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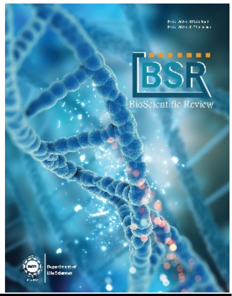

Title: **Effects of Fungal Fermented Feeds on Broiler Chicken Growth Performance, Gut Morphology, and Gastrointestinal Tract Microecology: A Review**

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Effects of Fungal Fermented Feeds on Broiler Chicken Growth Performance, Gut Morphology, and Gastrointestinal Tract Microecology: A Review

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ABSTRACT

Due to the shortage of poultry feed ingredients and the resultant continuous increase in their prices, researchers have been looking for their alternatives. Agricultural wastes and by-products (such as wheat bran, rice husk, sour cherry kernel, palm kernel, cassava pulp, rapeseed meal, and corn meal) are among such alternatives, although they cannot be used directly due to their high crude fiber content. Fermentation is an evolving process that converts complex substrates into simpler molecules using bacteria, fungi, and other microorganisms. It improves the nutrient content of feed ingredients. It is also used to ferment agricultural waste materials in order to improve their nutritional value, so they can be utilized as broiler feed. Moreover, it can provide an inexpensive feed source to poultry industry and convert waste materials into a useable product. Different bacterial and fungal strains are employed for fermentation. This review focuses on the use of fungal fermented agricultural waste-based feed and its effects on broiler chicken growth performance, gut morphology, and gastrointestinal tract microecology.

Keywords: agricultural waste, broiler chicken, fungal fermented feed, poultry industry

1. INTRODUCTION

The production of principal poultry products (eggs and meat) is increasing rapidly all over the world. This represents consumption driven by the consumer choice for high-quality products at a low price due to production efficiency [1]. The key goals of poultry industry include higher output, illness control, quality of product, and reasonable cost of production [2]. The poultry sector in Pakistan is a key and developing agricultural sector that contributes significantly to the GDP of the country (1.26% of GDP). Chicken farming at commercial level began in Pakistan in the 1960s and the public has been dependent on it to fulfil a significant portion of their daily protein requirement since then. The industry has profited from government incentives throughout the course of its development, although it has also encountered challenges such as disease breakouts and retail price changes. In Pakistan, the poultry sector is essential to fill the gap between protein supply and protein demand. Poultry production is one of the most vibrant and well organized industries of the country, providing for 26.8% of the total meat output, 5.76% of the agricultural sector, and 1.26% of the country's overall GDP. The poultry sector of Pakistan has experienced rapid expansion in recent years, providing employment to more than 1.5 million people. Despite showing remarkable potential and progress over the previous years, currently it is facing many challenges, such as disease outbreaks, low animal feed intake, poor quality, and high-priced feed [3].

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Grain crops have been exploited to produce biomass energy in recent years due to the energy crisis, resulting in higher prices for the key materials used in animal feed. Alternative crops or agricultural by-products can be used to address feed shortage as unconventional feedstuffs [4]. Agricultural by-products are affordable as compared to main grain crops, although they are mostly useless as feed for monogastric animals due to their high fiber content. The principal by-product of wheat flour processing is wheat bran (WB). It includes a significant amount of total dietary fiber (451 g/kg) [5]. However, it is widely understood that increasing fiber content has a negative relationship with animal feed intake and digestibility, thus decreasing the growth and production performance of the poultry industry. As a result, the use of wheat bran in animal feed has been generally limited [6,7]. Fermentation increases the nutritional value of feed materials, while lowering crude fiber content and harmful compounds [8]. Through fermentation, scientists have recently begun to employ fungal inoculum to increase the nutritional value of agricultural by-products [9]. Fermented feed ingredients show resistance to contamination by pathogens before they are fed to chickens [10]. The addition of fermented products into broiler feed can reduce the number of harmful microbes and support the growth of good microflora in the gut of broiler chicken [11]. Recently, several studies have been conducted regarding the addition of fermented feed into broiler diet to observe its effect on growth performance and gut health [12-14].

