# IMPACT OF ALTERNATIVE PROTEIN SOURCES ON GROWTH AND FEED EFFICIENCY IN BROILER BIRDS: A REVIEW

**KURUBA MANOHAR**

Faculty, Dept of Biotechnology,

Bharatiya Engineering Science & Technology Innovation University, Andhra Pradesh, India.

**Abstract**
Broiler production relies on high-quality protein sources to support rapid growth, efficient feed utilisation and optimal carcass yield. Soybean meal and fish meal have long dominated commercial diets due to their balanced amino acid profiles and digestibility; however, rising global demand, fluctuating prices, land-use limitations and environmental concerns have accelerated the search for sustainable alternatives. This review synthesizes verified research on insect meals, fermented plant proteins, leaf meals, single-cell proteins and agro-industrial by-products as emerging protein replacements in broiler nutrition. Black Soldier Fly Larvae meal, Tenebrio molitor meal, fermented soybean meal, Spirulina, Chlorella and bacterial protein meals have demonstrated the ability to maintain or improve growth performance, feed conversion ratio, nutrient digestibility, gut morphology and meat quality in controlled feeding trials. Mechanistic insights reveal improvements in villus height, crypt depth, enzymatic activity, antioxidant markers and immune function. Sustainability assessments highlight reduced greenhouse gas emissions, lower water and land demands and valorization of organic waste streams. Despite these advantages, constraints include anti-nutritional factors, chitin content, variable nutrient profiles, processing costs, regulatory barriers and consumer acceptance issues. This comprehensive review consolidates multidisciplinary evidence to guide the strategic inclusion of alternative proteins in broiler diets and identifies future research priorities for scaling these ingredients into commercial poultry systems.

**Keywords:** Commercial poultry systems, Tenebrio molitor meal, broiler birds, controlled feeding trials

## 1. Introduction

Poultry meat represents one of the fastest-growing animal protein sectors globally, driven by population expansion, urbanization, rising incomes and the comparatively low production cost of broiler meat. High growth rates of modern broiler strains demand nutrient-dense diets, with protein being a central component for muscle accretion, enzymatic systems, immune function and overall productivity. Soybean meal (SBM) is traditionally the predominant protein source due to its high crude protein (44-48%) and balanced amino acid composition especially lysine and consistent global supply chains. Fish meal, although less widely used today, remains a benchmark for protein quality due to its highly digestible amino acids and rich mineral profile.

However, both these conventional protein sources are increasingly constrained. Soybean cultivation requires significant land resources, and expansion in major producing regions is linked to deforestation and biodiversity loss. Global reliance on soybean imports creates price volatility for developing countries. Fish meal production, meanwhile, faces ecological limitations as marine stocks experience overexploitation, policy regulations and climate- driven fluctuations. The rising cost of soybean meal and fish meal accounts for a substantial proportion of total broiler production costs, often exceeding 60 to 70% of feed expenditure. These pressures necessitate exploration of alternative, sustainable and locally available protein sources.

Over the past decade, significant technological and biological advances have enabled evaluation of insects, fermented plant proteins, leaf meals, microalgae, yeast and bacterial protein meals as potential alternatives. Novel fermentation technologies improve digestibility and reduce anti-nutritional factors in plant-based meals. Insect farming has emerged as a circular bioeconomy model that converts organic waste into high-quality protein. Single-cell proteins, including microalgae and methane utilizing bacteria, offer the potential for scalable, environmentally efficient production with consistent amino acid profiles.

The suitability of these alternative proteins for broilers depends on their nutritional composition, amino acid balance, digestibility, presence of anti-nutritional compounds, processing technologies and effects on physiological and metabolic pathways. Beyond growth and feed efficiency, modern nutritional science demands a more holistic understanding of how these ingredients affect gut morphology, microbiota composition, immune responses, carcass characteristics, meat quality, environmental footprints and economic feasibility.

Therefore, the objective of this review is to synthesis global, peer-reviewed and research on alternative protein sources to:

- Evaluate their nutrient composition and functional attributes
- Assess their impact on broiler growth, feed conversion ratio and digestibility
- Discuss physiological responses including gut health, immunity and metabolism
- Examine carcass traits and meat quality outcomes
- Evaluate sustainability and economic implications
- Identify constraints, limitations and future research directions

## 2. Insect-Based Protein Sources

Insects have become one of the most intensively studied alternative protein categories for broiler nutrition. Their nutrient density, balanced amino acid profile and ability to convert organic waste into high-quality biomass make them central to sustainable feed development. The strongest experimental evidence comes from Black Soldier Fly Larvae (BSFL), *Tenebrio molitor* (mealworm) and housefly larvae meals. Studies show that insect meals can partially or fully replace soybean meal without impairing growth, feed efficiency or carcass yield.

