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Xylanase Supplementation Modulates Cecal E. Coli And Lactobacillus Populations In Broiler Chickens Fed Low-Energy Diets

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Abstract
This study was aimed to assess the impact of exogenous xylanase enzyme in reduced energy diet on carcass characteristics of broiler chickens. A total of 200 one-day-old Ross 308 broiler chicks were randomly allocated into foure groups (50 chicks per group), with two replicates for each group. The control group (T1) received a basal diet corn + soybeans meal. The second treatment fed a reduced energy diet (200/ kal) (T2). The third experiment fed xylanase enzyme (250g/ton) in reduced energy diet (200/kal) (T3). The fourth treatment fed a basal diet + xylanase enzyme (250g/ton) (T4). The bacterial count was increased significantly (p≤0.05) in the number of Lactobacillus in (T4) and (T3) groups compared with other groups. In addition; E. coli bacteria were decreased (p≤0.05) in (T4) and (T3) groups compared with other groups. In conclusion Increase in beneficial bacteria (Lactobacillus) compared to the control group. In addition to reducing, the number of harmful bacteria (E. coli) compared to the control group.
Keywords: *Xylanase, Broiler, Cecal Microbiota, Lactobacillus, E. coli*

INTRODUCTION

The gut microbiome, composed of diverse bacteria, yeasts, and protozoa, is a complex system. Commensal microorganisms in the intestinal tract are essential for digestion and immunity in poultry, as highlighted by (Seidavi et al., 2017).

The use of xylanase enzyme supplements in poultry feed has led to enhanced the growth of beneficial gut bacteria in broilers, leading to improved overall performance and improved nutrients digestibility and AME. The more beneficial bacteria there are, the less harmful bacteria there are and there are prevented the more pathogens from (Singh et al., 2021).

The use of a xylanase enzyme in a low-energy and/or protein diet resulted in a reduction in the level of potentially pathogenic bacteria, such as total anaerobic bacteria in the ileum and coliform bacteria in the cecum. This may be due to the minimal amount of nutrients available for fermentation by pathogenic bacteria (Mun et al., 2024).

The xylanase has the ability to degrade NSP such as arabinoxylan present in plant-derived feed ingredients to synthesize short-chain fatty acids (SCFA) in the distal intestinal region of broiler chickens (Courtin et al., 2008). The suggested that the derived SCFA may have created a favorable environment for lactic acid bacteria such as Lactobacillus spp. as shown by a nutrient-deficient diet supplemented with an exogenous enzyme mixture. The prevalence of lactic acid inhibits the proliferation of harmful bacteria such as coliforms and total anaerobic bacteria (Yaghobfar and Kalantar, 2017).

(Jozefiak et al., 2010) who observed a significant decrease in the number of coliforms in the intestine of broiler chickens due to dietary supplementation of xylanase. (Sharmila eat l., 2015), who reported a significant reduction in pathogenic intestinal bacteria (*Enterobacteria, E. coli* and *Salmonella* spp.) of broiler chickens by supplementing their diet with xylanase enzymes. Xylanase enzymes can reduce intestinal digesta viscosity, increase nutrient absorption and simultaneously increase rapidly fermentable substrates for normal gut microbiota (Tiwari et al., 2018). The use of enzymes in animal nutrition was first reported in 1925 (Hervey, 1925). The use of exogenous enzymes in the diet has been shown to increase nutrient utilization and thus improve growth performance in broilers. Feed enzymes have been widely used to improve nutrient digestibility and growth performance in animals (Bedford, 2018). In the literature, enzyme supplementation, especially in low-energy diets or diets containing high fiber and/or antinutritional factors, has been found to improve energy utilization for growth (Cooper and Ma, 2017).

Therefore, the objective of this study was to determine the efficacy of xylanase at 250 g/ton levels in reduced energy broiler diet (200 kcal/kg) and study effect on bacteria account of *Lactobacillus* and *E.coli*.

