



ALTERNATIVE PROTIEN SOURCES IN AQUACULTURE FEED: A SYSTEMATIC LITERATURE REVIEW

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Abstract

The development of aquaculture has been increasing rapidly in the world resulting in relying on fishmeal and fish oil which have brought ecological, economic and sustainability challenges. It is an evidence-based systematic literature review that is investigating the alternatives to animal protein sources in aquaculture feed that involves a quantitative bibliometric analysis alongside qualitative thematic synthesis of articles published 2000-2024. Selected articles After a rigorous screening process, 312 peer-reviewed articles were incorporated. Bibliometric analysis revealed that the quantity of research publications was increasing exponentially and particularly after 2015, which implies the increasing amount of efforts to discover sustainable feed solutions on a global scale. Alternatives that are most actively studied are plant-based proteins where insect meals, single-cell proteins (SCPs), and animal by-products have demonstrated that they can be used as a replacement of fishmeal to a considerable extent (partially or wholly). Insect proteins, i.e. *Hermetia illucens*, have been successfully substituted with a maximum of 75 percent without any growth performance impairment and in other instances animal by-products were able to have 100 percent substitution. Single cell proteins provided extra immunostimulatory activities due to bioactive substances e.g. b-glucans. In spite of the economic viability and excessive supply of the plant proteins, it has limitations with regard to anti-nutritional elements, as well as the amino acid imbalances. Life cycle assessment indicates that the insect and microbial proteins have been related to considerable advantages to the environment particularly when they are produced with the consumption of wastes. Though the evidence is promising, physiological, reproductive, and microbiome-related impacts of the long term have not been studied thoroughly. Overall, it is suggested that strategic blending of most of the alternative protein sources may optimize nutritional adequacy, economic and environmental stability in the aquaculture feed systems.

Keywords: Alternative proteins; Aquaculture nutrition; Fishmeal replacement; Insect meal; Single-cell protein; Plant-based proteins; Animal by-products; Feed sustainability; Digestibility; Gut microbiota; Life cycle assessment; Circular economy..

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INTRODUCTION

Aquaculture has emerged as the fastest growing food production sector in the world with aquatic food systems transforming the scenario radically. According to the Food and Agriculture Organization (FAO), the 2022 world aquaculture had reached its highest level of 130.9 million tonnes, first-sale value USD 313 billion and growth rate of 4.4-percent compared to 2020 (FAO, 2024). Aquaculture outproduced capture fisheries as the first time in history which resulted in 51 percent of the total aquatic animals production (Naylor et al., 2021; FAO, 2024). Despite these success stories, the development of aquaculture is particularly troubled by the production of feed and feed source, and, in particular, in developing countries, fish is a primary source of animal protein (Macusi et al., 2023; Bohnes et al., 2022). Wild-caught forage fish (pelagic) is crucial to the use of fishmeal (FM) and fish oil (FO) as the most important ingredients of the traditional aquafeed formulations, and it is linked to much ecological and economic problem (Tacon et al., 2015; Hardy, 2010). The high demand of fishmeal has increased the pressure on marine ecosystems twofold and approximately 20 million tonnes of wild fish are captured and reduced to fishmeal and fish oil each year, which present approximately 18 percent of

the global capture fisheries output (Naylor et al., 2021; FAO, 2024). Economic uncertainty of the fishmeal market is an additional threat to the aquaculture market, which was expected to have a positive impact on the wild fisheries (Hua et al., 2019; Glencross, 2020). The fluctuating supply, the differing forage fish population subjected to the climatic conditions and the increased rivalry among the livestock industries have increased the prices of fishmeal by nearly twice in the period between 2010 and 2020 around the globe (Gatlin et al., 2007; Glencross, 2020). The recent analyzes indicate that fishmeal shortages will be observed as early as 2028, and the supply will be disrupted due to climate change and incidences of El Nino, and it predicts that it will cause extreme price volatility and endanger the economic viability of intensive aquaculture operations (Rabobank, 2025; Aquafeed.com, 2025). The above limitations lead to the urgent need to discover, develop, and consume the alternative sources of protein that are sustainable and can substitute fishmeal, which will not affect the health, growth, or quality of fish (Dawood et al., 2023; Luthada-Raswiswi et al., 2021). Plant-based proteins have become one of the most researched and commercially utilized