### Nutritional Characteristics

BSFL meal typically contains 40-44 and crude protein with a favourable amino acid profile.

According to Makkar *et al.* (2014) [11], BSFL meets or exceeds essential amino acid requirements for poultry, particularly lysine and threonine, while offering high digestibility and metabolizable energy comparable to fish meal. Rumpold and Schlüter (2013) [18] further demonstrated that insect meals contain high levels of digestible protein and beneficial lipids, including lauric acid, which supports gut health by suppressing pathogenic bacteria. Mealworm (*Tenebrio molitor*) meal contains 50-55and crude protein. In the trial by Bovera *et al.* (2016) [5], mealworm protein showed amino acid availability similar to soybean meal and improved crude protein digestibility in broilers. These characteristics make insects a nutritionally competitive alternative to conventional protein sources.

### Growth Performance and Feed Efficiency Black Soldier Fly Larvae (BSFL)

Among insect proteins, BSFL is the most thoroughly validated. Schiavone *et al.* (2017) [20] reported that partially defatted BSFL meal replaced up to 25 and soybean meal without affecting final body weight, feed intake or growth rate. Dabbou *et al.* (2018) [6] demonstrated that broilers fed defatted BSFL had significantly improved FCR and higher digestibility of crude protein, ether extract and ash. Studies consistently show that moderate inclusion levels (5-15and) optimally support performance.

### Mealworm (*Tenebrio molitor*)

Evidence from controlled trials indicates similar or improved performance relative to conventional diets. Bovera *et al*. (2016) [5] found equal growth performance and improved feed digestibility when mealworm meal was included at 25 and. Biasato *et al.* (2018) [4] observed enhanced gut morphology and no negative impact on body weight or feed utilisation.

### Housefly Maggot Meal

Although fewer high-quality trials exist compared to BSFL or mealworms, Studies indicate strong potential. Pretorius (2011) and later evaluations show that maggot meal is rich in methionine and supports normal growth and FCR when replacing fish meal. It shows insect meals reliably maintain growth and frequently improve FCR when used at optimal levels.

**Digestibility and Gut Health**

Insect meals appear to act not only as protein sources but also as functional feed ingredients.

**Improvements in Digestive Morphology**

Biasato *et al.* (2018) [4] demonstrated increased villus height and goblet cell density in broilers fed *Tenebrio molitor*, indicating improved absorptive function. Dabbou *et al.* (2018) [6] reported similar findings with BSFL, including enhanced villus height to crypt depth ratio.

**Antimicrobial and Immunomodulatory Effects**

The lauric acid content highlighted by Makkar *et al.* (2014) [11] may reduce harmful bacteria such as *Clostridium perfringens*. Chitin, present in insect exoskeletons, acts as a natural prebiotic and may help stimulate beneficial microbiota. The consistent findings support the role of insect meals in improving intestinal function and nutrient utilisation.

**Carcass Traits and Meat Quality**

Carcass and meat quality parameters generally remain unchanged or show slight improvements. Schiavone *et al.* (2017) [20] reported no adverse effects on dressing percentage, thigh or breast yield when BSFL meal was included. Bovera *et al.* (2016) [5] observed no negative changes in carcass composition with mealworm diets. Some BSFL studies Dabbou *et al.* (2018) [6] reported increased polyunsaturated fatty acids in breast meat due to the larvae's lipid profile, though effects vary by processing method.

**Sustainability and Environmental Benefits**

Insect farming is environmentally efficient Rumpold and Schlüter (2013) [18] demonstrated insects require far less land, water and feed substrate than conventional crops. Van Huis (2013) [23] showed insects convert feed into protein 2-3 times more efficiently than poultry or fish. BSFL can be reared on organic waste streams, reducing environmental pollution and contributing to waste valorization. These

attributes align insect meals with circular agriculture and climate-smart livestock production.

**Economic Feasibility**

Current economic assessments show insects have strong long-term cost potential due to scalability and waste-based substrates. Short-term cost competitiveness varies by region, largely dependent on processing infrastructure. As insect production expands, economies of scale are expected to significantly reduce prices. Insects demonstrate strong economic promise, particularly BSFL and mealworm meals, which already have emerging industrial supply chains.

**3. Fermented Plant Proteins**

Fermentation has emerged as one of the most effective and scientifically validated methods to enhance the nutritional value of plant-based protein ingredients. The process improves amino acid availability, increases solubility of proteins, reduces anti-nutritional factors and enhances gut health. The strongest verified evidence comes from trials involving fermented soybean meal (FSBM), fermented rapeseed meal, fermented cottonseed meal, and fermentation/enzyme-treated Palm Kernel Cake (PKC).

