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Adding Multiple Enzymes to Diets Containing Wheat Distillers Dried Grains with Solubles Improves Broiler Performance by Reducing Viscosity

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ABSTRACT

This study aimed to investigate the effects of adding the multienzyme Apsazyme (glucanase, xylanase, galactosidase, mannanase) to diets containing different levels of wheat distiller's dried grains with solubles (WDDGS) on performance, ileal bacteria, intestinal viscosity, pH, and blood variables of broiler chickens. For this purpose, 300 one-day-old male and female broilers of the Ross 308 strain were used. The experimental diets included three levels of WDDGS (0%, 10%, and 20%) with and without multienzyme (125 g/ton). Data analysis was done using SAS statistical software as a factorial design. The use of 20% WDDGS reduced feed intake (FI) in the finisher period and total period compared to the control treatment (p<0.05). The use of 20% WDDGS level reduced body weight gain (BWG) compared to 10% WDDGS treatment (p<0.05). The feed conversion ratio (FCR) was significantly reduced in birds fed with 125 g/ton of multienzyme diet (p<0.05). The number of aerobes bacteria and coliform was higher in broiler chickens fed with 10% and 20% WDDGS diet compared to the control treatment (p<0.05). Also, the use of multienzyme increased the number of lactic acid bacteria in the ileum. Multienzyme supplementation significantly reduced the increase in intestinal viscosity and pH caused by the 20% WDDGS level (p<0.05). Using 20% WDDGS in the diet increased the serum albumin concentration compared to 10% and 0% WDDGS (p<0.05). Finally, it can be concluded that the use of multienzymes compensates for the negative effects of diets containing 20% WDDGS on performance by reducing intestinal viscosity and modulating the gut microbial population. Therefore, it is recommended to add a multienzyme to the diet when using a 20% WDDGS level.

Keywords: broiler; multienzyme; non-starch polysaccharides; WDDGS, viscosity

INTRODUCTION

The lack of main sources of poultry feed, such as corn and soybean meal in Iran and their increasing price shows the necessity of alternative and cheaper feed. Diets rich in non-starch polysaccharides (NSPs) cause an increase in the viscosity of the contents of the digestive tract and imbalance of the intestinal microbiota (Nguyen *et al.*, 2022). Wheat distillers dried grains with solubles (WDDGS) are a by-product of ethanol production. During alcohol production, starch is removed from the grain and converted into alcohol and carbon dioxide. With the decomposition of starch into alcohol, the rest of the nutrients remain in the grain, which is called the grain fermentation residue. Research on the nutritional value of WDDGS started 50 years ago and has continued (Youngji *et al.*, 2018). The content of high metabolizable energy, moderate crude protein, and digestible phosphorus have made WDDGS a valuable and inexpensive feed (Whiting *et al.*, 2018). The production process affects the nutritional value of WDDGS and heat can be a determining factor in the digestibility of amino acids (Monteagudo-Mera *et al.*, 2018).

When WDDGS partially substitutes for corn, soybean meal, and inorganic phosphorus sources in the broiler diet, the cost of the diet is frequently decreased (Jang *et al.*, 2022). On the other hand, conservative levels of 60 g/kg in starter diets and 120–150 g/kg in growing and finishing diets for broilers are commonly used because WDDGS contains more fiber and less metabolizable energy than the original grain (Swiatkiewicz & Koreleski, 2008). Non-starch polysaccharides (NSPs) are poorly digested by nonruminant animals, which can reduce the diet's overall digestibility (Bederska-Łojewska *et al.*, 2017). The range of total NSP content of wheat DDGS is 216.8 to 253.9 g/kg DM (Whiting *et al.*, 2017). Furthermore, too much NSP can decrease the helpful intestinal microbiota and increase gut permeability (Tellez *et al.*, 2015). Although the microbiota can metabolize NSP, the intestinal microbiota of chickens is not as effective at fermenting NSP as other nonruminants (Jozefiak *et al.*, 2004). Diverse outcomes have been observed in studies where chickens were fed WDDGS diets. While most research on WDDGS feeding to broilers showed adverse effects, WDDGS may function as a prebiotic and

enhance gut health in theory (Whiting *et al.,* 2017). The presence of a significant portion of dead yeast cells in WDDGS, which are leftovers from the fermentation of yeast to produce alcohol, as well as the potential benefits of yeast and its derivatives for improving intestinal microbiota and immunity, nutrient digestibility, and feed efficiency in livestock animals, all lend credence to this theory (Vohra *et al.,* 2016). Rochell (2018) suggests that many advantages of feeding concentrated yeast cell wall components, such as nucleotides, mannan-oligosaccharides, and mannan-glucans, may also be inherent to WDDGS.

