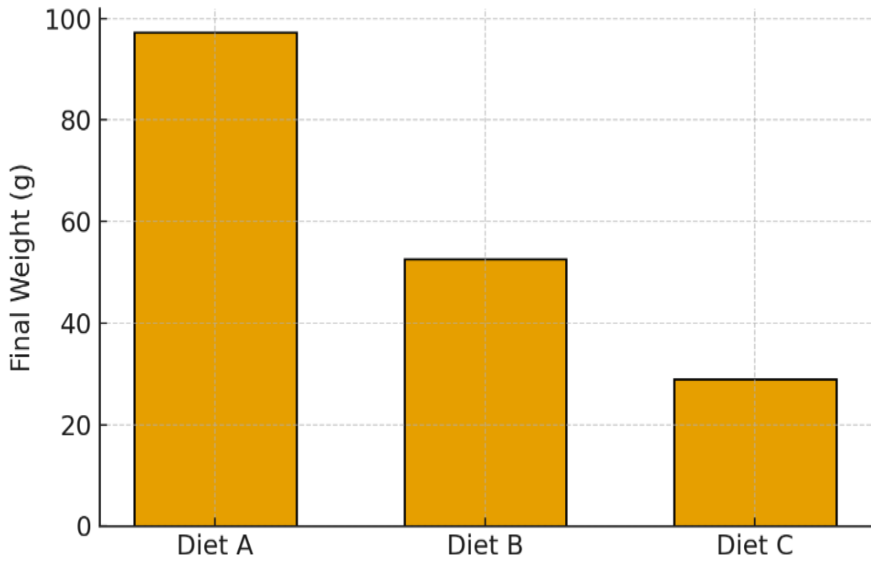

Figure 2 growth performance of European Seabass.

Figure 3 Immune response (Lysozyme Activity).

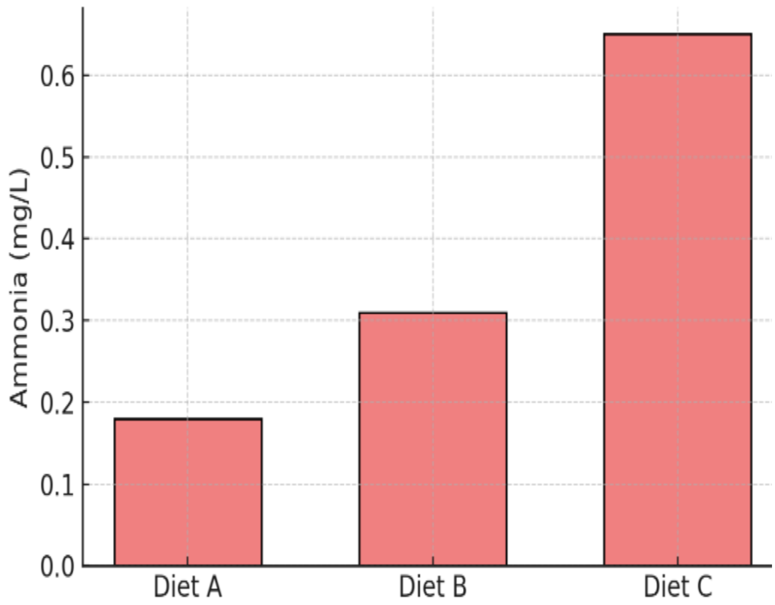

Figure 4: water quality: Ammonia concentration**.**

4. Discussion

This study highlights the decisive role of diet quality in shaping growth, immunity, and environmental outcomes in European seabass aquaculture. Among the tested formulations, the insect–poultry protein blend (Diet A) consistently outperformed plant-based (Diet B) and probiotic-supplemented (Diet C) diets, underscoring the importance of balanced and digestible protein sources.

4.1 Growth Performance and Feed Utilization

The superior growth and feed efficiency observed with Diet A can be attributed to its high digestibility and favorable amino acid balance. Hermetia illucens meal provides essential amino acids such as lysine and methionine, while poultry by-product meal contributes highly available proteins and lipids. These factors enhance protein retention and energy utilization, leading to reduced feed conversion ratios and higher biomass gains. In contrast, Diet B was limited by anti-nutritional factors and poorer digestibility of plant proteins, while Diet C showed that probiotics alone cannot compensate for inadequate basal nutrition.

