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REVIEW

Advances in enzyme-assisted microbial fermented feed for livestock and poultry production – a review

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ABSTRACT. Microbial fermentation followed by enzymatic hydrolysis is an innovative feed preparation approach. During this process, microorganisms metabolise feed components, while enzymes catalyse the breakdown of macromolecular compounds such as proteins and cellulose into smaller, more digestible molecules. This method offers several advantages over traditional feed preparation, including increased fermentation efficiency, improved product quality, and reduced nutritional loss. These benefits can greatly enhance the production performance and overall health of animals. Recent advancements in this field have demonstrated significant potential for improving animal production performance. This article describes the various types of strains and enzymes employed in the preparation of fermented feed and its adoption in animal farming, with a focus on livestock and poultry. The study also briefly discusses the advantages, limitations, and evolution of this technology. As animal husbandry progresses towards greater intensification, scale and technological innovation, the demand for efficient and sustainable feed solutions continues to grow. This review aims to provide practical recommendations for the production and application of fermented feed in modern animal farming.

Introduction

The livestock industry is currently facing new challenges in ensuring safe and sustainable production practices (Zhang et al., 2021). Consequently, there has been increasing focus on developing feed solutions that prioritise safety, efficiency, and environmental sustainability (Tang et al., 2023). Numerous studies indicate that biological feeds, especially those employing bacterial fermentation in combinations with enzymatic hydrolysis, offer promising solutions to current challenges in animal production (Lai et al., 2024). Feeds produced using these methods represent an innovative approach in animal nutrition that integrates fermentation engineering with enzymatic processing technologies. Bacterial fermentation combined with exogenous enzyme hydrolysis can address the problem of insufficient enzyme production during microbial fermentation. The synergistic action provides three key advantages: (1) more complete degradation of macromolecules into bioavailable nutrients, (2) significant reduction of anti-nutritional factors, and (3) enhanced conversionefficiency of specific substrate components (Chen et al., 2021). The composition of this feed consists of raw materials, enzymes, and microorganisms, prepared according to the current national legislation (Li et al., 2020). Compared to traditional fermentation methods, enzyme-enhanced fermentation offers distinct advantages including faster processing times, reduced production costs, and improved environmental sustainability (Sun et al., 2021). The synergistic action between microbial and enzymatic components increases nutrient bioavailability while modifying the feed matrix to improve digestibility. Furthermore, this approach provides significant benefits to animal health, including favourable modulation of intestinal microbiota and enhanced disease resistance (Sun et al., 2021; Song et al., 2022). The efficiency of enzyme-assisted fermentation can be assessed through multiple parameters, including fermentation products, fermentation rate, substrate conversion rate, cell growth, pH change and product quality (Zhang et al., 2024; Li et al., 2021). Studies demonstrate that enzyme-fortified bacterial fermentation effectively liberates bound nutrients in plant-derived feedstuffs, including glucosides, phytic acid complexes, and other nutrients in plant-based diets (Mukherjee et al., 2016). Feed processing using microbial fermentation and subsequent enzymatic treatment exerts a positive effect on livestock productivity and meat quality (Hu et al., 2020). This review provides a comprehensive overview of the various applications, effects, and emerging trends associated with the use of enzyme-assisted bacterial fermentation in animal production. Furthermore, it establishes a scientific foundation for the future development and integration of alternative feed resources in livestock farming.

Microbial and enzymatic components in synergistic feed fermentation systems

Enzyme-enhanced microbial fermentation

Microbial-enzymatic synergy in feed processing involves the coordinated action of microbial metabolism and enzymatic hydrolysis (Li et al., 2023). The combined action encompasses a range of substrates, strains, and enzymes, with modifications to strain types and fermentation conditions substantially influencing enzyme activity. In a cooperative fermentation system, enzymes play a crucial role in accelerating the breakdown of substrates, as well as increasing microbial fermentation efficiency by regulating lactic acid production and pH changes (Du et al., 2023). Microbial activity initiates the process by decomposing complex substrates into smaller molecules, which subsequently facilitates more efficient enzymatic hydrolysis (Gohar et al., 2024). The supplemented enzymes interact synergistically with microbial enzymes to optimize overall fermentation outcomes (Patel et al., 2023). For optimal results in enzyme-enhanced bacterial fermentation systems, enzyme selection should consider both substrate composition and microbial profile, with concentrations carefully titrated according to the principle of balanced supplementation (Li et al., 2021). The time point of enzyme addition depends on fermentation objectives, enzyme properties, and fermentation system. For growth promotion, enzymes are typically added during the early fermentation phase; for metabolite accumulation, addition occurs at mid-fermentation; and for product purification or stabilisation, enzymes are added during late fermentation stages (Vishnu Prasad et al., 2020).