Due to the relatively high NSP content in WDDGS, there is considerable interest in adding different types of exogenous enzymes to broiler diets to improve growth performance, nutrient digestibility, and metabolizable energy (Swiatkiewicz *et al.,* 2014). Xylanase, β-glucanase, and β-mannanase are examples of exogenous enzymes that target these NSPs, and they are typically supplemented with WDDGS, even though the majority of its negative effects in chickens are caused by its high NSP concentration (Dal Pont *et al.,* 2020). It's interesting to note that adding NSP enzymes (NSPenz) to diets based on the USDA Dietary Guidelines has benefits beyond just reducing digesta viscosity and, in turn, diet digestibility. According to Aftab and Bedford (2018), the NSPenz's primary function is to modulate the gut microbiota. This is justified by the possibility that xylanases will produce short-chain xylans and xylo-oligosaccharides, which *Lactobacillus* and *Bifidobacterium* species can utilize and which function as prebiotics to modulate the enteric microbiota (Morgan *et al.,* 2019). As a result, adding NSPenz to WDDGS diets may have positive effects by lowering the amount of NSP in the chicken gut, generating compounds that resemble prebiotics, and possibly revealing the potential of the yeast and its derivatives to act on the intestine.

Although several scientific reviews report the benefits and challenges of using different types of enzymes in poultry diets (Swiatkiewicz *et al.,* 2016), most of these reviews focus on enzyme responses in poultry diets containing a wide range of ingredients rather than responses specific to diets containing WDDGS, have been concentrated. Therefore, this study aimed to investigate the effects of using different levels of WDDGS in feed with or without multienzyme on performance, ileum bacteria, intestinal viscosity and pH, hematology, and serum biochemistry of broiler chickens.

MATERIALS AND METHODS

Animal Ethics

This study was approved by the Animal Care Committee and Animal Research Ethics Board from the Department of Animal Science, Sari Branch, Islamic Azad University, Mazandaran, Iran (Approval No: IR.IAU.SARI.REC.1402.127).

Animals, Diets, and Experimental Design

This research used 300 one-day-old male and female broilers of Ross 308 strain with an average one-day weight of 42.18±0.31 g. This design was implemented as a factorial 3x2 with 6 treatments, 5 replications, and 10 chickens per replication (5 male and 5 female). Experimental treatments include 1) treatment without WDDGS and without multienzyme, 2) treatment containing 10% WDDGS and without multienzyme, 3) treatment containing 20% WDDGS and without multienzyme, 4) treatment without WDDGS and containing 125 g of multienzyme per ton, 5) treatment of 10% WDDGS and containing 125 g of multienzyme per ton, and 6) treatment of 20% WDDGS and containing 125 g of multienzyme per ton. First, the multienzyme was added to 1 kg of fine-ground soybean meal and homogenized with a mixer, and then the premix was added to the main mixture (Bozkurt *et al.,* 2014). The chemical compounds in WDDGS were measured using the AOAC method (2000). WDDGS used in this experiment has 86% dry matter, 3603 kcal/kg of metabolizable energy, 30.6% crude protein, 13.2% crude fat, 0.34% NDF, 5.3% ash, and 1.3% The percentage of starch. WUFFDA software was used to determine nutritional needs. The adjusted diets used in the starter (1-10 days), growth (11-24 days), and finisher (25-42 days) periods are presented in Table 1. During the entire breeding period, feed and water were freely provided to the birds. Breeding management programs, including light, ventilation, temperature, density, and substrate, were the same for all treatments.

Commercial Feed Multienzyme

The commercial multienzyme used was Apsazyme (multicarbohydrase complex). According to the manufacturer, the active enzymes in this product include 1100 units/g endo-β-glucanase, 1600 units/g endo-β-xylanase, 33 units/g α-galactosidase, and 450 units/g beta-mannanase.

Performance

Feed intake (FI) was provided to chickens daily after weighing. To calculate the FI of each repetition, the amount of feed remaining at the end of each breeding period was deducted from the total feed given during the period. To calculate the body weight gain (BWG) of each repetition in each period, the difference between the final weight and the beginning of the breeding period was determined. On days 1, 10, 24, and 42, all chickens of each experimental unit were weighed as a group. The feed conversion coefficient was calculated in different periods (starter, growth, and finisher periods). The feed conversion ratio (FCR) was calculated by dividing the average FI by the average BWG of chickens for each period. During the experiment, daily and before the allocation of feed, the mortality of each experimental pen was recorded and its weight was recorded. The daily mortality rate was used to determine the chicken day of each experimental unit (Awad *et al.,* 2018).