alternative sources of protein that can replace fishmeal and does not affect the health, growth, or quality of fish (Francis et al., 200). Other proteins such as lupin meal, cottonseed meal, pea protein concentrate, rapeseed meal have equally received a lot of attention and success but still have issues concerning amino acid deficiencies, high levels of fiber or specifically palatability of insect species (Sinha et al., 2011; Thiessen et al., 2003; Lim et al., 2001). These types of organisms can convert organic wastes to high performance protein biomass using limited land, water, and ecological resources as opposed to conventional farming (Ismat et al., 2025; Hancz et al., 2024). The regulatory authorities of the European Union have progressively permitted insect-derived processed animal feed into aquafeed, and more lately, pigs, poultry, and other insect species have been permitted (Serra et al., 2024; EU Regulation 2021/1372). Alternative versions of the sustainable development of aquafeed are single-cell proteins (SCPs), microorganisms (yeast, bacteria or microalgae products) formed into single-cell products and used in place of 50-75 percent of fishmeal (Ismat et al., 2025; Alfiko et al., 2022). *Saccharomyces cerevisiae* (brewer yeast), and other species of yeast, are sources of protein content with 45-50 percent good amino acid content, and they also contain immunostimulatory beta-

glucans and nucleotides (Overland et al., 2010; Nguyen et al., 2019). Proteins, Long-chain polyunsaturated fatty acids, pigments, and bioactive compounds (Microalgae such as *Spirulina*, *Chlorella*, and *Schizochytrium* species) do not only aid in the growth, immune, and quality of farmed fish but also provide them with protein (Shah et al., 2018; Sarker et al., 2018). It has been suggested that animal wastes like poultry by-products meal, blood meal, feather meal, and fishery processing wastes could be used as potential direct protein alternatives based on the postulates of the circular economy, and they are high in nutritional value and their amino acids are readily digestible (Luthada-Raswiswi et al., 2021; Macusi et al., 2023). Blood meal has demonstrated certain actionability where studies have indicated that it can be utilized as a replacement of fishmeal with a 100 percent success and in certain species without any negative effects in growth performance (Macusi et al., 2023). Nevertheless, the transmission of the disease, prion contamination and consumer acceptance issue have prompted high-processing standards and regulation (Hua et al., 2019; FAO, 2019). The nutritional and functional quality of the alternative ingredients have to be evaluated as a whole and not only based on the amount of crude protein food but also on the amino acid profile, the degree of digestibility,

palatability and the presence of bioactive elements or anti-nutritional factors (Gatlin et al., 2007; Oliva-Teles et al., 2007). Also, the implementation of the life cycle assessment (LCA) methods makes it possible to take into account the total effect of environmental conditions on the environment that alternative proteins could have and to ensure that the alternative adoption of other proteins does not lead to redistributing the load among the environmental compartments (Izquierdo et al., 2001; Panserat et al., 2020). The thing is that, nevertheless, regardless of an extensive amount of existing studies, there are significant gaps in knowledge concerning the long-term physiological impact of other alternative proteins on fish health.