Common microbial strains in enzyme-supplemented feed fermentation

Microbial strains frequently employed in enzyme-enhanced feed fermentation primarily comprise yeasts, bacilli, and moulds (Li et al., 2021). Examples of these strains include *Lactobacillus*, *L. plantarum*, *L. acidophilus*, *Saccharomyces cerevisiae*, *Candida*, *Bacillus subtilis, B. coagulans* and others. Lactic acid bacteria are particularly effective in fermenting carbohydrates, generating significant quantities of lactic acid and other beneficial metabolites while exhibiting resistance to acidic conditions and bile salts (Ayyash et al., 2021). These products lower feed pH, inhibit the proliferation of undesirable microorganisms, enhance the nutritional value of the diet, and promote animal intake and immune function (Vieco-Saiz et al., 2019). In addition, yeasts are capable of metabolising carbohydrates and fats present in feed, as well as producing various bioactive compounds, including alcohol (Jach et al., 2018). Due to their capacity to modify proteins, nucleic acids, vitamins and polysaccharides, these mircoorganisms play a crucial role in animal diets (Patterson et al., 2023). Specific yeast strains, such as *Saccharomyces* and *Candida* contribute to the development of wine-like sensory characteristics of fermented feed, enhancing its palatability and flavour profile (Wang et al., 2022). Notably, *B. subtilis* secrets various extracellular enzymes, including amylases, proteases, lipases, and cellulases, which collectively degrade macromolecular feed components, significantly improving nutrient utilisation efficiency (Akintunde and Chukwudozie, 2021). In addition to enzymatic properties, *Bacillus* species are recognised for their effectiveness in controlling intestinal diseases and strengthening host immune responses (Palkovicsné Pézsa et al., 2022).

Common members of the genus *Bacillus* in feed fermentation include *Bacillus subtilis, B. licheniformis, B. coagulans,* and *B. cereus*. These *Bacillus* species are known to produce extracellular enzymes, such as proteases, haemicellulases, and cellulases, which efficiently degrade complex starches and proteins into simpler, more digestible compounds. The enzymatic activity not only improves the fermentation process but also the functional properties of feed substrates (Dumitru et al., 2022). These bacterial strains metabolise feed components while their enzymes break down complex nutrients, collectively improving digestion, nutrient absorption, and feed efficiency, thereby supporting animal growth and health.

Common enzymes applied in enzyme-enhanced microbial feed fermentation

In microbial feed fermentation with enzymatic supplementation, the most commonly used enzymes include non-starch polysaccharidases (e.g., cellulases, xylanases, glucanases), proteases, and amylases. Non-starch polysaccharidases catalyse degradation of structural polysaccharides such as cellulose and hemicellulose into simpler carbohydrates (Habte-Tsion et al., 2018). Proteases hydrolyse feed proteins into smaller peptides and free amino acids, significantly improving protein digestibility and assimilation. Supplementing proteases to bacteria-fermented feed has been shown to improve protein utilisation efficiency by up to 26% (Park et al., 2020). While amylases are not always central to these processes, their incorporation is highly beneficial, especially in starch-rich feeds, where they catalyse the breakdown of complex carbohydrates into readily absorbable sugars (Aderibigbe et al., 2020). Cellulases, xylanases, and glucanases are distinct enzymes that act complementarily to improve feed quality and nutritional value through targeted degradation of fibre components (Lamp et al., 2015). In microbial fermentation systems, the participating microorganisms naturally secrete various enzymes capable of modifying feed substrates. These microbiallyderived enzymes work in conjunction with supplemented enzymes to optimise substrate breakdown and nutrient release. Recent advances in feed formulations have identified optimal combinations of substrates, microbial strains, and enzymatic supplements for optimal fermentation outcomes. These key components are summarised in Table 1.

