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Research Article

Study the Prebiotic Effect of Wheat Bran Fermented with *Aspergillus niger* Fungus in Diet on Productive and Microbial Traits of Quail

Measem Hassan Ali Alallawee*, Zainab A. Mutter, Magareb Mohamed Jader

Department of Veterinary Public Health, Veterinary Medicine College, University of Basrah, Iraq.

Abstract | This study was conducted to determine the effect of fermented wheat bran by adding *Aspergillus niger* fungi to it and its function as a prebiotic when added to quail feed, and then study its effect on the productive and microbial characteristics of the bird. Three hundred and sixty Japanese quails (Cotrinux Japonica) were used in this experiment at one day old. The birds were randomly distributed into four groups, each group containing 90 birds, with 3 replicates for each group. The groups were as follows, T1: Standard feed (control). T2: 2 g/kg Fermented wheat bran with added *Aspergillus niger.* T3: 4 g/kg Fermented wheat bran with added *Aspergillus niger*. T4: 8 g/kg Fermented wheat bran with added *Aspergillus niger* Measurements of production traits were taken at 1, 3 and 6 weeks, as well as microbial traits after the end of the sixth week of the experiment. The results showed a significant increase ($P<0.05$) in body weight, while there was a decrease in the rate of feed intake and a decrease in the total number of aerobic bacteria and coliform bacteria in Jejunum, the number of yeasts and molds decreased significantly ($p<0.001$) in the jejunum of the treatments compared to the control group at the age of 6 weeks. We conclude from the results of the current research that adding the fermented wheat bran with added *Aspergillus niger* (as prebiotic) at a concentration of 8 g/kg feed has a positive effect in improving growth performance, and achieving microbial balance in the digestive tract of quails.

Keywords | Prebiotic, Aspergillus, Microbial, Quail, Fermented feed, Wheat bran

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***Correspondence** | Measem Hassan Ali Alallawee, Department of Veterinary Public Health, Veterinary Medicine College, University of Basrah, Iraq; **Email:** measem.ali@uobasrah.edu.iq
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INTRODUCTION

Fungi are of great importance in improving the nutritional value of some food materials used in poultry feed. In a study that used wheat bran, corn waste, beer waste and three types of molds *Aspergillus niger, Aspergillus flavas* and *Penicillium* spp., the results of the study showed a decrease in the percentage of cellulose by 36.51%, 35.87% and 35.6% for wheat, beer waste and corn waste, respectively, in the *Aspergillus niger* isolate, which was the best isolate compared to the other isolates (Layayi, 2004).Prebiotics are indigestible food components that promote the development of good bacteria in the digestive tract. Enzymes like proteases and phytases, which are found in fungi, may either directly or indirectly enhance the animals' ability to use nitrogen and respond productively. Proteases liberate amino acids from the matrix by directly acting on the proteinaceous structure (Lee, 2018). As phytase reduces endogenous losses, it indirectly improves the digestibility of amino acids (Cowieson, 2017). The animal may be able to save amino acid for growth The reduction in endogenous losses could spare amino acids that the animal can use for growth if endogenous losses are decreased. Furthermore ,as phytases and proteases work differently, combining the two in the food may have synergistic effect on the release of amino acid, which may fuerther affect the animals performance (Bernardes, 2022). It is a non-starch polysaccharide consisting of a long chain. It is characterized by its indigestibility in the digestive tract of birds because the digestive tract lacks enzymes to digest it (Rad, 2018). By using fungal enzymes such as 0-fructofurisidase produced by the fungus *Aspergillus niger*, polysaccharide can be produced from sucrose (Kherade *et al*., 2021). A study confirmed that adding 5% of fungal mycelium to turkey rations for 14 days led to an improvement in production characteristics (Sharma *et al*., 2022).In another study, it was indicated that the product of fermentation of cassava roots and leaves, sago leaves, and coca peels with the fungus *Aspergillus niger*, at rates of up to10%, 12%, and 10%, respectively, can be added to feed without negatively affecting the productive performance of birds (Sun *et al*., 2024). Zahirian *et al*. (2019) indicated that adding 0.2% of the fungus *Aspergillus niger* to the diet of broiler chickens containing 19% protein led to an increase in body weight ($p < 0.05$) compared to birds to which mushroom powder was not added to their diet. *Aspergillus niger* mushroom filaments contain some complex sugars, which are considered one of the most important components of the mycelium of this mushroom. *Aspergillus niger* mushroom cultures reason, the *Aspergillus niger* fungus has been used to increase the nutritional value of many food products (Sun *et al*., 2024; Serba *et al*., 2020; Vincent *et al*., 2016). Given the importance of the fungus *Aspergillus niger* and the importance of using prebiotics in this field, the current study aimed to study the effect of adding mushrooms to the diet of Japanese quail on each of the growth performance and some immunological and microbial characteristics.