**Nutritional improvements through fermentation** Plant proteins often contain anti-nutritional factors such as phytates, tannins, trypsin inhibitors and fiber-associated polysaccharides. Fermentation reduces these compounds and increases nutrient digestibility. Feng *et al.* (2007) [7] demonstrated that fermentation significantly increased the availability of small peptides, improved

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digestive enzyme activity and enhanced immune function in broilers fed fermented soybean meal. The meta-analysis by Irawan *et al.* (2022) [9] confirmed that FSBM consistently improved final body weight during the starter phase and tended to improve FCR when compared with conventional soybean meal diets. Fermented rapeseed meal improved ileal digestibility of essential amino acids, particularly lysine, methionine and threonine (Kim *et al*., 2016) [10]. Double-fermented soybean meal (using two microbial stages) completely replaced soybean meal and fish meal without reducing growth performance or carcass yield (Abdel-Raheem *et al.*, 2023) [1]. These findings indicate that fermentation not only preserves protein quality but may enhance it beyond the level of untreated soybean meal.

**Fermented Soybean Meal (FSBM)**

FSBM is the most widely studied fermented protein source for poultry. Feng *et al.* (2007) [7] found that birds receiving FSBM showed improved weight gain, increased amylase and protease activity, and enhanced intestinal morphology. Irawan *et al.* (2022) [9] verified through meta-analysis that FSBM improves body weight during early growth phases, highlighting its value in the starter diet when nutrient digestibility is crucial. Abdel-Raheem *et al.* (2023) [1] demonstrated that double-fermented soybean meal supported equal or higher weight gain, improved gut health scores and reduced feed cost per unit gain compared with conventional protein sources.

**Mechanisms of Improvement The benefits of FSBM arise from**

- Breakdown of complex proteins into easily absorbable peptides
- Reduced trypsin inhibitors
- Lower antigenicity, especially reducing glycinin and β- conglycinin
- Improved gut enzyme activity
- Increased probiotic microbial metabolites

FSBM has the strongest enhancing broiler performance among all fermented plant proteins

**Fermented Rapeseed Meal**

Rapeseed meal has high crude protein but contains glucosinolates, which limit its use. Fermentation reduces these compounds. Kim *et al.* (2016) [10] documented significant improvement in amino acid digestibility and reduction in anti-nutritional factors in broilers fed *Bacillus*- fermented rapeseed meal. Showed that fermentation markedly decreases glucosinolate content and improves growth rate.

**Functional Benefits**

- Improved ileal digestibility
- Reduced intestinal stress
- Enhanced villus height
- Better nutrient absorption

Thus, fermented rapeseed meal becomes a viable partial replacement for soybean meal.

**Fermented Cottonseed Meal**

Cottonseed meal contains gossypol, a toxic compound that limits its inclusion in broiler diets. Confirmed that microbial fermentation degrades free gossypol and increases digestibility. Fermentation enhances crude protein utilisation and reduces negative effects on liver enzymes, making cottonseed meal safer for poultry.

**Fermentation-or Enzyme-Treated Palm Kernel Cake (PKC)**

PKC is abundant in tropical countries but is limited by high fiber and low digestibility. Sundu *et al.* (2006) [22] demonstrated that enzyme treatment or microbial fermentation improved metabolizable energy values and supported better body weight gain compared with raw PKC. Treated PKC increases protein availability and reduces fibre-associated limitations. Given the low cost and wide

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availability of PKC in Asia and Africa, fermentation significantly increases its value in broiler diets.

**Gut Health Improvements from Fermented Proteins** Fermented proteins often have probiotic effects due to organic acids, enzymes and microbial metabolites. Mukherjee *et al.* (2016) [14] recorded improved villus height, lower crypt depth and reduced harmful bacterial counts when broilers were fed fermented rapeseed meal. Fermented soybean meal has been consistently associated with improved gut integrity, reduced inflammation and better intestinal enzyme activity (Feng *et al*., 2007; Kim *et al*., 2016; Abdel-Raheem *et al*., 2023) [7, 10, 1]. These physiological improvements contribute to better feed conversion ratio and overall health.

**Limitations of Fermented Plant Proteins Despite strong benefits, several constraints exist**

- Nutrient consistency depends on microbial strain and fermentation conditions
- Over-fermentation can degrade amino acids
- Production requires controlled facilities
- High initial processing cost
- Variability among commercial products affects diet formulation

Fermentation remains one of the most effective ways to upgrade low-cost plant meals into high-performance protein ingredients.

### 4. Leaf Meals and Forage-Based Protein Sources

Leaf meals and forage-derived proteins (for example, *Moringa oleifera, Azolla filiculoides*, and *Leucaena leucocephala*) offer low-cost, locally available alternatives for partial protein replacement in broiler diets. Experimental studies demonstrate that, when used at appropriate inclusion levels and after suitable processing, leaf meals can contribute to growth, antioxidant status and immune function, although their high fibre and anti-nutritional factors limit the safe inclusion level.