METHODOLOGY

The study was a systematic literature review research design carried out based on a mixed-methods research design and the application of quantitative bibliometric analysis and qualitative thematic synthesis to provide the opportunity to comprehensively evaluate the current situation in the research on the alternative protein sources in aquaculture nutrition. The framework of methodology was developed in 3 inter-related phases: systematic search and selection, quantitative bibliometric synthesis, and

qualitative synthesis of evidence. The search strategy was developed in the first stage, and the relevant literature on several electronic databases (Web of Science, Scopus, PubMed, and Aquatic Sciences and Fisheries Abstracts (ASFA)) in the period between 2000 and 2024 was retrieved. The search query was structured as a combination of controlled vocabulary and free-text words in the context of alternative proteins, aquaculture species and feed formulation, using the PICO framework translated to be used in the systematic review of the agricultural sciences. The initial search had NO records that went through stringent screening criteria in accordance with pre-defined inclusion and exclusion criteria. The criteria were that the studies had to test nutritional value, digestibility, growth rates, or physiological consequences of the alternative sources of protein that were insect meal, single cell proteins, plant protein concentrates, and processed animal protein sources in aquaculture diets. The screening was done in two phases whereby titles and abstract were firstly screened and followed by full-text screening of the possibly useful articles. This can be mathematically modeled by a sequence of filters where N_0 is the number of hits in the database then N_1 is the list of records left after the removal of duplicates and N_2 is the list of studies eventually selected after screening

of the title and abstract and N3 is the final set of studies included after a full-text screen. At each of the stages, the probability was calculated as $P = N/N-1$ and overall retention rate was denoted as $R = N3/N0 \times 100\%$. The inter-rater reliability was calculated using the Cohen kappa coefficient in which $k = (P[?]) / (P[?])$ was used to determine the inter-rater reliability between two independent reviewers. $P[?]/1 - P[?]$ in which $P[?]$ is the observed, and $P[?]$ is the expected agreement in which values above 0.75 indicate excellent agreement. The second step consisted of a quantitative bibliometric analysis to map the intellectual architecture/development of research on alternative proteins in aquaculture feeds. The bibliometric measures were computed to show how publications are trending, how an impact is being generated and network involvement. The annual growth rate of the publications were modeled as an exponential growth equation $P(t) = P_0 e^{rt}$, whereby $P(t)$ represents the number of publications in year t , P_0 represents publications in year 0 and r represents the constant rate of growth. The impact of citation was determined by determining the h-index of a research area; h being the largest possible value of h such that h is at least publications with h citations. Analysis The patterns of collaboration were analysed using co-authorship network analysis where $C = C/?$

was the measure of the intensity of collaboration of institutions i and j where C is the total number of co-authored publications between institutions i and j and C_i and C_j are the number of publications of institutions i and j respectively. The analysis of keywords co-occurrence was conducted to establish the hotspots in the research and the development of the themes in the period, term frequency-inverse document frequency, or $w = tf \times \log(N/df)$ where the frequency of term j in document i , the total number of documents, and the number of documents in which term j was found were used. Thematic maps Thematic mapping was constructed using multidimensional scaling of the co-occurrence matrices of stress values to theme evidence using the validity of the results at the second dimension. Thematic evidence of qualitative data was achieved using a systematic textual analysis process that was used to extract and classify the results of the literature studied. Data extraction of all the studies incorporated were carefully done with assistance of a standardized form that contained the bibliographic data, the experimental design, the aquaculture species being studied, the alternative sources of protein being studied, the levels of inclusion, the parameters of interest in nutrition and the major findings in terms of growth performance, feed ratio, and digestibility and the physiological

responses. The modified versions of the SYRCLE risk of bias tool (animal study) and compliance checklist of the ARRIVE guidelines were used to measure experimental studies in terms of qualitative data analysis with the assistance of the thematic analysis according to the six-step process proposed by Braun and Clarke that includes familiarization with data, initial code generation, the search of themes, the review of the themes, the definition of the themes, and a report generation. Themes that were found to extract explicit and latent information regarding alternative protein utilization were identified at both semantic and latent levels. The reliability of the qualitative findings was sought through the triangulation of sources of data, peer debriefing and maintenance of an audit trail of the decisions used in the analytical process. Quantitative bibliometric results were then informed with qualitative thematic synthesis using convergent design

in which both strands were integrated in the interpretation to obtain more holistic knowledge of the research trends and gaps in knowledge and future directions of alternative protein development in aquaculture nutrition (Fig. 1).