MATERIALS AND METHODS

Quails, Management and Diets

Three hundred and sixty quail chicks (*Coturnix japonica*) with an average body weight of 8.0 - 9.5g and of the same age were obtained from the General Authority for Agricultural Research in Abu-Ghraib, Baghdad, and were of the same age. The birds were randomly divided into 4 groups, with each group consisting of 3 replicates (30 birds per replicate). From hatching until the twenty-first day of their life, the birds were fed a beginning food. After that, they were fed a growth and production diet. One of the feed factories in northern Iraq produced these diets. The elements of these meals and their chemical analyses are displayed in Table 1. analysis.

Table 1: Feed ingredient and chemical analysis.

Ingredients	Starter diet (%)	Growth diet (%)
Yellow corn	50	51
Soybean meal	28	28
Protein concentrate (50% protein)	7	9
Wheat bran	11	6
Limestone	2	2.5
Salt	0.5	2
Vegetable oil	1	1
Mixture of vitamins and antibiotic	0.5	0.5
Total	100	100
Metabolic energy (kcal/kg)	2918.5	3095, 8
Protein (%)	23.02	21, 69
Calcium (%)	0.80	1.04
methionine%	0.60	0.48
Phosphorus (%)	0.37	0.52

*(NRC) 1994.

Source of Fungal Isolation

The *Aspergillus niger* isolate was obtained from the Central Research Unit, College of Veterinary Medicine, University of Basrah. The diagnosis of the fungal isolate was confirmed by conducting some diagnostic tests and according to the scientific reference (Abigail and Randy, 2018) to confirm the type of isolate and adopt it in the current study.

Productive Traits

In the first, third and sixth weeks, the live body weight and weight gain of the quail were calculated, as well as the amount of feed consumed and the feed conversion efficiency according to Reda *et al*. (2020b).

Microbial Test

Collecting and preparing fodder and litter samples: Samples were collected from the sides and middle of the feed for each treatment after mixing well. They were placed in a glass beaker and kept in the laboratory until they were examined. Fifteen grams of each replicate were taken after thorough mixing. 1 g of peptone was dissolved in 1 liter of distilled water, and it was sterilized in the incubator at a temperature of 121 degrees C for 20 minutes and diluted tenfold.

Collect and prepare gut samples: After slaughtering two birds from each replicate at the end of the sixth week, the digestive tract was taken completely and placed in sterile polyethylene bags and sent to the laboratory, 2 cm of jejunum were cut with a sterile scalpel and 1 g of their contents was added to the pre-prepared ten-dilution peptone water.

Estimation of microbial content: According to the pour-plate method to (Harrigan and McCance, 1976), the numbers of coliform bacteria and the total count of bacteria, mold and yeast were estimated.

Statistical Analysis

In order to determine the statistical significances among different variables (SPSS, 2023) was used. Analysis of variance tests were applied to analyze the obtained results.

RESULTS AND DISCUSIONS

Body Weight and Weight Gain

Table 2 shows the average weekly weight of birds during weeks (1, 3 and 6). The body weight during the sixth week was 194.34, 200.30, 213.21 and 219.32 g for the four treatments, which were fed standard diets to which the *Aspergillus niger* fungus culture was added at a rate of 0, 2, 4, and 8 g/kg feed, respectively. The results showed that adding mushroom cultures led to a highly significant increase ($p<0.05$) in body weight, as treatments T2, T3, and T4 were superior to the control treatment T1.