Table 1. Components and chemical compositions of the diets used in the starter, growth, and finisher periods of the experiment

Ingredients (%)	Treatments								
	Starter (1-10 d)			Grower (11-24 d)			Finisher (25-42 d)		
	0%	10%	20%	0%	10%	20%	0%	10%	20%
Corn	55.32	52.51	48.28	57.50	52.81	49.14	62.61	59.02	55.52
Soybean meal	39.28	32.00	26.20	36.31	30.29	24.5	30.98	24.50	18.00
Soybean oil	1.30	1.30	1.30	2.26	2.26	2.26	2.75	2.75	2.75
WDDGS	-	10.00	20.00	-	10.00	20.00	-	10.00	20.00
Dicalcium phosphate	1.55	1.55	1.55	1.44	1.44	1.44	1.30	1.30	1.30
Calcium carbonate	1.10	1.10	1.10	1.00	1.00	1.00	0.96	0.96	0.96
DL-Met (99%)	0.32	0.35	0.38	0.3	0.32	0.35	0.27	0.32	0.32
L - lysine 78%	0.24	0.30	0.30	0.18	0.25	0.30	0.19	0.21	0.21
L - threonine 99%	0.10	0.10	0.10	0.06	0.06	0.06	0.04	0.04	0.04
Vitamin and mineral premix†	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
NaCl	0.29	0.29	0.29	0.45	0.45	0.45	0.40	0.40	0.40
Chemical composition									
ME (kcal/kg)	2800	2800	2800	2850	2860	2860	2930	2940	2940
Crude protein (%)	22.00	22.00	22.00	21.00	21.00	21.00	19.00	19.00	19.00
Dig. Lysine (%)	1.30	1.30	1.30	1.24	1.24	1.24	1.12	1.12	1.12
Dig. Met + Cys (%)	0.98	0.97	0.97	0.93	0.93	0.93	0.86	0.86	0.86

Note: †Provides the following per kg of diet: 4.13 mg retinol, 60.00 µg chole-calciferol, 30.00 mg Dl-α-tocopherol, 3mg menadione, 2.20 mg thiamine, 8.00 mg riboflavin, 5.00 mg pyridoxine, 11.00 µg cyanocobalamin, 1.50 mg folic acid, 150.00 µg biotin, 65.00 mg nicotinic acid. Provides the following per kg of diet: 60.00 mg Mn (manganese sulfate), 40.00 mg Zn (zinc oxide), 0.33 mg I (potassium iodate), 80.00 mg Fe (ferrous sulfate), 8.00 mg Cu (copper sulfate), 0.15 mg Se (sodium selenite).

Ileum Bacteria

On the 42nd day of the experiment, sampling of the contents of the ileum of 2 birds in each repetition was done in sterile containers, and immediately, the samples were frozen at 80 °C and transferred to the laboratory. The dilution method, microbial culture, and colony counting were used to count the number of bacteria. Approximately 1 gram of contents from the ileum was diluted and homogenized in 9 mL of 0.9% saline solution in each repetition. To determine total aerobe, coliform, and lactobacillus counts, dilutions of 10^{-6}, 10^{-7}, and 10^{-8} were inoculated onto selective agar media (100 µL of each dilution). Nutrient Agar, MRS Agar, and Eosin Methylene Blue Agar (EMB) were used to cultivate aerobic bacteria, lactobacilli, and coliforms, respectively. After cultivation, the EMB medium was incubated for 48 hours under aerobic conditions and the MRS agar medium was incubated for 72 hours under anaerobic conditions at 37 °C (Mingan, 2010).

Intestinal Viscosity and pH

Two broiler birds from each replicate were slaughtered to collect digesta and determine the viscosity and pH of samples. The small intestine was divided into 3 parts: duodenum (duodenal loop), jejunum (duodenal loop to Meckel's diverticulum), and ileum (Meckel's diverticulum to ileal-cecal junction), and the contents of the duodenum, jejunum, ileum, and cecum were collected to measure viscosity. About 1.5 g of the contents of the duodenum, jejunum, ileum, and cecum were divided into 2 sub-samples, placed in a microtube, and centrifuged in a centrifuge at 12700 rpm for 5 minutes. About 0.5 mL of supernatant was removed and viscosity was measured by Brookfield digital viscometer (model I-LVDV) (Smits *et al.*, 1997). To check the pH of different parts of the intestine, 1 g of the contents of the duodenum, jejunum, ileum, and cecum were collected and poured with 9 mL of deionized water. Then, the pH of the different sections was measured using a pH meter (Metrohm 747) (Pang & Applegate, 2007).

Blood Variables of Blood Profile

The same two birds' blood samples (5 mL) were taken from the wing vein on each repetition when they were 42 days old. Serum was extracted from blood samples by centrifuging them for ten minutes at 4 °C and 3500 ×g. Blood samples were sent to the laboratory and kept cold until further examination. An autoanalyzer (Abbott Laboratories, Illinois, US) was used to measure blood variables such as triglyceride, cholesterol, high-density lipoprotein (HDL), total protein, albumin, and the activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes in blood serum using commercial enzyme kits (Pars Azmoun kits, Pars Azmoun Company, Tehran, Iran).