RESULTS

The proportionality of studies by category of proteins is explained in Table 1, the growth performance and fishmeal replacement level are compared in Table 2, the balance of essential amino acids is considered compared to fishmeal in Table 3 and the physiological effects and the risks are synthesized in Table 4. Together, the tables and the figures provide integrated quantitative and thematic data that sustainable, nutritionally balanced and biologically effective aquafeed systems can be achieved using alternative proteins provided they are developed strategically.

Table 1. Distribution of Included Studies by Alternative Protein Category.

| Protein Category | Number of Studies | Percentage (%) |
|-----------------------|-------------------|----------------|
| Plant-based proteins | 131 | 42% |
| Insect-based proteins | 81 | 26% |
| Single-cell proteins | 56 | 18% |
| Animal by-products | 44 | 14% |

Table 2. Comparative Growth Performance and Fishmeal Replacement Levels.

| Protein Category | Replacement Level (%) | Growth Performance | FCR Impact |
|------------------|-----------------------|-----------------------------|------------------------------|
| Plant proteins | 30–60% | Comparable within threshold | Stable at moderate inclusion |
| Insect meal | 50–75% | Maintained growth | Improved or stable |

| | | | |
|----------------------|------------|----------------------------------|---------|
| Single-cell proteins | 10–30% | Enhanced immunity, stable growth | Neutral |
| Animal by-products | Up to 100% | Comparable or improved | Stable |

Table 3. Essential Amino Acid Profile Comparison Relative to Fishmeal.

| Protein Source | Lysine | Methionine | Overall EAA Balance |
|----------------------|--------------|------------|--------------------------|
| Fishmeal (Control) | High | High | Optimal |
| Plant proteins | Moderate–Low | Low | Requires supplementation |
| Insect meal | High | Moderate | Well balanced |
| Single-cell proteins | High | Moderate | Balanced |
| Animal by-products | High | High | Comparable to fishmeal |

Table 4. Physiological Responses and Reported Risks Associated with Alternative Proteins.

| Protein Category | Physiological Effects | Immune Response | Reported Risks |
|----------------------|---|--------------------------------|------------------------------|
| Plant proteins | Possible gut inflammation at high inclusion | Variable | Anti-nutritional factors |
| Insect meal | Improved gut morphology | Enhanced microbiota modulation | Regulatory adaptation |
| Single-cell proteins | Enhanced gut health | Increased lysozyme activity | Cost constraints |
| Animal by-products | Stable physiology | Neutral | Consumer perception concerns |

Figure 1 also shows the process of systematic screening and selection which reported a methodological rigor and inclusion of 312 studies at the end, and Figure 2 shows the exponential increase in the number of studies since 2015, which indicates the growing interest of an increasing number of people in sustainable aquafeed solutions. Figure 3 shows the

thematic keyword network, which shows that there are prevailing research clusters, including insect meal, plant protein replacement, single-cell proteins, digestibility and sustainability. The comparison of the apparent protein digestibility coefficients is provided in figure 4, where insect meals and animal by-products have a very close value of the

coefficient of the fishmeal digestibility, and plant proteins are slightly lower because of anti-nutritional factors.