Table 1. Substrates, strains and enzymes commonly used in enzyme-enhanced bacterial feed fermentation

Substrates	Strains	Enzymes	References
Soybean meal	*Bacillus subtilis, Saccharomyces cerevisiae, Lactobacillus plantarum, Lactobacillus rhamnosus*	Proteases	Heng et al. (2022)
Rapeseed meal	*Saccharomyces cerevisiae, Aspergillus niger, Aspergillus oryzae, Bacillus subtilis, Bacillus cereus, Lactobacillus casei*	Alkaline protease, proteases, papain, neutral protease, endogenous enzyme complex, cellulase, pectinase, flavoured protease	Li et al. (2022)
Cottonseed meal	*Bacillus subtilis*	Papain	Sun et al. (2012)
Palm kernel meal	*Lactobacillus plantarum, Saccharomyces cerevisiae*	Mannanase	Rahim et al. (2022)
Peanut meal	*Lactobacillus plantarum*	Cellulase	Wang et al. (2022)
Miscellaneous meal (maize gluten-wheat bran)	*Lactic acid bacteria*	Acid protease	Jiang et al. (2021)
Corn stalks	*Lactobacillus plantarum*	Cellulase	Xu et al. (2020) Wang et al. (2022)
Sweet sorghum straw	*Prion-producing yeast, Aspergillus niger*	Xylanase, cellulase	Yue et al. (2021)
Pleurotus eryngii mushroom bran	*Bacillus subtilis, Pediococcus*	Cellulase	Zhang et al. (2024)
Mushroom bran	*Lactobacillus plantarum, Lactobacillus acidophilus, Lactobacillus buchneri,Prion-producing yeast, Aspergillus niger*	Cellulase, xylanase	Wang et al. (2022)
Tea residue	*Lactobacillus plantarum, Bacillus subtilis, Saccharomyces cerevisiae*	Cellulase, xylanase, β-glucanase, β-mannanase	Wu et al. (2022)
Potato residue	*Lactobacillus plantarum*	Cellulase	Xu et al. (2020)
Corn protein powder	*Bacillus subtilis*	Proteases	Fan et al. (2023)
Maize cob	*Lactobacillus fermentum, Saccharomyces cerevisiae, Bacillus subtilis*	Xylanase, β-glucanase, mannanase, cellulase, pectinase	Lin et al. (2021)
Wine lees bran mixture	*Lactobacillus plantarum, Bacillus subtilis, Saccharomyces cerevisiae*	Cellulase, xylanase	Zheng et al. (2023)
Bran	*Bacillus subtilis, Lactobacillus plantarum*	Cellulase	Wang et al. (2023) Wang et al. (2022)

Benefits of enzyme-enhanced microbial fermentation of animal feed

Improved feed nutritional value

Microbialfermented feed with hydrolysing enzyme supplementation has attracted significant attention from livestock producers due to its multiple benefits. During this process, microorganisms synthesise various compounds, including essential vitamins and amino acids, which support the growth and development of livestock and poultry. This technique also enhances the nutritional quality of the feed, enabling better absorption and utilisation of nutrients, thereby promoting overall growth and health. Li et al. (2020) found that *B. sphaericus* produced cellulase during fermentation, which broke down cellulose, a major component of the cell wall. This enzymatic process enhanced nutrient digestion and improved nutrient utilization in animals. Similarly, Li et al. (2018) showed that the combination of *Aspergillus riparius* fermentation with added fibrolytic enzymes improved lignocellulose degradation, while inclusion of *Enterococcus faecalis* significantly improved conversion efficiency, shortened fermentation time, and increased glucose and total reducing sugar content in the silage. Moreover, He et al. (2018) demonstrated that inoculating silage with lactic acid bacteria and adding cellulase significantly reduced condensed tannin content in the silage. This two-stage process of bacterial fermentation with subsequent enzyme treatment also generates a range of secondary metabolites. These include phenylpropanoids, terpenoids, steroids and alkaloids, which play important roles in plant growth and development, defence mechanisms, nutritional quality enhancement, and environmental adaptability. Beyond their roles in plants, these metabolites also provide significant benefits for human health and environmental protection (Zhao et al., 2024; Chen et al., 2024).

Improved feed palatability

The use of bacterial fermentation with enzyme supplementation has proven effective in degrading anti-nutritional factors and deleterious compounds in feed while improving palatability (bitter taste removal), which directly increases feed intake and feed conversion efficiency. Research by Cheng et al. (2019) demonstrated that fermented feed significantly reduced anti-nutritional factors in soybean meal, resulting in decreased expression of genes related to allergic inflammation, as well as lower serum IgG anti-soybean allergen levels in broiler chickens. Mukherjee et al. (2016) found that bacterial fermentation of soybean meal increased nutrient content, including free amino acids, small peptides, and unrefined proteins while successfully degrading anti-nutritional factors such as phytates, soybean trypsin inhibitors and oligosaccharides. Chuang et al. (2019) used yeast fermentation and phytic acid enzyme treatment in gluten feed, which effectively solved the problem of negative impact of phytic acid on nutrient absorption by animals and improved the digestibility of key amino acids such as lysine, methionine, cysteine, and threonine.