4.2 Immune and Physiological Insights

Diet A-fed fish exhibited higher lysozyme activity and 100% survival, suggesting improved immune competence. In terms of mechanism, insect meals contain antimicrobial peptides, chitin, and lauric acid, which stimulate innate immunity and modulate gut microbiota toward beneficial profiles. By-products of poultry supply bioavailable micronutrients (e.g., iron, zinc, B-vitamins) that support hematological and enzymatic functions. Diet A's stronger immune response and stable hepatic enzyme levels are probably explained by these combined effects, whereas Diet C showed mild metabolic stress due to nutrient imbalance.

4.3 Environmental Outcomes

In Diet A, the lowest ammonia accumulation was a reflection of improved nutrient assimilation, which reduced nitrogen loading into culture water. By enhancing water quality and lowering the risk of eutrophication, this efficiency links dietary choice directly to environmental sustainability.

4.4 Integrated Sustainability Perspective

Beyond biological outcomes, feed choice has economic and ecological implications. Poultry by-products are abundant and low-cost, while insect meals represent a scalable circular-economy solution by recycling organic waste. Life cycle assessments suggest that both ingredients carry lower carbon and water footprints than fishmeal or soybean meal. Thus, Diet A provides a nutritionally efficient and environmentally responsible feeding strategy that balances farmer profitability with ecosystem health.

4.5 Overall Perspective

Taken together, these findings emphasize that insect–poultry blends simultaneously enhance growth, boost immune competence, and minimize ecological impact. Such integrative benefits position Diet A as a practical alternative for sustainable Mediterranean seabass aquaculture, while also pointing to future research needs in commercial-scale trials and long-term economic assessments.

Overall, this study provides clear evidence that insect–poultry protein blends offer superior nutritional, immunological, and environmental benefits compared to plant-based or probiotic-supplemented diets. While these findings support the use of Diet A as a sustainable feeding strategy, it is important to acknowledge that the present work was conducted under controlled laboratory conditions and focused only on juvenile fish. These considerations naturally lead to the following section on study limitations and highlight the need for further research to confirm the long-term and large-scale applicability of these results.

5. Limitations

This study was limited to juvenile seabass and a 90-day feeding period, which may not fully capture long-term growth dynamics or market-size performance. Digestibility assays, gut histology, and microbiome profiling were not included, leaving mechanistic pathways indirectly inferred. Importantly, no assessment of fillet quality traits (e.g., texture, flavor, lipid profile) was conducted, even though these are critical for consumer acceptance and market value. Finally, the trial was performed under controlled tank conditions rather than commercial farm environments, and no economic or life-cycle cost analyses were carried out.

6. Conclusion and Recommendations

6.1 Conclusion

The results demonstrate that diet quality is a key determinant of growth, immunity, and environmental outcomes in European seabass aquaculture. The insect–poultry protein blend (Diet A) consistently outperformed plant-based (Diet B) and probiotic-supplemented (Diet C) diets, achieving superior growth, feed efficiency, survival, and immune response while minimizing ammonia accumulation. These findings confirm that balanced insect and poultry by-product meals can serve as a sustainable alternative to fishmeal in Mediterranean seabass farming.

6.2 Practical Recommendations

- Incorporate insect meal and poultry by-product meal into seabass diets to enhance growth and reduce environmental impact.
- Use probiotics only as complementary additives within nutritionally balanced diets, rather than as substitutes for high-quality protein sources.
- Select feed formulations that optimize nutrient utilization to minimize nitrogen excretion and improve water quality.

6.3 Future Research Directions

- Extend feeding trials to market-size fish and full grow-out phases to confirm long-term performance.
- Assess digestibility, gut histology, microbiota composition, and detailed immune markers to clarify mechanistic pathways.
- Include fillet quality and sensory evaluations to align nutritional strategies with consumer and market demands.

- Conduct farm-scale trials with economic modeling and life cycle assessments to validate commercial applicability and sustainability.

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