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Research article

Evaluation of *Aspergillus* meal prebiotic in productive parameters, bone mineralization and intestinal integrity in broiler chickens

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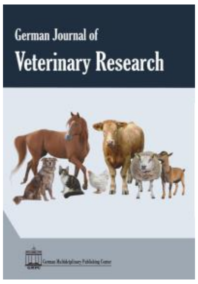

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Abstract

This study investigates the effects of *Aspergillus* meal prebiotic on a spectrum of parameters, including productive performance, bone mineralization, and intestinal integrity, in broiler chickens. Day-of-hatch Cobb 500 by-product male chicks (320 total) were randomly assigned to two experimental groups (G1 and G2) in floor pens (150×300 cm), each pen containing separate feeders and watering systems (8 replicates per treatment, 20 birds/pen). Chickens kept in G1 (control) were fed a basal diet, while those of G2 (treated) were fed a basal diet supplemented with 0.2% PRI-A-FERM. Performance parameters, including body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR), were evaluated weekly from the 7th day to the end of the trial at 42 d. Fluorescein isothiocyanate-dextran (FITC-d) was administered via oral gavage to 3 chickens/replicate (n = 24), and blood was collected on 21d and on 42d to determine serum concentration of FITC-d as a biomarker to evaluate intestinal permeability and peptide YY as an indirect biomarker to evaluate short chain fatty acids. Chickens that received the *Aspergillus* meal prebiotic showed a significant increase in BW, BWG, and FI on days 14, 21, 28, 35, and 42 ($P<0.05$) and a significant reduction in FCR on days 28, 35, and 42 of evaluation compared to control. Moreover, chickens that were supplemented with the *Aspergillus* meal prebiotic showed a significant reduction in serum concentrations of FITC-d on both days of evaluation, as well as a significant increase in serum concentration of peptide YY. Additionally, treated animals significantly increased bone strength, total bone ash, and calcium and phosphorous content at both evaluation times compared to the control. In summary, the results of this study suggest that *Aspergillus* meal prebiotic supplementation can positively influence productive parameters, bone mineralization, and intestinal integrity in broiler chickens. The observed improvements underscore the importance of gut health in overall poultry performance. The findings contribute to the growing body of knowledge on the benefits of prebiotics (0.2%, PRI-A-FERM) in animal nutrition and highlight the potential for their practical application in enhancing poultry production systems. Further research is warranted to elucidate the underlying mechanisms and optimize prebiotic utilization for improved broiler health and productivity.

Keywords: *Aspergillus* meal, Chickens, Performance, Bone mineralization, FITC-d, Peptide YY

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Introduction

In poultry farming, achieving optimal productive parameters while ensuring birds' well-being is paramount. Furthermore, maintaining bone health and intestinal integrity is vital to ensure efficient nutrient utilization and minimize potential health challenges (Shehata et al., 2022; Tellez-Isaias et al., 2023). Hence, evaluating interventions that could positively impact these factors holds great significance. As the demand for sustainable and efficient animal farming practices continues to rise, the outcomes of this investigation could have implications not only for poultry nutrition but also for broader agricultural practices. Broiler chickens are the world's most widely produced poultry species (Korver, 2023). They are raised for their meat, which is a high-quality source of protein and other nutrients. However, broiler chickens are also susceptible to several health problems, including gastrointestinal disorders, bone mineralization defects, and impaired intestinal integrity (Chowdhury et al., 2023).

Table 1: Ingredient composition and nutrient content of the corn-soybean diets used on as-is basis.