This review describes the use of fungal fermented agricultural waste-based feed and its effect on broiler chicken growth performance, gut morphology, and gastrointestinal tract microecology.

2. FUNGAL SPECIES EMPLOYED TO FERMENT SUBSTRATE

Scientists have recently begun to employ fungal inoculum to increase the nutritional value of agricultural by-products through fermentation. Filamentous fungi, such as Trichoderma species, are very well known cellulase producers [9]. The majority of commercial cellulase is produced from Trichoderma species. Filamentous fungi have a higher penetrating power into the insoluble substrates, making them better suited for the fermentation of lignocellulosic material. Researchers have found numerous strains of Trichoderma spp. as the most consistent and safe fungi for the synthesis of cellulase and hemicellulases. Fungi have a highly effective enzymatic system that allows them to breakdown lignocellulosic materials [13]. With solid-state fermentation, *Aspergillus niger* can create numerous enzymes, such as amylase, protease, tannase, cellulase, and lipase. These enzymes improve poultry growth and meat quality, while lowering the antinutritional components in the leaves of Ginkgo biloba, cottonseed meal, and olive leaves [14]. Filamentous fungi including *Aspergillus niger, Pleorotus ostreatus*, and Trichoderma spp. are typically employed for solid-state fermentation to increase the substrate's feed value. These fungi are presumably able to grow not only on the surface of substrate particles but are also assumed to penetrate them [15]. Table 1 shows the some examples of fungal strains used to ferment substrates added to broiler diets.

Table 1. Examples of Fungal Strains Used to Ferment Substrates Added to Broiler Diet

Fungal Strains	Substrates	References
Aspergillus niger, Trichoderma viride and *Pleorotus ostreatus*	Palm kernel meal	[15]
Trichoderma longibrachiatum (SF1)	Wheat bran	[13]
Acremonium charticola	Cassava pulp	[16]
Saccharomyces cerevisiae (and Lactobacillus acidophilus, *Bacillus subtilis*, and, *Lactobacillus rhamnosus*)	Wheat bran	[17]
Trichoderma pseudokoningii	Wheat bran	[18]
Cunninghamella echinulata ATHUM 4411	Wheat bran	[19]
Aspergillus niger strain (ATCC 200345)	Sour cherry kernel	[14]
Saccharomyces cerevisiae	A mixture of cereal grain flour, whey, and tomato pomace	[20]
Trichoderma pseudokoningii	Wheat bran	[12]
Trichoderma viride	copra meal	[21]
Saccharomyces cerevisiae (and *Bacillus subtilis Lactobacillus fermentum*, *Enterococcus faecium)*	Rape seed meal	[22]
Umbelopsis isabellina CCF2412	Corn meal	[23]
Laetiporus sulphureus	Wheat bran	[24]
Saccharomyces cerevisiae (and Bacillus amyloliquefaciens)	Wheat bran	[25]
Aspergillus niger (and Lactobacillus rhamnosus)	Cassava peels	[26]
White rot fungi	Wheat bran	[27]
Saccharomyces Cerevisiae and Aspergillus Oryzae	Wheat bran	[11]
Saccharomyces cerevisae (and Lactobacillus spp)	Cassava peels	[28]