**Nutritional composition and functional attributes** Leaf meals are typically rich in crude protein, vitamins and antioxidants but also contain higher crude fibre and variable mineral content compared with conventional meals. Moringa leaf meal provides considerable amounts of protein, carotenoids and vitamin C that act as antioxidants and may reduce oxidative stress in birds (Melesse *et al*., 2011) [12]. Azolla is comparatively rich in protein and carotenoids and supplies beneficial micronutrients when included at low levels (Basak *et al*., 2002) [2]. Leucaena has a high protein content but contains the secondary compound mimosine, which can limit utilisation unless detoxified by processing.

**Effects on growth performance and feed efficiency** Controlled trials indicate that modest inclusion of leaf meals (commonly 5-10 and of diet) maintains or modestly improves growth and feed efficiency, whereas higher levels often depress performance due to fibre and anti-nutrients. Melesse *et al.* (2011) [12] reported improved body weight and dressing percentage in broilers with 5-10 and moringa leaf meal inclusion, attributing benefits to improved antioxidant status and nutrient supply. Basak *et al.* (2002) [2] found that azolla meal at 5-10 and sustained growth performance and did not deteriorate feed conversion ratio. Conversely, studies synthesised by Sultana *et al.* (2014) [21] warn that high fibre and tannin contents in untreated leaf meals reduce apparent digestibility and therefore limit practical inclusion rates.

**Gut health, immunity and physiological responses** Leaf meals supply phytochemicals (polyphenols, vitamins, carotenoids) that can modulate immune function and antioxidant defenses. Trials have shown improvements in humoral immunity and reduced markers of oxidative stress in broilers receiving moringa or azolla at low-to-moderate inclusion levels (Melesse *et al*., 2011; Basak *et al*., 2002) [12, 2]. The improved antioxidant milieu can indirectly support gut integrity

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and nutrient utilisation, contributing to better FCR in some reports.

**Carcass traits and meat quality**

Where performance is maintained, carcass traits are generally unaffected or slightly improved; moringa

inclusion has been associated with better lean-to-fat ratios and improved oxidative stability of meat during storage (Melesse *et al.*, 2011) [12]. Azolla inclusion at recommended levels did not adversely affect dressing percentage or breast yield (Basak *et al*., 2002) [2].

**Processing, detoxification and practical considerations**

The principal limitation of leaf meals is high crude fibre and presence of anti-nutritional/toxic compounds (for example, mimosine in Leucaena). Processing methods: drying, grinding, ensiling, heat treatment, enzyme supplementation or sun-drying combined with microbial fermentation reduce fibre, deactivate toxins and improve palatability and digestibility. For Leucaena, simple drying and extended storage or microbial treatment reduce mimosine toxicity. Enzyme supplementation (cellulases, xylanases) can increase the metabolizable energy of leaf-based diets and thus permit slightly higher inclusion levels.

**Sustainability and local applicability**

Leaf meals are attractive for smallholder and regionally focused systems: they utilize locally grown trees and aquatic ferns, reduce reliance on imported soybean meal and contribute to circular nutrient flows when integrated into mixed farming systems. From an environmental perspective, leaf meals require no additional arable land if derived from existing tree crop systems (Melesse *et al.*, 2011) [12].

**Limitations and research gaps**

Despite encouraging results at low inclusion rates, large gaps remain: long-term feeding trials across commercial broiler genotypes, standardized processing protocols, quantitative digestibility data across seasons and regions, and comprehensive residue/toxicity analyses are still needed. The base summarized by Sultana *et al.* (2014) [21] highlights that uncontrolled variability in leaf meal composition leads to inconsistent performance outcomes.

**5. Single-Cell Proteins (Yeast, Bacterial Protein Meals, Microalgae)**

Single-cell proteins (SCP), produced from yeast, bacteria or microalgae, represent a technologically driven alternative to conventional feed proteins. SCPs offer high crude protein concentrations, favorable amino acid profiles, and in some cases, additional bioactive compounds (β-glucans, carotenoids, polyunsaturated fatty acids) that can enhance bird health beyond basic nutrition. This section synthesises verified evidence on SCPs from yeast (Saccharomyces and related products), bacterial protein meals grown on gaseous substrates, and microalgae (Spirulina, Chlorella), focusing on nutrient composition, effects on growth and feed efficiency, gut and immune responses, meat quality, and practical constraints.

**Nutritional and Functional Properties Yeast (*Saccharomyces* spp)**

Yeast products supply highly digestible protein, B-vitamins and functional cell wall components such as mannan- oligosaccharides and β-glucans. Reed & Nagodawithana, (1991) [17] describe yeast-based ingredients as lysine-rich and consistent in quality when produced under controlled fermentation. Yeast derivatives (autolysates, hydrolysates)

also provide peptides and nucleotides that can support intestinal development.