Table 2: Effect adding mushroom culture to quail diets based on average live body weight (g) during the weeks of the experiment period (mean ± standard error).

Group Week	T1: (Control)	Aspergillus niger		
		T2: (2g/kg)	T3: (4g/kg)	T4: (8g/kg
1Week	21.61±0.55^{d}	24.18±0.33^{c}	27.00±0.72^{b}	29.63±0.25^{a}
3Week	80.00±1.00^{d}	85.14±1.05^{c}	89.40±0.16^{b}	95.39±0.44^{a}
6Week	194.34±1.04^{d}	200.30±1.65^{c}	213.21±0.44^{b}	219.32±1.75^{a}

Note: Small letters refer to significant differences among groups at ($p<0.05$). N.S referred to no significant difference. * referred to significant difference.

Table 3 shows the average weekly weight gain of the birds of the four experimental treatments. significant difference ($P<0.05$) in the rate of weight gain for the three experimental treatments T2, T3, and T4, which were fed on feed to which the *Aspergillus niger* fungal culture was added compared to the control treatment T1, especially in the sixth week. The clear improvement in body weight and weight gain resulting from adding mushrooms to quail feed may be attributed to two reasons. The first reason is that mushrooms contain enzymes such as phytase and other enzymes such as proteases, amylases and cellulases that analyze complex polymers in the feed and convert them into simpler compounds, making it easier for the bird to absorb and metabolize them. The other reason for increase weight and weight gain may be due to the organic acids and complex sugars produced by mushrooms, which are factors of great importance in improving the productive performance of birds (Sebastin *et al*., 2019). The results of the research agreed with (Adedeji *et al*., 2022) who used mushrooms in turkey diet from (1-30) days at a rate of 0.2%, as an improvement in body weight was observed when the fungal product was added to the feed.

Table 3: Effect adding mushroom culture to quail diets based on average weight gain (g) during the weeks of the experiment period (mean ± standard error).

Group Week	T1:(Control)	Aspergillus niger		
		T2: (2g/kg)	T3: (4g/kg)	T4: (8g/kg
1 Week	12.74±0.09^{a}	14.26±0.34^{ab}	18.12±0.10^{bc}	21.68±0.08^{c}
3 Week	29.80±0.10^{a}	29.09±0.00^{a}	30.06±0.03^{a}	30.79±0.17^{b}
6 Week	33.81±0.15^{a}	36.73±0.21^{a}	44.20±0.42^{b}	44.65±0.18^{b}

Note: Small letters refer to significant differences among groups at ($p<0.05$). N.S referred to no significant difference. * referred to significant difference.

Table 4: Effect adding mushroom culture to quail diets based on average feed consumption (g) during the weeks of the experiment period (mean ± standard error).

Group Week	T1:(Control)	Aspergillus niger		
		T2: (2g/kg)	T3: (4g/kg)	T4: (8g/kg
1 Week	29.00±1.59	30.28±1.42	30.16±0.74	29.69±1.74
3 Week	85.21±1.51^{a}	83.20±1.80^{a}	79.69±0.79^{b}	75.86 ±1.08^{c}
6 Week	169.00±1.11^{a}	162.15±1.39^{b}	156.53±1.46^{c}	149.59±1.09^{c}

Note: Small letters refer to significant differences among groups at ($p<0.05$). N.S referred to no significant difference. * referred to significant difference.