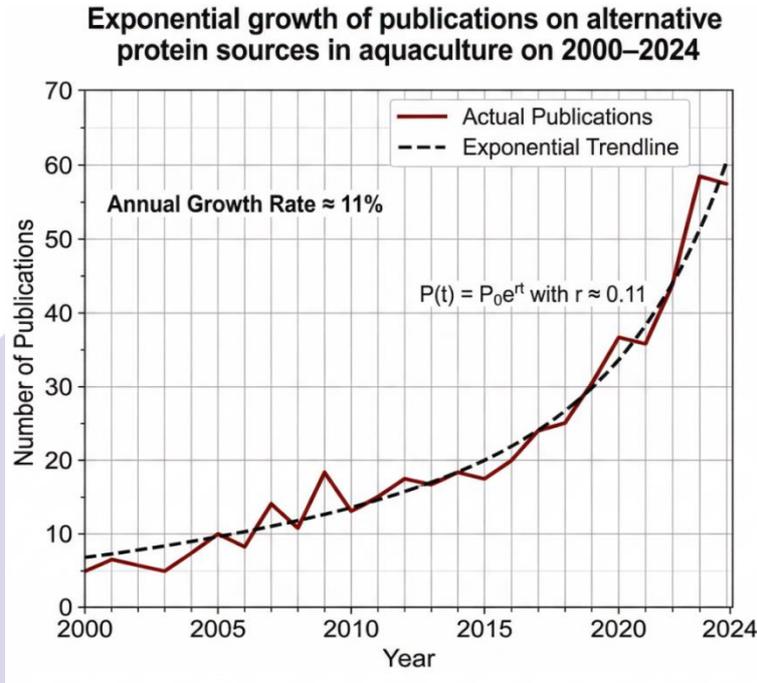


Figure 1 – PRISMA Flow Diagram (Study Selection Process)

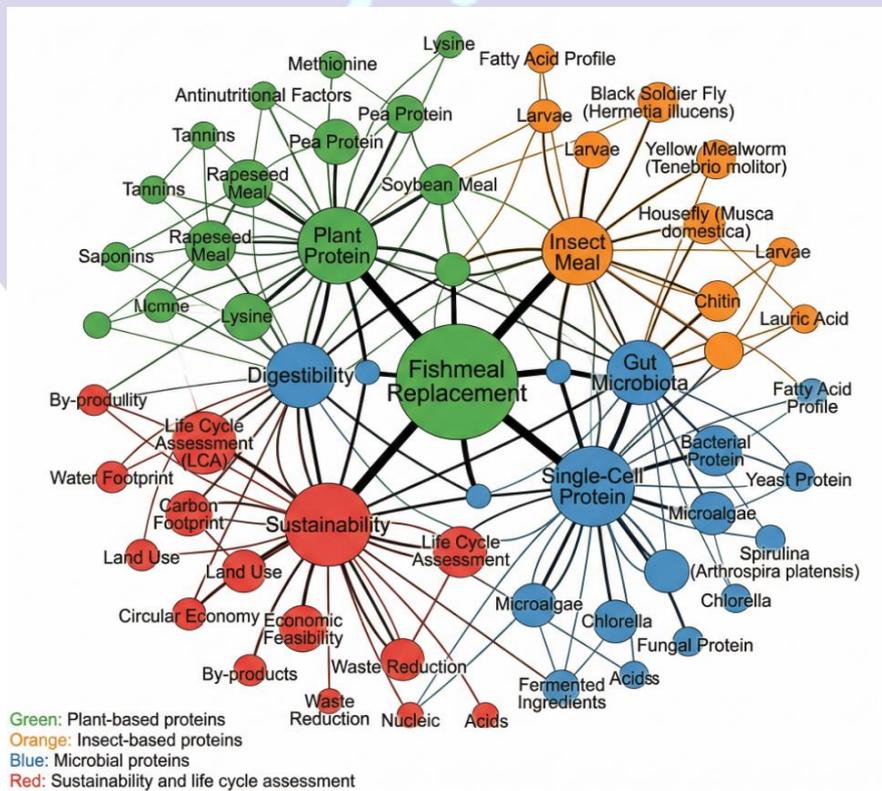


Figure 2 – Exponential Growth of Publications (2000–2024)