Item	Starter	Grower	Finisher
Ingredients (%)			
Corn	51.85	57.85	59.68
Soybean meal	37.66	31.62	27.23
DDGS 8.1% EE	4.00	4.00	6.00
Poultry fat	3.24	3.44	4.38
Limestone	1.08	1.06	1.03
Dicalcium phosphate	1.01	0.88	0.64
Salt	0.35	0.35	0.31
DL-methionine	0.29	0.25	0.22
L-lysine HCl	0.12	0.13	0.12
Mineral premix[a]	0.10	0.10	0.10
Vitamin premix[b]	0.10	0.10	0.10
L-threonine	0.08	0.09	0.09
Choline chloride	0.06	0.06	0.05
Sodium bicarbonate	0.04	0.05	0.03
Antioxidant[c]	0.02	0.02	0.02
Calculated analysis			
ME (kcal/ kg)	3015.00	3090.00	3175.00
Ether extract (%)	5.88	6.20	7.28
Crude protein (%)	22.30	20.00	18.70
Lysine (%)	1.18	1.05	0.95
Methionine (%)	0.59	0.53	0.48
Threonine (%)	0.77	0.69	0.65
Tryptophan (%)	0.25	0.22	0.20
Total calcium (%)	0.90	0.84	0.76
Total phosphorous (%)	0.63	0.58	0.53
Available phosphorus (%)	0.45	0.42	0.38
Sodium (%)	0.20	0.20	0.18
Potassium (%)	1.06	0.94	0.87
Chloride (%)	0.27	0.28	0.25
Magnesium (%)	0.19	0.18	0.17
Copper (%)	19.20	18.46	18.85
Selenium (%)	0.28	0.27	0.26
Linoleic acid (%)	1.0	1.13	1.16

[a]Mineral premix supplied the following per kg: manganese, 120 g; zinc, 100g; iron, 120 g; copper, 10-15 g; iodine, 0.7 g; selenium, 0.4 g; and cobalt, 0.2 g (Nutra Blend LLC, Neosho, MO 64850).
[b]Vitamin premix supplied the following per kg: vitamin A, 20,000,000 IU; vitamin D3, 6,000,000 IU; vitamin E, 75,000 IU; vitamin K3, 9 g; thiamine, 3 g; riboflavin, 8 g; pantothenic acid, 18 g; niacin, 60 g; pyridoxine, 5 g; folic acid, 2 g; biotin, 0.2 g; cyanocobalamin, 16 mg; and ascorbic acid, 200 g (Nutra Blend LLC, Neosho, MO 64850).
[c]Ethoxyquin

Prebiotics are non-digestible food ingredients that can beneficially affect the host by selectively stimulating the growth and activity of one or a limited number of bacteria (Roberfroid, 2007). The utilization of prebiotics in animal nutrition has gained considerable attention in recent years due to their potential to enhance gut health, improve nutrient absorption, and subsequently boost overall productivity (Morgan, 2023). Among these prebiotic options, *Aspergillus* meal prebiotic has emerged as a promising candidate. *Aspergillus* meal is a prebiotic that has been shown to have several beneficial effects in broiler chickens, including improving growth performance and gut health (Torres-Rodriguez et al., 2005; Huang et al., 2023).

This article delves into an assessment of the effects of this prebiotic on a spectrum of parameters, including productive performance, bone mineralization, and intestinal integrity, in broiler chickens. The assessment is conducted by analyzing serum concentrations of Fluorescein isothiocyanate-dextran (FITC-d) and peptide YY (PYY), both of which serve as reliable indicators of gut health and integrity. FITC-d is commonly employed to evaluate intestinal permeability, while PYY is an essential gut hormone associated with satiety and digestion regulation.

Materials and methods

Ethical approval

This study was carried out by the recommendations of the Institutional Animal Care and Use Committee (IACUC) at the University of Arkansas, Fayetteville, under IACUC-approved protocol #16084.

Aspergillus meal prebiotic

Pri-A-Ferm (APCO TRADING, INC., Laredo, TX USA) comprises *Aspergillus* meal derived from an active fermentation of a primary *Aspergillus niger*. It is the mycelium contained in this totally dead product that allows the monogastric expansion of its digestive capacity. The mycelium fiber expands the digestive capacity by establishing a healthy microbiota in the gastrointestinal tract of the animal, supporting the bacteria to propagate, producing increased levels of short-chained organic acids and slowing the gastrointestinal tract movement, allowing for longer passage, more metabolic activity, and absorption of nutrients.