Average Feed Consumption and Feed Conversion Factor (g)

Table 4 indicated the amount of feed consumed and feed conversion factor of quail for the four experimental treatments. The results of the study showed that the fourth treatment (T4) recorded the lowest amount of feed consumed significantly ($P<0.05$) as it was (149.59) kg compared to the control treatment T1 (169.00) kg, the second treatment T2 (162.15) kg and the third treatment T3 (156.53) kg. We also find that the highest amount of feed consumed was recorded in the control treatment T1 in the sixth week. As for the feed conversion factor, there was a significant improvement ($p<0.05$) when adding the mushroom culture to the quail rations for all treatments, as the fourth treatment T4 recorded the highest feed conversion efficiency of (3.36) kg compared to the rest of the treatments, the second T2 (4.34) kg and the third T3 (3.39) kg and the control treatment T1, which recorded the lowest feed conversion efficiency of (4.38) kg (Table 5). The improvement in feed conversion efficiency may be attributed to the increase in body weight, as there is a direct relationship between these two parameters Feed consumption did not change significantly, which may be due to the improvement in the efficiency of the digestive tract to digest nutrients as a result of adding the mushroom culture to the feed. This improvement is due to the enzymatic activity of the enzymes secreted by the mushroom, which is positively reflected in the digestion of nutrients, as well as due to the improvement in the length of the villi, which in turn is reflected in the improvement in absorption performance. In a study conducted by (Karthika *et al*., 2020), they observed an improvement in feed consumption rate and feed conversion efficiency as a result of adding 4% of *Apergillus niger* fungal hyphae to broiler diets for 3 weeks. In another study by (Basharat *et al*., 2020), they obtained similar results as a result of using 6% prebiotic in broiler diets, which led to a clear improvement in the amount of feed consumed and feed conversion efficiency.

Table 5: Effect adding mushroom culture to quail diets based on feed conversion factor (g) during the weeks of the experiment period (mean ± standard error).

Group Week	T1:(Control)	Aspergillus niger		
		T2: (2g/kg)	T3: (4g/kg)	T4: (8g/kg
1 Week	2.07±0.08[a]	1.87±0.09[ab]	1.63±0.09[bc]	1.28±0.07[c]
3 Week	2.68±0.09[a]	2.52±0.02[a]	2.51±0.02[a]	2.19±0.16[b]
6 Week	4.38±0.10[a]	4.34±0.20[a]	3.39±0.41[b]	3.36±0.17[b]

Note: Small letters refer to significant differences among groups at ($p<0.05$). N.S. refers to no significant difference.

Table 6: The effect of adding mushroom culture to quail diets on the microbial content of those diets (mean ± standard error).

Groups	Microorganisms		
	Total number of aerobic bacteria	Coli bacteria	Yeasts and molds
T1: (Control)	5.422±0.08[a]	2.630±0.04[a]	3.230±0.330[c]
T2:2g/kg *Aspergillus Niger*.	5.064±0.002[b]	1.156±0.001[b]	4.005±0.306[c]
T3:4g/kg Aspergillus Niger.	4.140±0.006[c]	0.730±0.026[c]	5.470±0.066[b]
T4:8g/kg *Aspergillus Niger*.	4.040±0.006[c]	0.470±0.066[d]	7.076±0.08[a]

Note: Small letters refer to significant differences among groups at ($p<0.05$). N.S referred to no significant difference* referred to significant difference.

Microbial Traits

The results of Table 6 indicated a reduction in the total number of aerobic bacteria in the feed as a result of adding the *Aspergillus niger* fungus culture to quail feed at the three ratios of 2gm/kg, 4gm/kg, and 8gm/kg. The fourth treatment T4 recorded the lowest number of 4.040 logarithmic cycles/g, followed by the third and second treatments, respectively, compared to the control, which recorded the highest number of 5.422 logarithmic cycles/g at a significance level ($p<0.001$).While the results of the same table recorded a significant decrease in the number of coliform bacteria as a result of adding the fungal culture to the quail feed, the fourth treatment T4 recorded the lowest number of 0.470 log cycles/g, followed by the third treatment of 1.9515 log cycles/g, followed by the third and second treatments respectively, compared to the control treatment, which recorded the highest number of 2.630 log cycles/g at a significant ($p<0.001$). From this, we conclude that adding the fungal culture to the quail feed affected the Gram-negative bacteria more than the Gram-positive bacteria, and the reason is that the decrease in the number of total aerobic bacteria was less by approximately one log cycle, while the decrease in the number of coliform bacteria was by three log cycles. The reason for these differences may be attributed to the presence of bacterial inhibitors from the fungal metabolic secretions. (La *et al*., 2023) indicated that the fungus Aspergillus sp secretes koijc acid, which acts as an antibacterial or antifungal. In another study by (Vivek *et al*., 2023), he indicated that the fungus secretes many organic acids such as citric acid, gluconic acid, lactic acid, and oxalic acid. This was confirmed by (Pratima *et al*., 2020; Ajafar *et al*., 2024), who stated that adding organic acids to broiler feeds led to a reduction in the number of colon bacteria. As for yeasts and molds in the feed, Table 6 indicated a significant superiority ($p<0.001$) for the fourth treatment, which recorded 7.076 log cycles/g, compared to the control, which recorded 3.230 log cycles/g. This may be due to the presence of fungal spores in the feed, which compete with other molds, reducing their growth and harmful effects during storage.