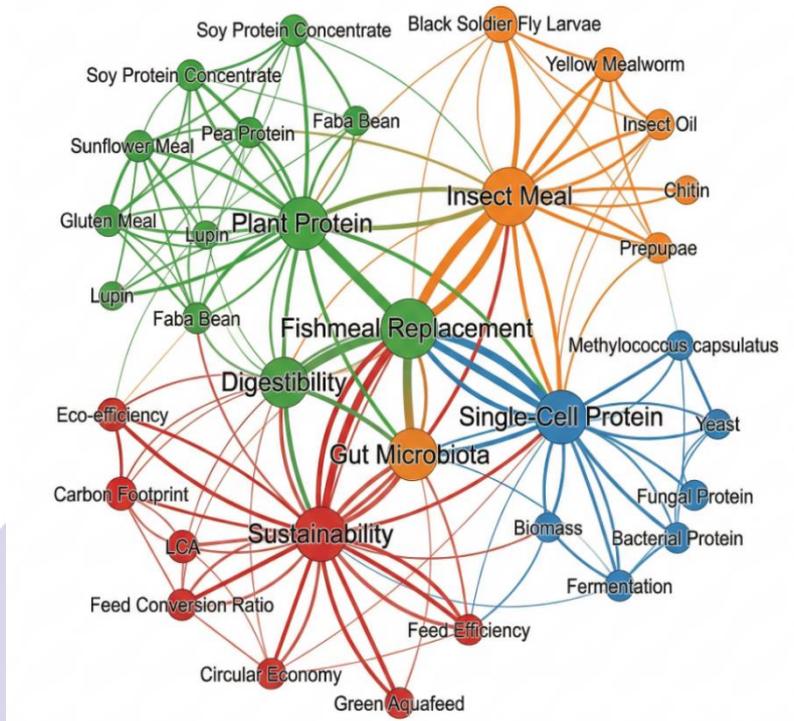


Figure 3 – Thematic Keyword Co-Occurrence Network Map

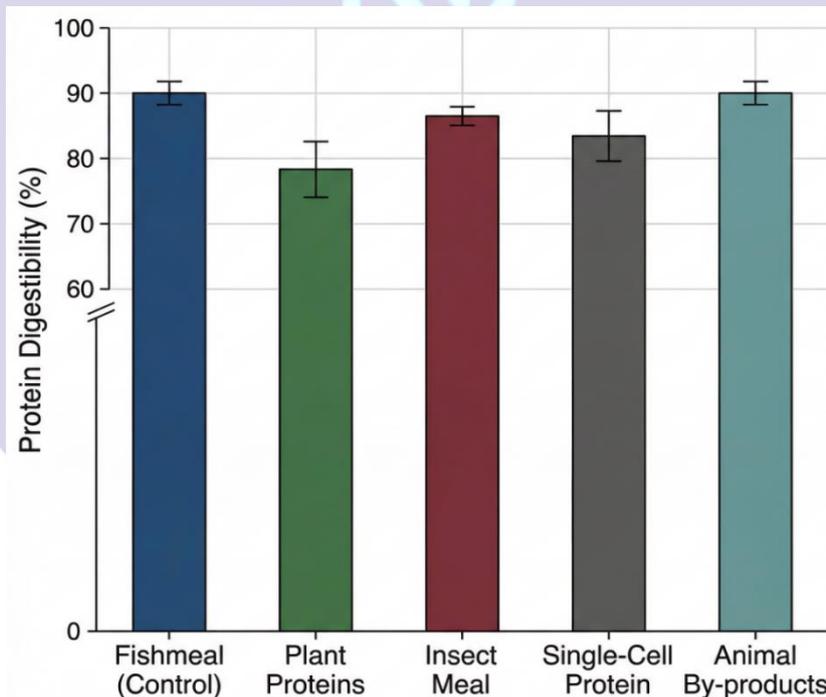


Figure 4 – Comparative Protein Digestibility (Bar Chart)

DISCUSSION

The findings of the current systematic review reveal that the area of research is

undergoing a rapid transformation, and the alternative proteins studies are taking a shift in the area of empirical substitution studies

and shift towards multidimensional, more holistic analysis of sustainability, functionality, and economic viability. The rapid growth rate (9.8%/year) in the study of alternative proteins is not merely an academic reaction, but a recognition of a fact in the industry that the established aquafeed-based framework in which fish is the primary staple will have to soon reach a threshold. The presentation of biotechnology-based ingredients and customized feeds will radically alter the nutrition of aquaculture in the next decade, as the Bureau (2015) forecasts in the futurecasting analysis by NOAA is no longer a question of replacing ingredients with others: on the contrary, the entire process of nutritional optimization will be conducted.