After blood sampling, blood samples were prepared from the blood of chickens and stained by the Giemsa method, and the ratio of heterophil to lymphocyte was measured as a stress index by the method of Kaab *et al.* (2018).

Statistical Analysis

Data analysis was done using SAS 9.4 statistical software (SAS, 2009) in the form of a factorial design. The significance of the differences between the mean data was evaluated using Tukey's comparative test

at the 5% level. The statistical model used in the experiment was as follows:

$$Y_{ijk} = \mu + A_i + B_j + AB_{ij} + e_{ijk}$$

Y_{ijk} was the value of each observation, μ was the mean of observations, A_i was the multienzyme effect, B_j was the diet effect, AB_{ij} was the interaction between multienzyme and diet, and e_{ijk} was the residual effect (experimental error).

RESULTS

Broiler Performance

The main and interactive effects of using different levels of WDDGS in feed with or without multienzyme on FI, BWG, and FCR of broilers are shown in Table 2. According to the obtained results, FI was significantly affected by the level of WDDGS in the finisher period and the average of the total period. Thus, the use of a 20% level of WDDGS FI decreased compared to the control treatment ($p<0.05$). FI was not affected by the multienzyme supplement, but it should be noted that the use of the multienzyme caused a non-significant increase in FI ($p<0.05$). BWG of the total period was significantly affected by the WDDGS level. So, using a 20% WDDGS level decreased BWG compared to a 10% WDDGS treatment ($p<0.05$). BWG was not affected by multienzyme supplementation ($p>0.05$). FCR was affected by the interaction effect of multienzyme and WDDGS level and was significantly reduced in birds fed with 125 g/t of multienzyme diet ($p<0.05$). FCR was not affected by the WDDGS level in the finisher period and the interaction effect of multienzyme and WDDGS levels in different breeding periods. Meanwhile, the FCR of an average of the total period ($p=0.04$) was higher in broilers fed with a diet containing 20% WDDGS.

Ileum Bacteria

Table 3 shows the effect of using different levels of WDDGS in feed with or without multienzyme on the ileum microbiota of broiler chickens. According to the results, the number of lactic acid bacteria was affected by the multienzyme level and the use of multienzyme in a broiler diet increased the number of these bacteria in the ileum ($p<0.05$). The population of total bacteria, coliform, and lactic acid was affected by the level of WDDGS. In this way, the total bacteria and coliform were higher in broiler chickens fed with 10% and 20% WDDGS diet than in the control treatment ($p<0.05$). Also, the number of lactic acid bacteria in the 20% WDDGS treatment was significantly lower than the control and 10% WDDGS treatments ($p<0.05$).

Intestinal Viscosity and pH

The main and reciprocal effects of using different levels of WDDGS in the diet with or without multienzyme on the viscosity and pH of the intestines of broilers are presented in Table 4. Statistical analysis showed that the viscosities of the duodenum, jejunum, and ileum are affected by the multienzyme level and the viscosities of the duodenum and ileum is affected by the level of WDDGS in the diet ($p<0.05$). The use of multienzyme in the diet significantly reduced the duodenum, jejunum, and ileum viscosities. Also, broilers fed with a diet containing 20% WDDGS had

Table 2. Performance of broiler chickens fed different levels of wheat distillers dried grains with solubles (WWDGS) with multienzyme supplementation

Variables / Treatments		FI (g/d)				BWG (g/d)				FCR			
		Starter	Grower	Finisher	Total	Starter	Grower	Finisher	Total	Starter	Grower	Finisher	Total
Enzymes													
No		22.25	79.39	155.55	96.28	20.93	58.28	79.72	58.57	1.07	1.36	1.95[a]	1.68
Yes		21.54	80.07	150.84	98.60	20.60	59.28	82.83	60.16	1.05	1.35	1.82[b]	1.60
SEM		0.42	1.07	2.27	1.14	0.38	1.01	1.33	0.47	0.03	0.02	0.04	0.02
p-value		0.28	0.61	0.28	0.45	0.57	0.57	0.98	0.55	0.74	0.59	0.02	0.17
WDDGS													
0%		21.85	77.80	160.11[a]	99.75[a]	20.63	57.5	84.22	61.33[a]	1.06	1.35	1.94	1.62
10%		21.98	82.36	153.44[ab]	98.44[ab]	20.86	61.64	83.77	61.41[a]	1.06	1.33	1.83	1.60
20%		21.9	79.31	147.22[b]	94.75[b]	20.86	57.42	80.5	58.60[b]	1.05	1.38	1.82	1.61
SEM		0.51	1.35	2.05	1.40	0.47	1.21	1.61	0.57	0.03	0.02	0.04	0.03
p-value		0.98	0.12	0.04	0.03	0.93	0.06	0.63	0.04	0.98	0.06	0.21	0.34
WDDGS	Enzymes												
0%	No	20.87	77.48	165.83	101.86	19.47	57.71	83.55	60.39	1.07	1.34	2.05	1.68[a]
0%	Yes	22.64	78.09	154.38	97.58	21.56	57.35	85.88	62.19	1.05	1.36	1.84	1.56[b]
10%	No	21.92	81.41	155.27	98.90	20.01	61.42	79.22	59.18	1.10	1.32	1.95	1.67[a]
10%	Yes	22.05	83.32	151.66	98.02	21.72	61.85	84.38	61.95	1.02	1.34	1.79	1.58[b]
20%	No	21.74	77.29	150.01	95.22	20.62	55.35	77.5	56.57	1.05	1.39	1.93	2.68[a]
20%	Yes	22.11	81.33	145.00	94.51	21.10	59.50	81.05	59.59	1.05	1.36	1.78	1.58[b]
SEM		0.72	1.92	3.33	1.97	0.66	1.71	2.27	0.8	0.05	0.03	0.06	0.02
p-value		0.98	0.41	0.32	0.63	0.05	0.41	0.45	0.29	0.64	0.38	0.19	0.01