MATERIALS AND METHODS

Experiment design and dietary treatment

Two hundred one-day-old unsexed broiler chicks (Ross-308) obtained from a commercial hatchery divided into four groups, each group with 50 birds. Each group was divided into two replicates with 25 birds in each. Control group (T1) is fed basal diet a corn soybean diet. Treatment (T2) fed reduced energy diet (200 kcal/kg). Treatment (T3) fed xylanase enzyme (250 g/ton) in reduced energy diet (200 kcal/kg). Treatment (T4) fed basal diet with xylanase enzyme (250 g/ton). At 35 days old, six birds from each treatment were selected at the end of the experiment.

Table (1) Ingredients and nutrients composition of broiler starter diets from 1-15 days and grower from 16-35 days

Ingredient (%)	Starter	Starter Reduced energy diet	Grower	Grower Reduced energy diet
Yellow corn	52.1	51.2	59.1	60.3
Soybean meal	40.2	40.5	32.6	32.4
Xylanase [1]	T4 25g	T3 25	T4 25g	T3 25 g
Vegetable oil	2.9	0	4	0
Limestone	1.6	1.6	1.3	1.3
MCP[2]	0.6	0.6	0.4	0.4
Premix[3]	2.5	2,5	2.5	2.5
Antitoxin	0.1	0.1	0.1	0.1
Sand	0	3.5	0	3
Total	100	100	100	100
Calculated chemical composition				
energy	2900	2700	3000	2800
Crude protein (%)	23	23	23	23
Calcium	0.96	0.96	0.96	0.96
Av. Phosphorus	0.54	0.54	0.54	0.54
Av. Lysine	1.3	1.3	1.3	1.3
Av. Methionine	0.65	0.65	0.65	0.65
Av. Threonine	0.87	0.87	0.87	0.87
Electrolyte	289	289	289	289

1. MCP1 GREENPHOSP/22.7% (Mono Calcium phosphate) cal 16% from BAF (An MCP1 GREENPHOSP/22.7% (Mono Calcium phosphate) cal 16% from BAF(Animal feed company), ADANA, Turkey animal feed company), ADANA, Turkey.
2. Composition of Premix2: vitamin, 6,000,000 IU; vitaminD3,1,500,000 IU; vitamin E, 15,000mg; riboflavin, 3,00mg; pantothenic acid, 7,000mg; nicotinic acid, 25,000mg; folic acid,500mg; vitamin

B12,15,000mg (Vit-VORM 6/1.5, supplied by Animedica, Horb, Germany). 4 compositions of trace elements premix supplied per kilogram of premix: Mn, 120,000 mg; Zn, 80,000 mg; Fe, 90,000; Cu,15,000 mg; I, 1,600 MG; Se, 500 mg (Spu"rElevor SGI, supplied by Animedica, Horb, Germany.

Bacterial Culturing and account

At 35 days of age, cecal swab samples were collected from six randomly selected birds in each treatment group for the enumeration of total bacteria, *Lactobacillus* spp., and *Escherichia coli*. Total bacterial counts were performed using Nutrient Agar or Tryptic Soy Agar (TSA). *E. coli* was selectively cultured on MacConkey agar, while *Lactobacillus* spp. were cultured on MRS agar (de Man, Rogosa, and Sharpe Agar), a selective medium for *Lactobacillus*."

Bacterial count were done by using Calculate CFUs (Colony Forming Units):

$$CFU/g = \frac{\text{Number of Colonies} \times \text{Dilution Factor}}{\text{Volume Plated (mL)}}$$

Statistical analysis.

Data was analyses by using the SPSS 22.0 software was used to analyze the data using the general linear model (GLM) method and one-way ANOVA (Corp, 2011). Using a "protected" Duncan's analysis at the 0.05 significance level, four treatment means were determined.