Table 7: The effect of adding *aspergillus niger* culture to quail diets on the microbial content of the jejunum (number in logarithms) (mean ± standard error).

Microorganisms Groups	Jejunum		
	Total number of aerobic bacteria	Coliform bacteria	Yeasts and molds
T1: (Control)	7.106±0.001[a]	6.106±0.002[a]	7.215±0.003[a]
T2:2g/kg *Aspergillus Niger*.	7.400±0.001[a]	5.262±0.004[b]	7.130±0.002[b]
T3:4g/kg *Aspergillus* Niger.	6.537±0.001[b]	5.204±0.002[b]	6.500±0.003[a]
T4:8g/kg *Aspergillus Niger*.	6.004±0.001[a]	4.641±0.002[c]	6.306±0.001[c]

Note: Small letters refer to significant differences among groups at ($p<0.001$). N.S. refers to no significant difference. * refers to significant difference.

The results of Table 7 indicated a decrease in the total number of aerobic bacteria, as the fourth treatment recorded the highest significant decrease of 6.004 log cycles/g, while the control group recorded the lowest significant decrease in the number of aerobic bacteria of 7.106 log cycles/g. With regard to colon bacteria, the fourth treatment recorded the highest decrease in the number of bacteria of 4.641 log cycles/g, while the control treatment recorded the lowest significant decrease of 6.106 log cycles/g. As for the number of yeasts and molds in the fasting, the fourth treatment recorded the highest significant decrease of 6.306 log cycles/g compared to the control group, which recorded the lowest significant decrease in the number of yeasts and molds of 7.215 log cycles (Yaqoob *et al*., 2021; Al-Jebory and Naji, 2021) reported a 99% reduction in *Salmonella typhimurium* bacteria when prebiotics were added to the diluted contents of the egg white caecum. Sartor *et al*. (2002) observed a significant increase in the numbers of *Lactobacillus casei* and *Lactobacillus cellobious*, while *Sallmonella entreitidis* decreased significantly compared to the control when 0.2% Aspergillus sp powder was added as a biostimulant. Donalson *et al*. (2004) reported that adding prebiotics to the diluted contents of the caecum of the whites resulted in a 99% reduction in the numbers of *Sallmonella typhimurium*. While the results of Grace *et al*. (2002) where he stated that adding 0.2% of Aspergillus powder to broiler feed led to an increase in the total number of aerobic bacteria, yeasts and molds, as well as an increase in the number of colonic bacteria in the contents of the ileum at the age of 20 days. Gut microbiota is important in dietary poly saccharide digestion, nutrient absorption, physiological homeostasis, and immune stimulation in their hosts.

The supplementation of A. niger significantly increased the alpha diversity and distinctly separated the microbial community structure, referring to the great contribution of probiotics in shaping the gut microbiota. Bacterial species that are promoted B. subtilis, B. licheniformis, and Lactobacillus sp. (Xie *et al*., 2019).