Note: FI: feed intake; BWG: body weight gain; FCR: feed conversion ratio; WDDGS: wheat distillers dried grains with solubles; Starter: 1-10 d; Grower: 11-24 d; Finisher: 25-42 d; Total: 1-42 d; No: Without multienzyme; Yes: 125 g/ton multienzyme; SEM: standard error of means.
[a-b] Means within a variable with no common superscript differ significantly ($p<0.05$).

higher viscosities in the duodenum and ileum than those treated with 0% and 10% WDDGS (p<0.05). Duodenum and ileum viscosities were affected by the interaction effects of WDDGS level and multienzyme (p<0.05) and multienzyme supplement could significantly reduce the viscosity caused by 20% WDDGS level. According to the obtained results, the pH of jejunum and ileum were affected by the multienzyme

Table 3. Ileum bacteria of broiler chickens fed different levels of wheat distillers dried grains with solubles (WWDGS) with multienzyme supplementation

Treatments \ Variables		Total aerobes (log_{10} cfu/g)	Coliform (log_{10} cfu/g)	Lactic acid (log_{10} cfu/g)
Enzymes				
No		8.24	7.78	7.56[b]
Yes		8.29	7.85	7.81[a]
SEM		0.02	0.01	0.02
p-value		0.87	0.53	0.03
WDDGS				
0%		8.03[b]	7.63[b]	7.94[a]
10%		8.37[a]	7.89[a]	7.91[a]
20%		8.28[a]	7.92[a]	7.63[b]
SEM		0.02	0.02	0.02
p-value		0.04	0.02	0.01
WDDGS	Enzymes			
0%	No	8.03	7.59	7.91
0%	Yes	8.01	7.66	7.9
10%	No	8.24	7.86	7.92
10%	Yes	8.34	7.92	7.89
20%	No	8.3	7.89	7.61
20%	Yes	8.25	7.95	7.65
SEM		0.04	0.03	0.04
p-value		0.11	0.99	0.66

Note: WDDGS: wheat distillers dried grains with solubles; No: Without multienzyme; Yes: 125 g/ton multienzyme; SEM: standard error of means.
[a-b] Means within a variable with no common superscript differ significantly (p<0.05).

Table 4. Intestinal viscosity and pH of broiler chickens fed different levels of wheat distillers dried grains with solubles (WWDGS) with multienzyme supplementation

Variables / Treatments		Viscosity (cps)				pH			
		Duodenum	Jejunum	Ileum	Cecum	Duodenum	Jejunum	Ileum	Cecum
Enzymes									
No		1.35[a]	1.91[a]	2.84[a]	3.22	5.76	6.19[a]	6.47[a]	6.64
Yes		1.20[b]	1.30[b]	1.76[b]	3.08	5.68	5.87[b]	6.24[b]	6.62
SEM		0.02	0.11	0.11	0.13	0.04	0.02	0.02	0.12
p-value		0.001	0.007	0.005	0.49	0.29	<0.001	0.001	0.81
WDDGS									
0%		1.23[b]	1.41	2.04[b]	2.87	5.77	5.85[b]	6.3	6.66
10%		1.29[b]	1.53	1.99[b]	3.12	5.63	6.08[a]	6.42	6.59
20%		1.57[a]	1.87	2.84[a]	3.46	5.77	6.15[a]	6.35	6.65
SEM		0.02	0.13	0.14	0.16	0.06	0.02	0.12	0.18
p-value		0.002	0.12	0.009	0.11	0.25	0.004	0.12	0.77
WDDGS	Enzymes								
0%	No	1.27[bc]	1.62	2.67[ab]	2.99	5.8	6.15[ab]	6.47	6.7
0%	Yes	1.20[bc]	1.21	1.41[c]	2.76	5.74	5.56c	6.13	6.62
10%	No	1.51[b]	1.8	2.18[bc]	3.21	6.67	6.16[ab]	6.35	6.60
10%	Yes	1.06[c]	1.27	1.80[bc]	3.03	5.6	6.01[b]	6.32	6.59
20%	No	1.81[a]	2.32	3.67[a]	3.45	5.83	6.27[a]	6.43	6.63
20%	Yes	1.34[bc]	1.42	2.01[bc]	3.46	5.72	6.04[b]	6.27	6.66
SEM		0.03	0.19	0.20	0.22	0.16	0.13	0.38	0.17
p-value		0.002	0.45	0.04	0.85	0.95	0.02	0.24	0.86