**Bacterial Protein Meal**

Bacterial SCPs produced from methane or natural gas (for example, *Methylococcus* spp. derivatives) can yield high protein biomass with predictable composition. Øverland *et al.* (2010) [15] reported that broilers fed bacterial protein produced on natural gas achieved body weights and FCR comparable or superior to soybean meal-based diets, demonstrating bacterial SCP's practical viability.

**Microalgae**

Microalgae contain 40-65and crude protein (depending on species and processing) and are rich in pigments (carotenoids), essential fatty acids, vitamins and antioxidants. Spirulina (*Arthrospira platensis*) and Chlorella spp. are the most studied for poultry applications; Ghasemi *et al*. (2020) [8] and Saeid *et al*. (2018) [19] document beneficial nutritional and functional effects.

**Effects on Growth Performance and Feed Efficiency Yeast**

Yeast inclusion especially as a functional yeast culture or autolysate has been associated with improved nutrient digestibility and performance stability under challenge conditions (Reed & Nagodawithana, 1991) [17]. Yeast improves crude protein utilisation partly via enhanced gut microbial balance and enzyme stimulation.

**Bacterial SCP**

Øverland *et al.* (2010) [15] provided controlled trial evidence that bacterial protein meal could replace a substantial portion of conventional soybean protein without compromising body weight gain; in some cases, FCR was improved. Follow-up work on meat fatty acid composition and oxidative stability confirmed that bacterial SCP did not impair meat quality.

**Microalgae**

Ghasemi *et al*. (2020) [8] found that dietary Spirulina supplementation improved weight gain and FCR in broilers at practical inclusion levels, benefits are often linked to improved antioxidant status and modulation of gut health. Saeid *et al*. (2018) [19] similarly reported that Chlorella enhances antioxidant capacity and can improve meat quality parameters. Microalgae can partially substitute soybean meal when diets are balanced for limiting amino acids.

Across SCP categories, the consensus from Studies is that SCPs can maintain or improve growth and feed efficiency when diets are correctly formulated, and inclusion levels are optimized to account for differences in amino acid digestibility and non-protein constituents (nucleic acids, pigments, cell wall polysaccharides).

**Gut Health, Immunity and Physiological Effects Immune Modulation**

Yeast cell wall components (β-glucans, mannan- oligosaccharides) exert immune-stimulatory effects, enhancing macrophage activity and antibody responses; Reed & Nagodawithana (1991) [17] describe these functional properties, and applied work shows improved resilience to enteric challenges in yeast-supplemented flocks.

**Microbiota and Mucosal Integrity**

Bacterial and yeast-derived products can stabilize intestinal microbiota, increasing beneficial taxa and reducing opportunistic pathogens, thereby improving villus morphology and nutrient absorption. Ghasemi *et al.* (2020) observed improvements in gut health markers with Spirulina supplementation, while Øverland *et al.* (2010) [15] reported no negative gut histology with bacterial SCP.

**Antioxidant Status**

Microalgae are potent sources of natural antioxidants (phycocyanin, carotenoids) that increase systemic antioxidant biomarkers and reduce lipid oxidation in meat. Saeid *et al.* (2018) [19] documented enhanced antioxidant capacity in birds fed Chlorella. These physiological effects translate into more efficient nutrient utilisation and resilience, which underpin gains in feed conversion ratio observed in several trials.

**Carcass Traits and Meat Quality**

Studies indicate SCPs do not adversely affect carcass yield and often provide quality benefits:

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Reported acceptable fatty acid profiles and oxidative stability in meat from birds fed bacterial SCP. Ghasemi *et al.* (2020) [8] and Saeid *et al.* (2018) [19] found improved meat pigmentation, oxidative stability and sometimes improved sensory traits due to microalgal pigments and antioxidants.
Formulation attention is required because pigments from microalgae (for example, carotenoids) can alter meat color usually an acceptable or desirable change (more yellow/orange hues) depending on market preferences.

**Economic and Production Considerations Production Costs**
At present, SCPs particularly microalgae tend to be more expensive than soybean meal on a per-kg basis, largely due to cultivation and drying costs. Bacterial SCP grown on methane/natural gas can be cost-competitive in regions with available gas resources and industrial infrastructure (Øverland *et al.*, 2010) [15].

**Scalability**
Yeast products are already produced at scale for feed applications, while scaled industrial production of bacterial SCP and microalgae is increasing but remains regionally variable. Technological advances and co-product valorisation (pigments, nutraceuticals) are improving SCP economics.

**Sustainability**
SCPs offer sustainability advantages: bacterial SCP can utilise methane (a potent greenhouse gas) as a substrate, converting emissions into protein (Øverland *et al.*, 2010) [15]. Microalgae cultivation can use wastewater and $CO_2$ streams, enhancing circularity (Saeid *et al.*, 2018) [19].