Enhanced livestock immunity

Beneficial microorganisms in enzyme-enhanced fermented feed produce health-promoting compounds that improve animal immunity. When these probiotics colonise the gut of livestock and poultry, they competitively inhibit the development of harmful bacteria and restore microbial balance, strengthening immunity and lowering disease incidence. Research has confirmed that the addition of cellulase to animal feed significantly reduces pathogenic *E. coli* population, simultaneously promoting the growth of *Lactobacillus*. This helps to effectively mitigate gut-related illnesses and lower the incidence of diarrhoea (Long et al., 2020). Furthermore, Su et al. (2018) demonstrated that the combined application of protease and probiotics in soybean meal fermentation significantly improved bacterial viability, accelerated protein degradation, and increased lactic acid production. In addition, this treatment reduced pH and sugar levels, while demonstrating antibacterial properties against *Staphylococcus aureus* and *E. coli*. Similarly, Zhang et al. (2017) reported that piglets fed fermented soybean meal had a considerably higher average daily weight gain and feed intake compared to those on antibiotic-based diets. Additionally, the study observed lower mRNA levels of pro-inflammatory markers in the jejunum and ileum of piglets consuming the fermented soybean meal, implying that the combined action of bacterial fermentation and enzyme supplementation could reduce intestinal inflammation while improving growth performance.

Improved livestock and poultry production performance

The use of enzyme-assisted microbialfermented feed significantly improves livestock and poultry performance, such as weight gain and laying rates. The improvement is a result of increased nutrient solubility in fermented feed, facilitating their easier utilisation by the animals. Additionally, beneficial bacteria in the feed contribute to improved digestive and metabolic efficiency of animals. Jiang et al. (2020a) demonstrated this by fermenting maize protein meal with lactic acid bacteria and adding acid protease, which increased peptide content and antioxidant properties. When fed to weaned calves, this enzyme-aided fermented feed improved growth and reduced oxidative stress. On the other hand, Jazi et al. (2017) observed that feed fermented by bacteria with enzyme addition significantly enhanced broiler growth while promoting the development of beneficial gut microbiota and improving intestinal structure. Similarly, Boroojeni et al. (2017) reported that enzyme-enhanced bacterial fermentation of pea-based feed increased its nutritional value, resulting in higher growth rates and improved ileal digestibility in broilers.

Increased feed protein sources

The combined process of bacterial fermentation with enzyme supplementation enhances the nutritional quality of alternative feed materials through complementary biological actions. Beneficial microorganisms actively ferment feed substrates while added enzymes target specific nutrient breakdown, working together to degrade toxins and anti-nutritional factors while improving palatability and protein availability. A study by Zhang et al. (2022) has demonstrated that this approach significantly improves the feeding value of unconventional feedstuffs, creating new possibilities for protein resource utilisation. The addition of protease-producing bacterial strains or exogenous protease preparations during the fermentation of cake meal feedstuffs decompose macromolecular proteins in raw materials into functional small peptides and amino acids, improving protein digestibility and creating a more balanced nutritional profile (Kim et al., 2019). Boroojeni et al. (2018) demonstrated that the fermentation of low-quality single protein feeds using *B. subtilis*, supplemented with pectinase and protease enzymes, resulted in balanced amino acid ratios in the final feed product.

Enzyme-bacteria antagonistic effects during feed fermentation

Certain combinations of bacteria and enzymes may exhibit antagonistic interactions. For instance, *Lactobacillus* may conflict with certain proteases, yeast with some lipases, and bacteria like *Bacillus* with specific cellulases. These antagonistic effects may stem from differing optimal conditions for bacteria during aerobic fermentation, macromolecule breakdown in anaerobic fermentation, competition for nutrients in fermentation substrates, or suboptimal conditions during initial enzymatic hydrolysis (van Schie et al., 2021). Despite these challenges, the combined use of bacteria and enzymes in feed fermentation can achieve significant benefits, including 40–60% reduction of anti-nutritional factors, 25–35% improvement in protein digestibility, and enhanced palatability through metabolite production. Standardised evaluation methods have been developed to assess key performance indicators such as toxin degradation efficiency, nutrient retention rates, and microbial/enzyme activity profiles (Leelasuphakul et al., 2006).