Experimental design

Day-of-hatch Cobb 500 male chicks (320 total) were randomly assigned to two experimental groups in floor pens (150×300 cm), each pen containing separate feeders and watering systems (8 replicates per treatment, 20 birds/pen). Group 1 (G1); controlled the basal diet and Group 2 (G2) treated group, which consisted of a basal diet plus 0.2% PRI-A-FERM. Fluorescein isothiocyanate-dextran (FITC-d) was administered via oral gavage to three chickens/replicate (n = 24) and blood was collected on 21d and on 42d to determine serum concentration of FITC-d as a biomarker to evaluate intestinal permeability as described by Baxter et al. (2017), and PYY as an indirect biomarker to evaluate short chain fatty acids post-mortem as describe by Tellez et al. (2020).

One hour before humanely euthanizing the chickens by CO_2 inhalation, 24 broiler chickens from each group (3 chickens/replicate) were given an oral gavage dose of 8.32 mg/kg FITC-d. Blood samples were collected from the femoral vein and centrifuged (1000 ×g for 15 min) to collect serum for FITC-d and PYY measurement. The left tibia from each chicken was removed to evaluate breaking strength (kg) on 21d and 42d, as described below. Performance parameters body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were evaluated weekly from 7 d to the end of the trial at 42 d. Starter, grower, and finisher mash feed diets with no antibiotics or coccidiostats (Table 1) used in this experiment were formulated to approximate the nutritional requirements of Cobb 500 broiler chickens according to the breeders' guidelines (Cobb-Vantress, 2018).

Bone parameters

Bone parameters were assessed using Zhang and Coon (Zhang and Coon, 1997) techniques. The right tibia was conventionally assayed for determination of the content of ash, calcium, and phosphorus according to the standard methods (AOAC, 2000), and the left tibia was broken to evaluate strength using an Instron 4502 (Norwood, MA) material testing machine with a 509 kg load cell. The loading rate was kept constant at 20 mm/min, collecting 10 data points per second using Instron's Series IX Software (Norwood, MA).

Data and statistical analysis

All data were analyzed by a one-way analysis of variance (ANOVA) using the General Linear Models procedure of SAS (SAS Institute Inc., 2002). Means were further separated using the Student's T-test with significance at $P < 0.05$.

Results

The results of the evaluation of the effects of *Aspergillus* prebiotic supplementation on performance parameters (BW, BWG, FI, and FCR) in broiler chickens are summarized in Table 2.

Chickens that received the *Aspergillus* meal prebiotic showed a significant increase in BW, BWG, and FI on days 14, 21, 28, 35, and 42 ($P < 0.05$) and a significant reduction in FCR on days 28, 35, and 42 of evaluation compared to the control non-treated chickens. No significant differences in total mortality (%) between control or treated groups were observed (Table 2).

Table 3 shows the results of the evaluation of *Aspergillus* meal prebiotic on serum concentrations of FITC-d and PYY on days 21 and 42 in broiler chickens. Chickens that were supplemented with the *Aspergillus* meal prebiotic showed a significant reduction in serum concentrations of FITC-d at both days of evaluation, but they showed a significant increase in the serum concentrations of PYY compared with control-non-treated chickens (Table 3).

The results of the evaluation of *Aspergillus* meal prebiotic on bone parameters on days 21 and 42 in broiler chickens are summarized in Table 4. Chickens that received the prebiotic showed a significant increase in bone strength, total ash, calcium, and phosphorous at both evaluation times compared with control chickens (Table 4).

The present study aimed to investigate the potential effects of *Aspergillus* meal on various aspects of broiler chicken health and productivity, including productive parameters, bone mineralization, and intestinal integrity. The findings of this study contribute to our understanding of the potential benefits of using *Aspergillus* meal as a dietary supplement in broiler chicken production systems.