**Constraints and Research Gaps**
- **Nucleic Acid Content:** High RNA/DNA in SCPs can be a metabolic load if included at very high levels; processing steps (heat, enzymatic) may mitigate this.
- **Processing and Consistency:** Standardised production methods are needed to ensure consistent amino acid digestibility and product stability.
- **Cost Barriers:** Capital and operating costs for microalgae and specialized bacterial SCP remain a barrier in many regions.
- **Regulatory and Consumer Acceptance:** Novel feeding substrates and microbial origins require clear regulatory frameworks and consumer communication.

**Recommendations**
- Use SCP as partial replacement (e.g., 5-15and inclusion) while formulating diets to meet essential amino acid requirements.
- Prefer yeast autolysates and bacterial SCP in starter and grower diets where digestibility improvements and functional immune benefits are most valuable.
- Combine SCPs with enzyme supplementation and amino acid balancing to maximise FCR gains and avoid nutrient imbalances.
- Consider microalgae for value-added carcass traits (pigmentation, antioxidant status) where markets accept such changes.

## 6. Agro-industrial by-products, gut physiology synthesis, and comparative summary

**Agro-industrial By-products as Protein Sources**
Agro-industrial co-products (distillers' dried grains with solubles-DDGS, oilseed cakes, sunflower/copra cakes, brewery waste, palm kernel cake-PKC) are widely available, low-cost protein and energy sources. Their effective use depends on processing, inclusion level and enzyme/fermentation treatments to improve nutrient availability.

**Nutritional features**
- **DDGS**: moderate to high crude protein (20-30and), high fiber and variable fat; amino acid profile differs from SBM, with limiting lysine; energy and nutrient composition vary with

ethanol plant processing.

- **Oilseed cakes (sunflower, copra, cottonseed)**: variable protein (20-40 and), often higher fibre and some anti-nutritional factors (free gossypol in cottonseed).
- **Palm kernel cake (PKC)**: lower protein (~15- 20and) and high fiber; low metabolizable energy in raw form.

Murray *et al.* (2018) [13] reviewed DDGS use in broiler diets and reported that DDGS up to about 10-15and can be included without depressing growth or carcass traits when diets are formulated for amino acids and energy; variability in DDGS batches requires proximate analysis for accurate formulation. Sundu *et al.* (2006) [22] showed that enzyme or fermentation treatment of PKC significantly increased its metabolizable energy and supported better body weight gain relative to untreated PKC, making treated PKC a viable partial protein/energy source in tropical regions.

**Recommendations**

- Limit DDGS to 10-15and in typical broiler diets unless amino acid supplementation and energy adjustments are made.
- Pre-treat PKC (enzyme or fermentation) to increase usable energy before higher inclusion.
- Monitor batch-to-batch variability (especially DDGS) and correct diets for lysine and methionine deficits.

**Sustainability and economics**

Agro-byproducts lower feed cost and valorise waste streams, contributing to circular agriculture. Their local availability often makes them economically attractive for regionally focused systems.

**Gut Physiology: Integrated Synthesis from Studies** Gut morphology, digestive enzyme activity, microbial composition and mucosal immunity are central to understanding how alternative proteins affect growth and feed efficiency. Synthesizing verified evidence.

**Villus-crypt architecture and nutrient absorption** Villus height and villus height: Crypt depth ratio are reliable markers of absorptive surface and feed utilization. Biasato *et al.* (2018) [4] found *Tenebrio molitor* (mealworm) inclusion increased villus height and goblet cell density, suggesting enhanced nutrient absorption. Dabbou *et al.* (2018) [6] reported BSFL diets improved villus height: crypt depth ratio in broilers, correlating with higher digestibility and improved FCR.

**Digestive enzyme activity**

Fermented proteins increase endogenous enzyme activity (amylase, protease). Feng *et al.* (2007) [7] documented higher amylase and protease activities in broilers fed fermented soybean meal, which supports improved starch and protein digestion and thus growth. Irawan *et al.* (2022) [9] meta-analysis reinforces that FSBM enhances early-phase digestive capacity in broilers.

**Microbiota modulation and immune responses** Alternative proteins modulate gut microbiota composition and mucosal immunity: Chitin and medium-chain fatty acids in insect meals (Makkar *et al*., 2014; Dabbou *et al*., 2018) [11, 6] can suppress pathogenic bacteria and encourage beneficial taxa, contributingto lower intestinal inflammation and improved nutrient uptake. Fermented proteins supply organic acids and microbial metabolites that suppress pathogens and support barrier integrity (Kim *et al.*, 2016; Mukherjee *et al*., 2016) [10, 14]. Microalgae and yeast components (Ghasemi *et al*., 2020; Reed & Nagodawithana, 1991) [8, 17] enhance antioxidant status and immune markers, indirectly protecting gut mucosa.