Application of enzyme-enhanced bacterial fermented feed in livestock and poultry production

Enzyme-enhanced bacterial fermented feed in poultry production

Research demonstrates that feed fermented by bacteria with enzyme supplementation significantly improves intestinal health, growth performance, and immune function in poultry. Boroojeni et al. (2017) found that bacterial fermentation of pea-based feed enhanced by enzymatic hydrolysis substantially increased its nutritional value. When incorporated into broiler diets, this fermented feed improved growth rates and ileal nutrient digestibility. Chuang et al. (2019) reported that the addition of brewer's yeast-fermented bran supplemented with phytase in broiler diets reduced ileal *Clostridium perfringens* populations while downregulating key pro-inflammatory markers (interleukin-1 beta, inducible nitric-oxide synthase, interferon-γ) in peripheral blood mononuclear cells. Li et al. (2022) investigated the effects of microbial fermentation, enzymatic digestion, and a combined bacterial fermentation with enzyme supplementation of rapeseed meal in yellow-feathered broiler diets. Their results showed that the integrated approach of bacterial fermentation followed by enzymatic treatment improved the antioxidant function and production performance of the broilers. The findings indicate that while standalone fermentation or enzymatic digestion each have limitations in feed processing, their combined application creates synergistic benefits for nutritional value.

Enzyme-enhanced bacterial fermented feed in swine production

The combined use of bacterial fermentation and enzyme supplementation enhances piglet feed through complementary mechanisms. Bacterial fermentation helps stabilise intestinal microbiota and reduces diarrhoea incidence, while enzyme treatment improves nutrient digestibility and absorption. This integrated approach leads to better growth performance and immune function in piglets. Long et al. (2021) showed that supplementing weaned piglet diets with multiple enzymes, including protease and xylanase, improved intestinal morphology by increasing the ileal villus to crypt ratio, and promoting nutrient digestion and absorption. Zhu et al. (2018) observed that incorporating fermented soybean meal to the diets of weaned piglets significantly improved their intestinal microecological environment by reducing *E. coli* and colonic bacteria populations, while effectively enhancing immune function and overall health. Huang et al. (2019) showed that probiotic-fermented feed combined with mycotoxin-degrading enzymes protected intestinal integrity by increasing porcine jejunal epithelial cell viability, mitigating mycotoxin damage, and upregulating tight junction proteins and B-cell lymphoma-2 (*Bcl*-2) expression to prevent epithelial apoptosis. Liu et al. (2024) found that mycobacterial enzymes and fermented herbal compounds in piglet diets improved growth performance, enhanced immune and antioxidant capacity, and elevated serum growth hormone levels.

Enzyme-enhanced bacterial fermented feed in cattle production

The inclusion of bacterial fermentation with enzyme supplementation in cattle diets improves growth performance, meat quality, and nutrient digestibility, and plays a positive role in regulating rumen fermentation parameters and immune properties. Kim et al. (2018) reported that fermented total mixed rations (FTMR) significantly increased dry matter intake in Korean beef cattle while reducing blood albumin and lactate dehydrogenase levels, which indicated improved metabolic efficiency and disease resistance. Similarly, Hu et al. (2020) showed that replacing fresh maize stover with probiotic-enzyme fermented material substantially modified the structure of the rumen flora in beef cattle. This approach significantly increased the abundance of bacterial groups and elevated metabolite concentrations related to amino acids, carbohydrates, cofactors and vitamin metabolism in the rumen, improving feed energy conversion efficiency.

Enzyme-enhanced bacterial fermented feed in sheep production

Supplementing sheep diets with bacterial-fermented feed preparations enriched with exogenous enzymes improves growth performance, meat quality, serum antioxidant function, and the apparent digestibility of nutrients. Alsersy et al. (2015) demonstrated that enzyme-treated quinoa silage reduced neutral detergent fibre and acid detergent fibre content while increasing total digestible nutrient intake due to improved digestibility and rumen fermentation in sheep. Jiang et al. (2020a) showed that bacterial-enzyme treatment of buckwheat straw significantly enhanced feed conversion efficiency in Tan sheep by promoting cellulose-degrading bacterial populations and upregulating related metabolic genes. Further research by Jiang et al. (2021) showed that combining enzyme- and bacteria-treated buckwheat straw with alfalfa hay in Tan sheep diets increased average daily weight gain, lowered feed conversion ratios, and improved multiple carcass characteristics, including slaughter rate, lean meat yield, and meat colour quality.

Conclusions

Enzyme-enhanced bacterial fermented feed offers significant advantages for livestock and poultry production, including enhanced nutritional value, immunity, and production performance. It also contributes to reducing environmental impact, improving feed palatability, and expanding protein resources. As a result, it holds significant potential for widespread application in livestock and poultry farming.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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