3. FERMENTATION METHODS

Fermentation is an evolving process that converts complex substrates into simpler molecules and involves microbes, substrates, and ambient conditions [10]. Solid state fermentation (SSF) and submerged fermentation (SmF) are the two main types of fermentation processes, depending on the type of substrate [29]. These techniques are used to ferment agricultural waste materials to supplement broiler feed. SSF uses solid substrates – for example wheat bran, rice, grains, and rice bran (without free-flowing liquid). It is commonly used to make fermented dried up feed that could be crushed or powdered and added to basic diet mixtures, such as whole grain. Due to the lower moisture level, only a few microorganisms can perform the SSF method. Primarily, these include fungi such as Rhizopus spp. and Aspergillus spp., though bacterial species such as Lactobacillus species can also be utilized.. Still, SSF has attracted greater attention since it produces better yields and products with improved quality than SmF. Furthermore, the greater moisture content in SmF's fermented feeds may make poultry production difficult in the context of actual feeding and litter quality [30]. SSF has been proved to be a promising tool for recycling the agricultural and industrial wastes used in animal diets[31]. It enhances the digestibility of nutrients, increases the number of good bacteria (lactobacilli) in the large intestine of broilers, improves the morphology of their small intestine, and reduces the cost of poultry farming [22]. Aside from efficient feed storage, the fermentation technique can enhance the nutritional value of animal feed ingredients [13]. It improves the quality of feedstuffs by producing enzymes, eliminating harmful chemicals, and by improving the aroma [15].

Fermentation technique has been used to improve the quality of several feed ingredients, for instance, wheat bran [12,18,27] , cassava peel [16,26,28], palm kernel meal [15], rapeseed meal [22], copra meal [21], and sour cherry kernel [14].

4. UNCONVENTIONAL FEEDS

The poultry sector is currently experiencing swings in feed pricing as a result of the rising prices of conventional feedstuffs. Consequently, chicken nutritionists are looking for new feed supplements [16]. Wastes and by-products of various industries, such as palm kernel meal of palm oil industry [15] and cassava pulp of tapioca industry [16] can be used as unconventional feed sources in boiler diets. Such use, however, is limited due to their high fiber and low protein content, though fermentation can improve their nutritional value and decrease their crude fiber content [8]. The use of fungi to ferment a low quality feedstuff can improve its quality [15].

5. EFFECT ON GROWTH PERFORMANCE

Fermentation improves the nutritional value of feed ingredients but decreases the crude fiber content and toxic compounds [8], which can affect the growth of broiler chicks. The parameters used to measure their growth are body weight, weight gain, feed consumption, and feed to gain ratio [31]. According to a study, 10% fermented wheat bran group of broiler chickens had a reduced feed consumption and a higher feed to gain ratio than control group, which resulted in their improved growth [12]. Likewise, when comparing the average live weight of birds fed with fermented wheat bran (1640 g) to control group 1 (1147.7 g) and control group 2 (1363.5 g), the statistical analysis revealed a significant increase in the average live weight of birds fed with fermented wheat bran, while feed conversion ratio (FCR) was also the best of the group fed with fermented wheat bran [13]. Furthermore, the *Acremonium charticola* fermented Cassava pulp (AC-FCP) and AC-FCP + Antibiotic Growth Promotors birds had reduced feed costs per kg and live weight gain than that of the control and control + Antibiotic Growth Promotors birds [16].

Moreover, increased growth and FCR were observed in broilers fed on fermented wheat bran by bacillus and saccharomyces spp [25]. Likewise, broilers fed on wheat bran fermented by *Cunninghamella* spp. showed improved meat quality and health indicators [19]. According to [18], in comparison to the control group, the groups supplemented with the enzyme powder of *Trichoderma pseudokoningii* showed that body weight gain and FCR improved during the starting phase (1–21 day). The findings of [17] suggested that wheat bran is a good dietary supplement as it did not affect the growth of broilers.

Other than wheat bran, sour cherry kernel fermented by *Aspergillus niger* can also be added to broiler diet up to 2%, without having any negative effects on their growth [14]. Broilers fed on rapeseed meal fermented by *Bacillus subtilis, Lactobacillus fermentum*, and *Enterococcus faecium* showed improved FCR and weight gain [22]. Birds fed on unfermented palm kernel meal consumed less feed than other experimental groups [15]. Table 2 shows some examples of fermented agricultural waste-based feeds and their studied effects on broiler growth.