Discussion

The beneficial effects of *Aspergillus* meal prebiotic on productive performance can be attributed to its prebiotic properties. Prebiotics are non-digestible food ingredients that promote beneficial gut bacteria growth (Fei et al., 2023). These beneficial bacteria produce short-chain fatty acids (SCFAs), which have several beneficial effects on the host, including improving gut health, increasing nutrient absorption, and stimulating the immune system (Leone and Ferrante, 2023). *Aspergillus* meal prebiotic is a substrate for SCFAs production, which is likely to be one of the mechanisms by which it improves productive performance in broiler chickens (Liu et al., 2023). A healthier gut lining with improved integrity can lead to better nutrient absorption. This can positively impact the broiler's overall health, growth, and performance (Stadnicka et al., 2023).

Prebiotics like *Aspergillus* meal can selectively stimulate the growth of beneficial bacteria in the gut. A balanced and diverse gut microbiota can contribute to the proper functioning of the digestive system and overall gut health. A healthy gut lining and microbiota can enhance the absorption of nutrients from feed. This could lead to better nutrient utilization, potentially affecting broiler chickens' production and growth (Bouiche et al., 2023).

The results of the current study revealed significant improvements in productive parameters among broiler chickens supplemented with *Aspergillus* meal prebiotic. This observation is consistent with previous research

Table 2: Evaluation of the effects of *Aspergillus* meal prebiotic supplementation on body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) in broiler chickens.

Age (days)	Control non-treated	*Aspergillus* meal prebiotic
	Body weight, g/broiler	
d 0	41.48±0.22	41.15±0.25
d 7	195.57±2.42	215.57±2.42
d 14	505.79±5.93^{b}	625.79±5.93^{a}
d 21	965.08±15.17^{b}	1,018.84±18.45^{a}
d 28	1,363.88±26.10^{b}	1,607.63±21.98^{a}
d 35	1,897.26±51.29^{b}	2,205.32±33.39^{a}
d 42	2,537.26±80.82^{b}	2,981.48±63.52^{a}
	Body weight gain, g/broiler	
d 0 to 7	154.01±0.98	164.21±0.87
d 7 to 14	310.14±3.42^{b}	412.04±3.72^{a}
d 14 to 21	460.84±10.23^{b}	553.60±9.62^{a}
d 21 to 28	378.89±25.86^{b}	429.39±12.46^{a}
d 28 to 35	434.72±38.62^{b}	598.09±20.48^{a}
d 35 to 42	640.56±40.91^{b}	776.89±58.44^{a}
	Feed intake, g/broiler	
d 0 to 7	150.31±1.42	160.45±1.22
d 7 to 14	480.47±4.42^{b}	500.34±4.71^{a}
d 14 to 21	1,205.07±8.35^{b}	1,218.44±7.85^{a}
d 21 to 28	2,101.06±21.71^{b}	1,965.36±20.67^{a}
d 28 to 35	3,041.39±46.53^{b}	2,910.98±35.39^{a}
d 35 to 42	4,731.43±82.04^{a}	4,695.75±75.00^{b}
	Accumulated feed conversion ratio	
d 0 to 7	0.76±0.001	0.74±0.002
d 0 to 14	0.95±0.002	0.80±0.001
d 0 to 21	1.24 ±0.02	1.19±0.02
d 0 to 28	1.54±0.02^{a}	1.22±0.02^{b}
d 0 to 35	1.60±0.05^{a}	1.31±0.02^{b}
d 0 to 42	1.86±0.06^{a}	1.57±0.03^{b}
Total mortality (%)	5.62	3.75

Data expressed as mean±SE. Eight replicates per treatment (n=20 chickens per replicate).
$^{a-b}$Values within rows with different superscripts differ significantly ($P<0.05$).

Table 3: Evaluation of *Aspergillus* meal prebiotic on serum concentrations of FITC-d and PYY in broiler chickens.