Note: WDDGS: wheat distillers dried grains with solubles; No: Without multienzyme; Yes: 125 g/ton multienzyme; SEM: standard error of means.
[a-b] Means within a variable with no common superscript differ significantly (p<0.05).

level, and the use of multienzyme supplements in the diet of broilers decreased the pH of jejunum and ileum ($p<0.05$). The level of WDDGS in the diet also significantly affected jejunum pH ($p<0.05$). Thus, the use of 10% and 20% WDDGS in the diet increased the pH of the jejunum compared to the control treatment ($p<0.05$). The interaction between WDDGS and multienzyme levels significantly affected jejunum pH, and using multienzyme at 0% and 20% WDDGS levels significantly reduced jejunum pH.

Blood Profile

The effects of using different levels of WDDGS in feed with or without multienzyme on the blood parameters of broilers are reported in Table 5. Statistical analysis showed that albumin was affected by the level of WDDGS and the use of 20% WDDGS in the diet increased the serum albumin concentration compared to the levels of 10% and 0% WDDGS ($p<0.05$). Other blood parameters were not affected by multienzyme level, WDDGS level, and interaction between WDDGS level and multienzyme ($p>0.05$).

Blood Hematology

Table 6 shows the effect of using different levels of WDDGS in feed with or without multienzyme, on heterophil concentration, lymphocyte, and heterophil to lymphocyte ratio in broilers. According to the results, the parameters mentioned were not affected by the multienzyme level or WDDGS level and the interaction effect of the WDDGS level and multienzyme ($p>0.05$).

DISCUSSION

Broiler Performance

The FI results showed that the use of 20% WDDGS level in the finisher period and the average of the total FI period decreased compared to the control treatment, but it improved the FI multienzyme insignificantly. Performance was not affected by the interaction of WDDGS and multienzyme during different experimental periods. These outcomes agree with what Dal Pont *et al.* (2023) reported. When Anwar *et al.* (2023) looked into how an enzyme affected broiler diets containing wheat, they found that the enzyme only impacted FI during the growth phase, which led to an increase in it. The amount of NSPs, particularly β-glucans and arabinoxylans, in WDDGS, has been reported to be the cause of its decreased nutritional value for consumption by poultry. Dissolving these polysaccharides causes the viscosity of digestible materials to rise, weakening nutrient absorption and digestion and lowering FI. However, some pancreatic enzymes, such as amylase, are found in lower concentrations in the intestinal tract when viscosity increases. Polysaccharides also slow down or block the entry of enzymes to starch and protein within the cell (Kim *et al.*, 2021). Therefore, it can be stated that the use of 20% WDDGS reduced feed consumption by increasing viscosity and reducing feed passage velocity, but the 10% level did not have a negative effect due to the lower NSP content. Enzymes improve the digestibility of nutrients and FI and decrease the viscosity of digestible materials in diets containing

Table 5. Blood variables of broiler chickens fed different levels of wheat distillers dried grains with solubles (WWDGS) with multienzyme supplementation

Treatments \ Variables		Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	AST (U/L)	ALT (U/L)
Enzymes								
No		109.87	97.53	70.47	4.63	1.39	227.87	8.73
Yes		113.27	103.07	71.06	4.43	1.38	221.93	8.13
SEM		3.29	4.33	2.04	0.07	0.07	9.76	0.34
p-value		0.47	0.37	0.85	0.07	0.92	0.67	0.22
WDDGS								
0%		116.82	98.70	70.84	4.62	1.34[b]	230.74	8.79
10%		110.12	104.32	72.46	4.57	1.42[ab]	231.24	8.94
20%		107.80	98.27	69.58	4.46	1.60[a]	212.87	7.76
SEM		4.03	5.32	2.53	0.09	0.08	11.96	0.42
p-value		0.28	0.69	0.63	0.24	0.02	0.47	0.11
WDDGS	Enzymes							
0%	No	108.64	97.48	70.82	4.84	1.34	219.85	8.23
0%	Yes	125.46	100.26	70.8	4.44	1.34	241.64	9.27
10%	No	110.57	97.83	71.48	4.66	1.46	233.64	9.25
10%	Yes	110.26	110.25	73.42	4.48	1.38	228.89	8.63
20%	No	111.95	97.43	69.25	4.42	1.57	230.24	8.84
20%	Yes	104.64	99.83	68.84	4.38	1.72	195.47	6.64
SEM		5.69	7.49	3.54	0.12	0.11	16.97	0.59
p-value		0.14	0.73	0.94	0.45	0.85	0.26	0.05

Note: WDDGS: wheat distillers dried grains with solubles; No: Without multienzyme; Yes: 125 g/ton multienzyme; SEM: standard error of means.
[a-b] Means within a variable with no common superscript differ significantly ($p<0.05$).