Table 2. Examples of Fermented Agricultural Waste-Based Feeds and their Effects on the Growth Performance of Broilers

Fungal Strain	Substrate	Results	References
Trichoderma pseudokoningii	Wheat bran	Improved growth and intestinal structure of broilers.	[12]
Trichoderma longibrachiatum (SF1)	Wheat bran	Improved growth, feed intake, and feed conversion ratio.	[13]
Acremonium charticola	Cassava pulp	Feed cost reduced per kg and live weight gain of broiler chickens.	[16]
Saccharomyces cerevisiae (and Bacillus amyloliquefaciens)	Wheat bran	Compared to the control group, all the fermented wheat bran treatments showed the potential to lower broiler serum cholesterol.	[25]
Trichoderma pseudokoningii	Wheat bran	Improved growth, intestinal structure, gut microflora, and oxidative properties of broiler chickens.	[18]
Cunninghamella echinulata ATHUM 4411	Wheat bran	Fermented feed enriched with agrimony extract improved meat quality and health indicators.	[19]
Aspergillus niger strain (ATCC 200345)	Sour cherry kernel	Fermented cherry kernel can be added to broiler feed up to 2%, while raw cherry kernel can be used in broiler feed up to 1% without negatively impacting growth.	[14]
Saccharomyces cerevisiae	A mixture of cereal grain flour, whey,	Improved growth performance by supplementing broiler diet	[20]

Fungal Strain	Substrate	Results	References
	tomato pomace	with 5g and 10g per kilogram yeast fermented supplements.	
Trichoderma viride	Copra meal	Improved the protein content of feed.	[21]
Aspergillus niger, Trichoderma viride and Pleorotus ostreatus	Palm kernel meal	Fermentation improved the quality of palm kernel meal and ultimately growth performance.	[15]
Umbelopsis isabellina CCF2412	Corn meal	Improved growth performance and the quality of meat.	[23]
Laetiporus sulphureus	Wheat bran	Improved the growth and gut microflora of broiler chicken as compared to control group.	[24]
White rot fungi	Wheat bran	Wheat bran fermented by WRF may boost lignocellulolytic enzyme activity.	[27]
Saccharomyces cerevisiae, (and Bacillus subtilis, Lactobacillus fermentum, Enterococcus faecium)	Rapeseed meal	Enhanced the digestibility of nutrients, increased the number of lactobacilli in the large intestine, and reduced the cost of poultry farming.	[22]
Saccharomyces Cerevisiae and Aspergillus Oryzae	Wheat bran	There was no apparent change in the growth of broilers fed on diet supplemented with fermented product and control group.	[11]
Saccharomyces cerevisiae (and Lactobacillus spp.)	Wheat bran	Wet fermented wheat bran did not have a negative effect on the growth of broiler chicks.	[17]

6. EFFECTS ON BROILER CHICKEN GUT MORPHOLOGY

An increase in villus height results in a large surface area, which leads to an increase in the absorption of nutrients and also improves the health of gut tract [32]. Furthermore, it's possible to think of the crypt as a villus assembly line; indeed, a large crypt indicates faster tissue renewal and a higher energy demand during tissue development [33]. The low depth of crypt is considered good to save energy for animal growth [34]. To measure the absorption capacity of the small intestine, the ratio of villus height to crypt depth (VH:CD) is an important parameter [22]. A study reported the effects on the gut morphology of broiler chicken when 10% of basal diet was replaced with *Trichoderma pseudokoningii* fermented wheat bran. A significant increase was detected in the VH:CD ratio in the ileum, although no significant difference was observed in the jejunum [12]. Another study demonstrated an increase in the height of the villus and the VH:CD ratio in the ileum and the jejunum of broiler chickens fed with the fermented rapeseed meal during the starter phase [22]. Another study reported the effects on broiler chickens fed with *Trichoderma longibrachiatum* (SF1) fermented wheat bran. Normal villus height and histological structures were observed [13]. Table 3 shows some examples of agricultural waste-based fermented feed ingredients and their studied effects on the gut morphology of broiler chickens.