**Mechanistic linkage to FCR and growth**

Improved gut morphology + higher enzyme activity + favourable microbiota = increased nutrient

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extraction per unit feed → improved feed conversion ratio and growth. Trials (Feng *et al*., 2007; Dabbou *et al*., 2018; Biasato *et al*., 2018; Mukherjee *et al*., 2016) [7, 6, 4, 14] consistently show this mechanistic pathway.

**Comparative Summary Table**

Below is a comparative summary using only verified sources from our list. Use this as a quick reference for inclusion levels, main benefits and limitations.

| Protein source | Recommended inclusion (broiler diets) | Benefits (Year) | Limitations (year) |
|---|---|---|---|
| BSFL meal | 5-15% | Maintains weight; improves FCR and digestibility (Dabbou 2018; Schiavone 2017) [6, 20] | Chitin, lipid variability; substrate- dependent composition (Makkar 2014) [11] |
| Mealworm (*T. molitor*) | Up to 25% (many trials use 10-25%) | Comparable growth, improved gut histomorphology (Bovera 2016; Biasato 2018) [5, 4] | Cost and scale limitations in some regions (Bovera 2016) [5] |
| Fermented soybean meal (FSBM) | Replace SBM variable; starter phase focus | Improves early weight gain, enzyme activity, gut health (Feng 2007; Irawan 2022; Abdel-Raheem 2023) [7, 9, 1] | Batch variability, processing requirements (Kim 2016) [10] |
| Fermented rapeseed / PKC | PKC: treated 5-15%; rapeseed: partial replacement | Reduced anti-nutrients, improved digestibility (Kim 2016; Sundu 2006) [10, 22] | Processing needed to reduce glucosinolates/fibre (Sundu 2006) [22] |
| Leaf meals (Moringa, Azolla) | 5-10% | Improved antioxidant status, moderate growth support (Melesse 2011; Basak 2002) [12, 2] | High fibre, anti-nutrients restrict higher inclusion (Sultana 2014) [21] |
| Microalgae (Spirulina/Chlorella) | 1-5% (for functional effects) | Improved antioxidant status, meat pigmentation, growth support (Ghasemi 2020; Saeid 2018) [8, 19] | Higher cost; pigment effects change meat colour (Ghasemi 2020) [8] |
| Bacterial SCP | Partial replacement (varies by product) | Comparable/superior BW and FCR in trials (Øverland 2010) [15] | Production infrastructure, regulatory approval (Øverland 2010) [15] |
| DDGS / agro-byproducts | DDGS: ≤15and typical; PKC treated higher | Cost reduction; acceptable performance at controlled inclusions (Murray 2018; Sundu 2006) [13, 22] | Variable nutrient content; high fibre; lysine limiting (Murray 2018) [13] |

**Practical formulation notes**

- Analyse each batch of alternative protein for proximate composition and digestible amino acids before formulating (Murray 2018; Makkar 2014) [13, 11].
- Balance limiting amino acids (lysine, methionine) when replacing SBM, using crystalline amino acids as needed (Murray 2018) [13].
- Consider enzyme blends (protease, cellulase, xylanase) or prior fermentation for high-fibre by-products (Sundu 2006; Kim 2016) [10, 22].
- Start with partial replacements (5-10%) and monitor performance and carcass traits before scaling inclusion.

## 7. Integrated discussion, comparative meta-insights, economics, regulatory issues and consumer acceptance

### 7. Integrated Discussion

The collective evidence from insect-based proteins, fermented plant proteins, leaf meals, single-cell proteins (SCP) and agro-industrial by-products reveals consistent patterns that help explain why many of these ingredients can effectively replace soybean meal or fish meal in broiler diets. Across Studies, three mechanistic pillars repeatedly explain improvements in growth and feed efficiency.

**Nutrient Digestibility and Enzyme Activity** Fermentation enhances nutrient digestibility through degradation of anti-nutritional factors and generation of bioavailable peptides. Feng *et al.* (2007) [7] and the Irawan *et al.* (2022) [9] meta-analysis demonstrated increased digestive enzyme activity (amylase, protease) and improved pre-cecal digestibility from fermented soybean meal.

Similarly, defatted BSFL improved digestibility of dry matter, crude protein and ether extract (Dabbou *et al*., 2018) [6]. These improvements directly translate to better feed conversion ratios as more nutrients are captured per unit feed.

**Gut Morphology and Microbiota Stabilisation**

The intestinal villi and crypts show consistent positive responses: Biasato *et al.* (2018) [4] and Bovera *et al*. (2016)5] showed enhanced villus height and goblet cell density in birds fed mealworm meal. Mukherjee *et al.* (2016) [14] recorded improved villus architecture and reduced harmful bacterial counts with fermented rapeseed meal. Enhanced villus structures increase nutrient absorption, while beneficial microbial shifts favour intestinal resilience.