RESULTS:

Effect of xylanase enzyme on the bacteria count

The results of the experiment on the bacterial count of the cecal content showed that the best results were achieved in the (T4) and (T3) groups, where the number of *Lactobacillus* bacteria increased and the number of E. coli bacteria decreased in the same two groups compared with other groups as show in table (2). E. coli counts were significantly higher ($P < 0.05$) in the control group (T1: 8.20 ± 0.04 log₁₀ CFU/g) compared to other treatments. A progressive reduction was observed in T2 (7.50 ± 0.04), T3 (6.50 ± 0.04), and T4 (5.80 ± 0.04), with T4 (basal diet + xylanase) showing the lowest counts ($P < 0.05$). on the other hand, Lactobacillus populations were significantly lower ($P < 0.05$) in T1 (5.58 ± 0.08) and T2 (5.3 ± 0.48) compared to T3 (6.05 ± 0.06) and T4 (7.5 ± 0.09). The highest counts were recorded in T4 (basal diet + xylanase), followed by T3 (reduced-energy diet + xylanase).

Table (2) Effect of xylanase enzyme in a reduced energy diet on the bacteria count of broiler chickens CFU/g (Mean±SE):

Group CFU/g	T1	T2	T3	T4
E. coli	8.20 ± 0.04 a	7.50 ± 0.04 b	6.50 ± 0.04 c	5.80 ± 0.04 d
Lactobacillus	5.58±0.08 c	5.3±0.48 c	6.05±0.06 b	7.5±0.09 a

Different letters among treatment show significant difference (p≤ 0.05)

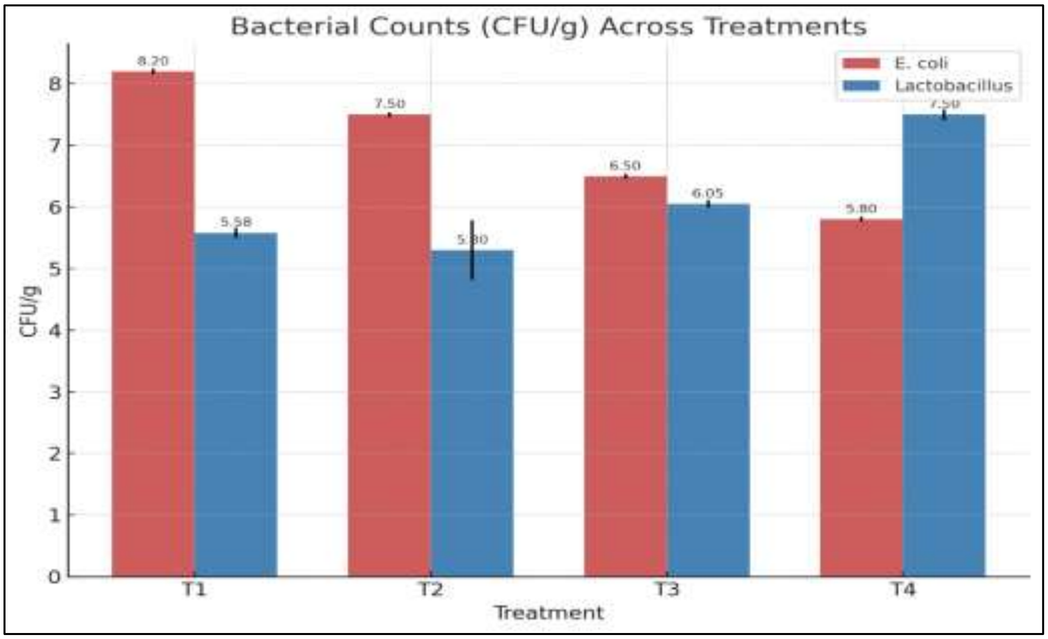

Figure 1: Comparison of E. coli and Lactobacillus populations in different treatment groups

DISCUSSION:

Adding the enzyme xylanase to poultry feed improves intestinal health, as the enzyme breaks down indigestible material concentrated in the ceca (Rodríguez-Soriano et al., 2025). This will reduce the growth environment for bacteria such as *Clostridium perfringens*, which causes necrotic enteritis. It will also reduce the presence of *Salmonella* and *E coli* bacteria in the intestines (coliform bacteria) (Abd El-Ghany, 2024) and (Mckounnen et al., 2024). Therefore, the fewer harmful bacteria, the better the intestinal health, with increased absorption area, longer villi, fewer diseases, increased immunity, and weight gain.