Table 3. Fermented Feeds and their Studied Effects on the Gut Morphology of Broiler Chickens

Fermented Feeds	Outcomes	References
Fermented rapeseed meal	A significant increase in the height of the villus and VH:CD ratio in ileum and jejunum within the starting phase.	[22]
Fermented wheat bran	An increase in the height of the villus and VH:CD ratio in the ileum.	[12]
Fermented wheat bran	No significant difference in villus height.	[13]
Fermented sour cherry kernel	There was an increase in the length and weight of the gastrointestinal tract of broiler chicken.	[14]
Fermented palm kernel meal	There was no significant increase in the length of small intestine.	[15]
Fermented wheat bran	There was a significant increase in the VH:CD ratio.	[18]

7. EFFECTS ON THE MICROECOLOGY OF GASTROINTESTINAL TRACT

The microflora of gut tract has a significant role in gut morphology, physiology, microbiology, immunology, and nutrition. Fermented feeds enhanced gut microbial ecosystems in pigs by reducing the number of enteric bacteria, such as coliform bacteria and salmonella [35]. The host's intestinal microflora is the first line of defense against diseases induced by pathogen colonization in the gastrointestinal tract [36]. Bacteria, such as lactobacilli, are capable of inhibiting the growth of pathogenic bacteria [22]. The use of fermented feeds, which has the potential to affect gut bacterial ecology, is receiving more scholarly attention. The effects of fermented feeds on gut ecology are mostly apparent in the flora and metabolites of the gastrointestinal tract. Due to its unique properties, SSF feeds cause the acidification of the upper part of the gastrointestinal tract and provides ideal circumstances for the development of bacteria that are beneficial to animals [36]. Short-chain fatty acids are produced by the beneficial microbial population, which lowers gut pH. This also serves as a natural protective barrier against infectious diseases and pathogenic microorganisms. Fermented feeds have a large quantity of lactic acid bacteria and a high quantity of lactic acid [10]. Fermented diets, because of essential features such as low pH, large number of lactobacillus species, high quantity of acetic and lactic acid, and a low number of enterobacteria, might also be helpful to maintain healthy gut microecology in broiler chickens [30]. According to a study, the number of lactic acid bacteria was higher in the colon of broiler chickens fed with diet 10% of which was replaced by solid state fermented rapeseed meal than control group broilers, which led to a reduced number of pathogenic microbes in gut [22]. Similarly, broiler chickens fed on fermented wheat bran showed a significant increase in good microflora (lactic acid bacteria) count in the gut [18,25]. The number of coliform bacteria was reduced in the gastrointestinal tract of broilers fed on fermented feeds [12,13,24]. Table 4 shows some examples of fermented feeds and their effects on gastrointestinal tract microecology.

Table 4. Fermented Feeds and their Studied Effects on Gut Microecology

Fermented Feeds	Results	References
Fermented wheat bran	No significant difference in the bacterial count of caecum among all experimental groups. The number of coliform bacteria was reduced in the ileum of broiler chickens.	[12]
Fermented wheat bran	Significant reduction in the number of coliform bacteria in caecum. Lactobacilli number increased significantly in caecum.	[13]
Fermented rapeseed meal	Lactobacilli count was higher in colon and caecum than control group.	[22]
Fermented wheat bran	Lactic acid bacteria count increased in the caecum of 21-day old chicken. Coliform number was reduced.	[18]
Fermented wheat bran	The number of coliform bacteria was significantly reduced in the ileum of experimental group as compared to control group.	[24]
Fermented wheat bran	There was a significant increase in lactic acid bacteria count of ileum.	[25]

8. CONCLUSION

Fermentation is a low-cost technique that can improve the nutritive value of feed ingredients obtained through unconventional feedstuffs. It is evident by the literature that such fungal fermented feed ingredients obtained from agricultural waste materials and by-products can improve the broiler chicken growth performance, gastrointestinal tract morphology, and microecology without having any deleterious effects. Further study is needed to evaluate the effects of fungal fermented feeds on broiler chicken growth and gut health; however, this process can provide a cheap feed source to poultry which may eventually contribute to the potential growth of poultry industry in developing countries. Moreover, it may also provide a way to convert agricultural waste materials into a useful product.

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