Age (days)	Control non-treated	*Aspergillus* meal prebiotic
	Serum fluorescein isothiocyanate-dextran (FITC)-d (ng/mL)	
Day 21	502.71±18.06^{a}	226.85±7.37^{b}
Day 42	612.41±28.16^{a}	316.15±17.07^{b}
	Serum peptide YY (ng/mL)	
Day 21	2.40±0.16^{b}	5.55±0.69^{a}
Day 42	3.64±0.26^{b}	6.15±0.45^{a}

Data expressed as mean±SE. Eight replicates per treatment (n=24 serum samples).
$^{a-b}$Values within rows with different superscripts differ significantly ($P<0.05$).

Table 4: Evaluation of *Aspergillus* meal prebiotic on bone mineralization in broiler chickens

Age (days)	Control non-treated	*Aspergillus* meal prebiotic
	Tibia strength load at yield (kg/mm^2)	
Day 21	2.36±0.12 ^{b}	4.54±0.23^{a}
Day 42	4.36±0.56 ^{b}	7.76±0.12^{a}
	Total ash from tibia (%)	
Day 21	47.61±0.81^{b}	62.17±0.75^{a}
Day 42	52.63±0.12^{b}	75.76±0.12^{a}
	Calcium (% of ash)	
Day 21	32.35±0.07^{b}	54.31±0.46^{a}
Day 42	61.56±0.52^{b}	87.26±0.11^{a}
	Phosphorus (% of ash)	
Day 21	25.35±0.42^{b}	34.77±0.39^{a}
Day 42	42.36±0.15^{b}	52.10±072^{a}

Data expressed as mean±SE. Eight replicates per treatment (n=24 tibias).
$^{a-b}$Values within rows with different superscripts differ significantly ($P<0.05$).

demonstrating the positive influence of prebiotics on growth performance, feed efficiency, and carcass characteristics in poultry (Karar et al., 2023). The mechanism underlying these effects may involve promoting beneficial gut microbiota populations, enhanced nutrient utilization, and modulation of gut health (Abdel-Raheem et al., 2023). The increased body weight and improved feed conversion ratio observed in the prebiotic-supplemented group indicate a potential for enhanced economic returns for poultry producers. *Aspergillus* meal could potentially promote the growth of beneficial gut bacteria, leading to a balanced and diverse gut microbiota composition (Nakano et al., 2023). A healthy gut microbiota is associated with improved intestinal health and well-being in broiler chickens (Fathima et al., 2023).

The assessment of intestinal integrity is a vital aspect of evaluating the health of broiler chickens, as the gastrointestinal tract plays a central role in nutrient absorption, immune response, and defense against pathogens. In this study, serum concentrations of FITC-d and PYY were utilized as indicators of intestinal barrier function and gut hormone secretion, respectively. A balanced gut microbiota and improved gut barrier function can help reduce intestinal inflammation. Chronic inflammation can compromise the integrity of the intestinal lining, leading to increased permeability (Tellez-Isaias et al., 2023).

By promoting a healthier gut environment, *Aspergillus* meal may indirectly reduce serum FITC-d concentrations (Shehata et al., 2022). The significant decrease in serum FITC-d levels in the prebiotic-treated group suggests improved intestinal barrier integrity, potentially due to the maintenance of tight junctions between enterocytes (McGilloway et al., 2023). This finding aligns with the hypothesis that prebiotics can positively modulate gut health by influencing gut microbiota composition and immune function. Prebiotics may help strengthen the intestinal barrier by promoting the growth of beneficial bacteria that produce short-chain fatty acids. SCFAs maintain tight junctions between intestinal epithelial cells, reduce gut permeability, and prevent harmful molecules' leakage into the bloodstream (Goes et al., 2022). Moreover, some prebiotics, including those derived from fungal sources like *Aspergillus*, may have antioxidant properties (Derakhshan et al., 2023).