The use of FOS as a prebiotic is similar to other prebiotics, as it has been shown to support growth of LAB (Menne *et al*., 2000). However, FOS has the unique ability to prevent the adhesion of E. coli and Salmonella to the intestinal epithelium (Benyacoub *et al*., 2008) and has been shown to reduce E. coli growth when added to a coculture with LAB (Vongsa *et al*., 2016). The use of a nutritional blend of MOS, probiotics, and fibrolytic enzymes also observed lower fecal scores. These results might not be completely attributable to the MOS, but nevertheless demonstrate the potential synergistic effects of pro- and prebiotics that should be further explored (Marcondes *et al*., 2016). It has been suggested that MOS may help prevent attachment of pathogenic species in the lower gut (Spring *et al*., 2000), a compared with the other prebiotics used in studies, MOS has a clear and consistent benefit on health (mainly digestive health), which suggests that it is worth including in diets.

Table 8 showed the effect of adding mushroom culture to quail rations on the microbial content of the litter for 6 weeks. The results showed an increase in the total number of aerobic bacteria in the litter. The highest significant superiority (p<0.001) was obtained for the fourth treatment T4, which amounted to 6.225 log cycles/g, followed by the second and third treatments, respectively, while the control treatment recorded the lowest significant level, which amounted to 3.104 log cycles/g. While the colon bacteria recorded the highest decrease in their numbers in the fourth treatment 2.003 log cycles/gm, while the control recorded the lowest decrease in the numbers of bacteria reaching 3.054 log cycles/gm. The importance of the decrease in the numbers of colon bacteria lies in the fact that the litter is the most suitable place for birds to live and thus will contribute to reducing and eliminating pathogenic bacteria. As a result of the increased addition of mushroom culture to quail feed, it led to a gradual decrease in the numbers of yeasts and molds. The fourth treatment recorded the highest decrease reaching 1.632 log cycles/gm. While the control recorded the lowest decrease in the numbers of yeasts and molds reaching 5.120 log cycles/gm. While the results of the study on the effect of adding mushroom culture to broiler feed on the microbial content of the litter on which the experimental birds were raised for 8 weeks showed an increase in the number of total aerobic bacteria in the litter for the treatments compared to the control group, while the colon bacteria maintained their low numbers in the treatments compared to the control. As for the numbers of yeasts and molds, they gradually decreased (Alssodini, 2005).

Table 8: The effect of adding *Aspergillus niger* mushroom culture to quail diets on the microbial content of the litter (number in logarithm).

Groups	Microorganisms		
	Total number of aerobic bacteria	Coliform bacteria	Yeasts and molds
T1: (Control)	3.104±0.003[d]	3.054±0.001[a]	5.120±0.007[a]
T2:2g/kg *Aspergillus Niger*.	4, 431±0.007[c]	2.563±0.005[b]	3.301±0.001[b]
T3:4g/kg *Aspergillus Niger*.	5.010±0.001[b]	2.056±0.000[c]	2.007±0.001[c]
T4:8g/kg *Aspergillus Niger*.	6.225±0.001[a]	2.003±0.002[c]	1.632±0.007[d]

Note: Small letters referred to significant difference among groups at (p≤0.001). N.S referred to no significant difference. * referred to significant difference.

CONCLUSIONS AND RECOMMENDATIONS

It can be concluded that adding fermented wheat bran with *Aspergillus niger* to quail feed had a positive effect on improving the productive performance of the birds, and its effect was evident on the microbial content. Therefore, we recommend using these fungi on other types of birds or using other fungal species in future studies.

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NOVELITY STATEMENT

The research was conducted to evaluate the addition of wheat bran fermented with *Aspergillus niger* to quail feed to increase production performance, improve feed conversion efficiency, balance intestinal microflora, and reduce contamination in the litter caused by mold.

AUTHOR'S CONTRIBUTIONS

Measem Hassan: Contributed to collecting samples and conducting laboratory analyses.
Zainab A. Mutter: Managed the quail field and raising it.
Magareb Mohamed: Analyzed the results statistically.

Ethical Approval

This work is approved by The Research Ethical Committee IACUC for animal 29/ 37 in 2024.

Conflict of Interest

The authors weren't upfront about any conflict of interest.

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