**Functional Bioactive Compounds**

Alternative proteins are not merely protein sources; many contain immunomodulatory compounds: Lauric acid in BSFL (Makkar *et al.*, 2014) [11] β-glucans and mannan- oligosaccharides in yeast (Reed & Nagodawithana, 1991)
[17] Phycocyanin and carotenoids in Spirulina and Chlorella (Ghasemi *et al*., 2020; Saeid *et al.*, 2018)
[19] Polyphenols in Moringa (Melesse *et al*., 2011) [8, 12].

These compounds enhance oxidative status, modulate immunity and improve resilience against enteric pathogens.

**Comparative meta-insights across protein categories Insect Meals vs Fermented Plant Proteins**

Insect meals show more consistent improvements in FCR (Dabbou 2018; Schiavone 2017) [6, 20] than most plant alternatives. Fermented plant proteins demonstrate stronger early-growth (starter phase) benefits due to superior digestibility (Feng 2007; Irawan 2022) [7, 9].

**Leaf Meals vs Single-Cell Proteins**

Leaf meals improve antioxidant and immune profiles at low inclusion but cannot fully replace soybean meal due to high fibre (Sultana 2014; Melesse 2011) [21, 12]. SCPs (yeast, bacterial, microalgae) reliably maintain growth and often improve meat quality (Øverland 2010; Ghasemi 2020) [15, 8].

**Agro-Byproducts vs High-Tech SCP/Insects**

By-products like DDGS and PKC are cost-effective but nutritionally variable (Murray 2018; Sundu 2006) [13, 22]. SCPs and insects offer superior protein quality and digestibility but require investment in controlled production systems. Overall, the evidence suggests that blended strategies (e.g., insects + fermented meals) may yield optimal economical and nutritional outcomes.

**Economic Modelling and Cost Considerations Cost of protein per unit gain**

DDGS, PKC and leaf meals remain the cheapest per-kg options. Insects and SCPs cost more per kg but often reduce feed required per kg gain (due to improved digestibility and gut health).

**Break-even analysis (based on verified trial outcomes)** BSFL becomes cost-competitive when priced within 15- 20and of soybean meal because improved digestibility offsets higher cost (Dabbou *et al*., 2018) [6]. Fermented soybean meal reduces starter-phase feed costs by improving early growth efficiency (Feng 2007; Irawan 2022) [7, 9]. Bacterial SCP becomes economical in regions with low methane/natural gas cost (Øverland 2010) [15].

**Long-term sustainability economics**
Insects and SCPs have potential for large-scale automation, reducing costs by 40-60and over the coming decade (by Makkar 2014; Van Huis 2013) [11, 23]. Microalgae remain premium but offer value-added benefits (meat pigmentation and antioxidant capacity).

**Regulatory Framework and Constraints Insect Protein Regulations**
Many regions (EU, US, India) still regulate insects cautiously for poultry feed. BSFL is the most approved insect species globally due to safety data (Makkar *et al*., 2014) [11].

**Fermented Proteins and SCPs**
Fermented SBM and rapeseed meal are routinely accepted worldwide. Bacterial SCP derived from methane is approved in some countries after performance trials (Øverland, 2010) [15].

**Leaf Meals and By-products**
Leaf meals face minimal regulation but require safe processing (especially Leucaena). DDGS regulations vary due to ethanol plant variation (Murray 2018) [13]. Regulation often evolves more slowly than science, causing delays in adoption even when evidence supports safety and performance.

**Consumer Acceptance and Market Perceptions Insects**
Consumer acceptance is higher for insect-fed poultry than for insects as food. BSFL-fed broiler meat generally shows no negative sensory change (Schiavone 2017; Dabbou 2018) [20, 6].

**Microalgae**
Spirulina-fed chicken meat has more yellow pigmentation, which is advantageous in markets preferring golden- coloured skin (Ghasemi 2020) [8]. Health-oriented consumers may prefer poultry raised on sustainable SCPs.

**Fermented Proteins**
Generally well-accepted, perceived as natural and safe. Consumer acceptance ultimately depends on communication about sustainability and health benefits.

**8. Summary**
- Insects and SCPs provide the highest-quality protein alternative to soybean meal.
- Fermented plant proteins significantly enhance nutrient digestibility and gut morphology.
- Leaf meals offer immune and antioxidant benefits but must be limited to low levels.
- Agro-byproducts are economical but nutritionally variable.
- Improvements in villus height, digestive enzyme activity and microbiota are the strongest predictors of performance gains.
- Successful adoption depends on regulatory permissions, cost reduction and quality assurance systems.

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