Antioxidants can help mitigate oxidative stress in the gut, often associated with intestinal inflammation and increased permeability. By reducing oxidative stress, the prebiotic may help maintain gut health and reduce the levels of serum FITC-d (Ogbuewu et al., 2022). In addition, stressors, such as dietary changes and biological, physical, chemical, or environmental factors, can negatively impact gut health and increase intestinal permeability (Basiouni et al., 2023). Prebiotics have been studied for their potential to alleviate stress-induced disturbances in the gut, which could contribute to reducing serum FITC-d concentrations. PYY is a gut hormone produced by cells lining the small intestine and caecum (Conlon, 2002). It plays a role in appetite regulation and slowing down gastrointestinal motility, affecting digestion and absorption (Aoki et al., 2017). The elevated levels of PY in prebiotic-supplemented broilers suggest a potential enhancement in appetite regulation and nutrient utilization, supporting the observed improvements in growth performance.

Bone health is a crucial aspect of poultry production, as skeletal integrity influences locomotion, weight-bearing capacity, and overall welfare. The significant enhancement in bone mineralization observed in broilers receiving the *Aspergillus* meal prebiotic suggests its positive impact on calcium and phosphorus metabolism, key components of bone formation (Shehata et al., 2022). This result aligns with existing literature indicating that prebiotic supplementation can influence mineral absorption and deposition, thereby contributing to skeletal development and strength in poultry. The positive effects of *Aspergillus* meal prebiotic on bone mineralization can also be attributed to its prebiotic properties (Selim et al., 2022). Prebiotics have been shown to increase calcium absorption and other minerals from the gut, which is important for bone health (Egbu et al., 2022). Additionally, prebiotics can stimulate the production of bone-building hormones, such as IGF-1 (Jaiswal et al., 2023). *Aspergillus* meal prebiotic is likely to have these effects in broiler chickens, which is why it improved bone mineralization in this study.

The mechanisms underlying the observed effects of *Aspergillus* meal on productive parameters, bone mineralization, and intestinal integrity warrant further investigation. Prebiotics are known to selectively stimulate the growth and activity of beneficial gut bacteria, producing short-chain fatty acids and other metabolites that can impact host physiology. The modulation of gut microbiota composition and activity may have contributed to the observed improvements in growth, bone health, and gut barrier function. However, the specific bacterial taxa and metabolic pathways involved require deeper exploration.

The findings of this study hold practical implications for the poultry industry, where enhancing productivity and maintaining animal health are paramount. Using *Aspergillus* meal prebiotic (0.2%, PRI-A-FERM) as a dietary supplement could offer a sustainable and cost-effective approach to improving broiler chicken performance. Further research is needed to elucidate gut microbiota dynamics and meat quality. While this study provides valuable insights into the potential benefits of *Aspergillus* meal, several limitations should be acknowledged. The present study did not explore the underlying molecular mechanisms through which the prebiotic exerts its effects. Future research should employ advanced techniques such as metagenomics and metabolomics to better understand the microbial and metabolic changes induced by prebiotic supplementation.

In summary, the results of this study suggest that *Aspergillus* meal prebiotic supplementation (0.2%, PRI-A-FERM) can positively influence productive parameters, bone mineralization, and intestinal integrity in broiler chickens. The observed improvements underscore the importance of gut health in overall poultry performance. The findings contribute to the growing body of knowledge on the benefits of prebiotics in animal nutrition and highlight the potential for their practical application in enhancing poultry production systems. Further research is warranted to elucidate the underlying mechanisms and optimize prebiotic utilization for improved broiler health and productivity.

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Conflict of Interest. The authors declare no conflict of interest.

Authors Contributions. KM, RLAF, JDL, and GT-I conceptualized the study. KM, LL, MEC, LL, LSG, RS-C, RFRM, AS, IL and IC-H handled the methodology. KM, LL, MEC, LL, LSG, RS-C, RFRM, AS, and IL performed data collection. JDL and GT-I validated the study. KM and LL performed the formal data analysis. GT-I prepared and wrote the original draft. XH-V, JDL, SE-A, EA-O, and GT-I contributed to the writing, reviewing, and editing of the final manuscript. JDL and GT-I oversaw the project administration and funding acquisition. All authors contributed to the article and approved the submitted version.

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