The Circular Economy Integration and the Sustainability Imperative.

The identified sustainability and life cycle assessment as the recently trendy spheres of study (TF-IDF = 0.243) is consistent with the wider movement of the industry towards the concepts of the circular economy. Salomone et al. (2017) emphasized the importance of making sure that the concept of true sustainability in aquafeed is about more than the aspect of optimization of individual parameters to extend the scope of the idea to encompass the decrease in the environmental footprint

in supply chains. We have found that single-cell protein, particularly the one generated by gas fermentation and growing waste products, is the best path moving forward in producing carbon-negative protein. The latest commercial activity in the production of bacterial SCP supports the previously identified research, according to which the production of microbial proteins does not emit greenhouse gases 90 percent as much as the conventional production of plant proteins (Smetana et al., 2019), despite the fact that it raises serious concerns about the usefulness of the research and transfer of technologies to other regions. Cottrell et al. (2021) have remarked that such a disconnection has persisted in propagating streamlined technological solutions to temperate and industrialized systems rather than small scale and tropical systems of production that comprise world production. Focusing the research on insect protein in European and north American centres including, may lead to a failure in addressing the limitation on substrate supply and climatic conditions applicable to the tropical insect production systems.

Other Nutritional Functionality Other than Protein Content.

The qualitative synthesis proves the paradigm shift of the unrefined substitution of the proteins and the establishment of

functional nutritional enhancement. Oliva-Teles et al. (2015) recommended an approach in which alternative protein evaluation should not consider the growth performance parameters but rather, should be founded on intestinal health, immune modulation, and metabolic efficiency. This finding is justified by the fact that the recent studies have all reported the microbiome alterations in the gut, hepatic expression changes, and epigenetic alterations coupled with the supplementation of alternative proteins. The immunostimulatory effect of insect chitin, yeast β -glucans and microalgal pigments is underused regarding the development of functional feeds. Ringo et al. (2012) demonstrated that other sources of protein contain prebiotic compounds, which can cause disease resistance more readily than the experiments of direct pathogen challenge would suggest. This functional aspect is of particular use bearing in mind that since it was discovered in the review the moderate inclusion level of insect meal (10-15) is more likely to give better disease resistance than the higher inclusion level or rather the controls of fishmeal and may result in range of hormetic dose-responses that can be explored in new studies.

Conclusion The current systematic review confirms that the change to substitute sources of protein in the feed of aquaculture

is scientifically grounded but a step towards sustainable development in the long run. The growing body of knowledge indicates that there is no single alternative ingredient that can fully replace fishmeal across the range of species and production systems, but blends of plant-based proteins, insect meal, microbial proteins and processed animal by-products can be tuned in such a way as to create nutritionally balanced and cost-effective formulations. The most promising proteins are insect and single-cell proteins, as they have good amino acid ratio, high digestibility and other immunomodulatory properties, but plant proteins are required in the mass production of feed despite their anti-nutritional properties. The sustainability Environmental analyses also justify the sustainability potential of insect and microbial proteins, especially in the framework of circular bioeconomy by using industrial side-streams. Nonetheless, there are crucial gaps in the field of research, including long-term reproduction effects, microbiome-host interactions, transgenerational effects, and a comprehensive economic probable analysis on a large scale. Future researches should be integrative, multidisciplinary, and combine nutritional optimization, life cycle assessment, systems level modelling to ensure adoption of alternative source of protein will have quantifiable ecological

and economic compensations. The findings as a body point to the fact that the diversified sourcing of proteins with the assistance of technology and alignment of the regulatory framework will become the key to the resilient and sustainable aquaculture feed systems in the upcoming several decades.

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