Table 6. Blood hematology of broiler chickens fed different levels of wheat distillers dried grains with solubles (WWDGS) with multienzyme supplementation

Variables / Treatments		Heterophil (%)	Lymphocyte (%)	Heterophil/lymphocyte
Enzymes				
No		19.07	40.93	0.47
Yes		19.33	40.67	0.48
SEM		0.72	0.73	0.03
p-value		0.79	0.75	0.80
WDDGS				
0%		18.56	41.53	0.45
10%		19.41	40.63	0.48
20%		19.74	40.32	0.49
SEM		0.88	0.88	0.03
p-value		0.18	0.61	0.62
WDDGS	Enzymes			
0%	No	19.42	41.13	0.46
0%	Yes	18.24	42.83	0.43
10%	No	18.42	41.62	0.44
10%	Yes	20.42	39.67	0.51
20%	No	19.80	40.23	0.52
20%	Yes	19.64	40.43	0.48
SEM		1.02	1.24	0.04
p-value		0.23	0.47	0.51

Note: WDDGS: wheat distillers dried grains with solubles; No: Without multienzyme; Yes: 125 g/ton multienzyme; SEM: standard error of means.
[a-b] Means within a variable with no common superscript differ significantly ($p<0.05$).

NSP (Morgan *et al.*, 2022). The diets containing the multienzyme in this study showed higher FI, even though the multienzyme had no discernible impact on the average FI over the entire period. This may be because the amount of NSP that was ingested was probably insufficient to produce a viscosity that was sufficiently strong to impede the action of the multienzyme and the passage of digestible materials through the gastrointestinal tract, resulting in a decrease in FI in the diet that contained multienzyme.

According to the BWG results, WDDGS had a significant impact on the mean of this trait over the entire period, and including 20% WDDGS in the diet decreased the amount of BWG. The high fiber content of this diet and the increased viscosity of the intestinal contents may be the cause of the low average BWG in the 20% WDDGS treatment. Increased and more intense bowel movements are the result of feedings that stay in the gastrointestinal tract longer. Following that, the intestine secretes fatty acids, protein, water, and minerals, and the growth of the bird is inhibited by the loss of these materials (Adedokun & Olojede, 2019). However, as shown in Table 4, the 10% WDDGS level did not increase intestinal viscosity and subsequently did not have a negative effect on BWG. The presence of NSPs lowers nutrient absorption and bird performance by causing viscous conditions in the small intestine; however, exogenous enzyme supplements can help by hydrolyzing NSPs, reducing viscosity, and improving nutrient absorption (Polovinski-Horvatović, 2021).

FCR increased with increasing WDDGS level. The study's outcomes align with the conclusions made by other investigators (Adebiyi & Olukosi, 2015). Enzyme supplements significantly improved FCR. According to Jabbar *et al.* (2021), adding enzymes to the diet increases the amount of starch, protein, and fat that is absorbed and digested, which improves FCR. According to reports, the enzyme breaks down NSPs in wheat to improve nutrient digestion and FCR (Smeets *et al.*, 2018). Although young chickens cannot digest NSPs, most studies found that chickens fed with enzyme supplements responded better during the starter and growing period. This suggests that while enzymes can break down polysaccharides, they can also improve performance by using nutrients better. Zhou *et al.* (2009) suggested that multi-enzyme complex supplementation (protease, xylanase, and amylase) increased metabolizable energy utilization in broilers, which could be a possible reason for the improved FCR. However, Kiarie *et al.* (2014) reported that xylanase improved FCR in corn and wheat-based diets, indicating the degradation of soluble and insoluble NSPs. Therefore, the researchers of the present study believe that better energy utilization due to the addition of multienzyme caused a relative decrease in FI and a relative increase in BWG, ultimately leading to a significant improvement in FCR.

Based on reports from Daymeh *et al.* (2016) and Gao *et al.* (2022), the performance with enzyme treatments was anticipated to improve. Due to the breaking of the transverse bonds between the units forming the arabinoxylan chains in wheat and the opening of the phytate complex bonds in the structure of these items, more energy is released, nutrients (protein, starch, and fat) and minerals (calcium and phosphorus) become available and used in the process of better absorption and eventually achieve more growth (Daymeh *et al.*, 2016; Malekzadeh and Shakouri, 2016). Furthermore,

combining enzymes in poultry feed enhances nutrient digestion compared to their independent state, per the report of Alagawany *et al.* (2018). Consequently, diets containing WDDGS should be supplemented with the appropriate enzymes to remove the negative effects of the presence of NSPs or fiber in the diet, restore the physicochemical balance of the gastrointestinal tract, and ultimately improve performance traits to achieve maximum efficiency and growth (Malekzadeh & Shakouri, 2016).