(Nusairat et al., 2018) reported the positive impact of xylanase in reducing Salmonella incidence in broilers at 21 and 42 days. Additionally, xylanase supplementation alone has demonstrated the ability to decrease *E. coli* counts and increase *Lactobacillus spp*. counts in the ceca of 35-day-old broilers (Van Hoeck et al., 2021). Therefore, the observed reduction in microbial load could be attributed to the effect of xylanase. The decrease in pathogen counts in the ceca suggests less contamination of the environment. This, in turn, helps control the spread of infection within the flock and subsequently to other flocks. The addition of xylanase has the potential to enhance the digestion of non-starch polysaccharides, thereby providing the necessary substrates for the proliferation of probioticbacteria. Xylanase can facilitate the breakdown of starch, a process that might potentially restrict the availability of substrates for pathogenic bacteria. If the mechanisms of enzymes and probiotics can reach their full potential, it logically follows that the combination of these products may contribute to enhancing intestinal health and achieving a balanced microbial environment in the gut. This, in turn, could lead to improved growth performance and feed efficiency in broilers (Flores et al., 2016). Previous studies have shown, that xylanase addition could promote the improvement of beneficial bacteria and play a role in intestinal protection. Consistently, it has been reported that adding xylanase can improve the microflora structure of broilers. By promoting the generation of propionate, acetic, and butyrate acid in beneficial bacteria, such as *Phascolarctobacterium, Parabacteroides, Faecalibacterium, Lactobacillus, Eubacterium_hallii*_group, and others, the content of butyrate-producing beneficial bacteria *Barnesiella and Fournierella* (Liu et al., 2023) ,(Ni et al., 2023), (Singh et al., 2023) was decreased. These findings indicate that the addition of xylanase could change the microflora structure to a certain extent and promote the production of SCFAs within a reasonable range. SCFAs play various roles, including promoting mucin production, regulating gene expression, stimulating enterocyte proliferation, and maintaining intestinal stability (Wu et al., 2023). The researchers (Wang et al., 2024) indicate that the addition of xylanase increased Verrucomicrobiota at the phylum level. Recent studies have documented that Verrucomicrobiota possesses enough glycoside hydrolase genes to degrade carbohydrates so that they release monosaccharides and amino acids that power other beneficial bacteria (Orellana et al., 2022). Akkermansia muciniphila is the mostrepresentative bacterium in Verrucomicrobiota, playing a role in mucin degradation regulation to protect the mucosal layer, promoting the production of prebiotics and SCFAs, and contributing to intestinal health and nutrient absorption (Cani et al., 2022).in conclusions**:** there was an increase in beneficial bacteria (Lactobacillus) compared to the control group. In addition to reducing, the number of harmful bacteria *E.coli* compared to the control group.

Novelty Statement

This study demonstrates, for the first time, that dietary xylanase supplementation in energy-deficient broiler diets significantly enhances cecal Lactobacillus populations while suppressing E. coli, suggesting a novel strategy to improve gut health under dietary stress. These findings reveal xylanase's dual microbial modulation capability, offering a practical solution to counteract energy-related dysbiosis in poultry production without reliance on antibiotics or probiotics

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Author Contributions

Hayder Ali: Conceptualization, broiler chicken preparation, nutritional management (diet and water formulation), and daily animal care, Yaser Jamel Jamel: Experimental design, dietary treatment formulation, statistical analysis, data interpretation, and manuscript review as well as Ali Jasim: Bacterial culture isolation and identification (*Lactobacillus* and *E. coli*), microbiological analysis, and validation of results.

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