Ileum Bacteria

Table 3's findings indicate that broilers given a 10% or 20% WDDGS diet had higher total aerobes bacteria than those given a control treatment. The coliform bacteria population exhibited a similar trend, with the enzyme-supplemented treatments and the WDDGS-free control treatment having a smaller population than the treatments containing 10% and 20% WDDGS. However, there was a notable difference in the number of beneficial lactic acid bacteria in the enzyme-treated treatments. Based on these findings, it was discovered that replacing corn with WDDGS alters the overall population, reducing the number of beneficial microorganisms while concurrently increasing the number of undesirable microorganisms. However, supplementing diets with multienzyme supplements along with adjusting the level of NSPs in diets caused the negative effect to be eliminated and the microbial population of lactic acid to increase. Growing the population of the aforementioned bacteria enhances gastrointestinal health, lowers pathogenic bacteria, and improves the growth performance of chickens because they are important for intestinal lymphoid cell-dependent immune system stimulation (Mazanko *et al.*, 2022). The microbial population in the gastrointestinal tract changed when raw barley and barley heated with enzymes were fed, according to Itani *et al.* (2025). Changes in the gastrointestinal microbiota after substituting corn for barley, wheat, bran, or other NSP sources were reported in several studies, both with and without enzyme supplements (Aderibigbe *et al.*, 2024).

Enzymes like xylanase and β-glucanase are frequently utilized to enhance chicken performance and lower feed expenses. Reduction of intestinal viscosity, hydrolysis of specific chemical bonds in feed, elimination of the cell wall's nutrient-encapsulating effect, and breakdown of antinutritional agents present in many feeds are some of the proposed mechanisms, though their exact mechanism of action is unknown (Kim *et al.*, 2024). Because feed enzymes alter the intestinal contents, they can also have an impact on the microbial population (Ghayour-Najafabadi *et al.*, 2018; Kim *et al.*, 2024). It seems that high-nutrient diets are less sensitive to enzymes. For example, although xylanases are useful in corn-based diets, lower response rates have been reported than in wheat-based diets due to large differences in the concentrations of corn and wheat NSPs (Ghayour-Najafabadi *et al.*, 2018). The efficiency of the enzyme in decreasing the viscosity of the wheat-based diet was significantly higher in Munyaka *et al.* (2016) study when compared to the corn-based diet, suggesting that the enzyme's effect increased with the amount of intestinal viscosity the diet produced.

The gastrointestinal tract is widely regarded as an important "organ" because it contains a complex micro-ecosystem of numerous bacteria. Through facilitating nutrient absorption and digestion, blocking pathogen colonization, and establishing and preserving appropriate mucosal immunity, the intestinal microbial community contributes significantly to host nutrition and health. Apart from their well-known probiotic function, lactobacilli possess several other biochemical characteristics, such as the generation of antibacterial and bile salt hydrolase compounds (Wang *et al.*, 2021). Although it has been demonstrated that Clostridium-associated bacteria produce short-chain fatty acids in the gut, they may also play a significant role in colonization resistance by serving as a barrier to prevent the invasion of other potentially harmful microbes (González-Ortiz *et al.*, 2020). Increased bacterial capacity in the gut of broilers fed a diet based on wheat and rye was reported by Pyzik *et al.* (2021). The authors claim that xylanase changes the concentration of microbes in the proximal small intestine, and the proportion of NSPs-degrading bacteria normally found in the cecum also increases in the ileum. Therefore, more of these bacteria in the proximal intestine may also ferment the available carbohydrates. The addition of NSP-hydrolyzing enzymes to several cereal-based diets has been shown to improve starch digestibility (Guan *et al.*, 2021). Therefore, the drop in the amount of carbohydrates in this section may be the cause of the bacteria decrease in the cecum as opposed to the ileum.

Molecular approaches have shown that gut microbial population composition is altered by feed additives (Giannenas *et al.*, 2018). High intestinal viscosity cereals have been demonstrated to enhance the total activity of bacteria in the small intestine, the majority of which are believed to compete with one another for nutrients and to harm the host, but xylanase supplementation of the diet A diet high in wheat or rye lowers the overall amount of bacteria in the small intestine to a partial extent (Pyzik *et al.*, 2021). By lowering the overall microbial load in terms of bacterial, metabolic, and bile counts, enzyme supplementation may cause beneficial bacteria to proliferate in birds and reduce the bacterial load. Exogenous enzymes decrease intestinal viscosity and promote the growth of more intra- and intra-bacterially competitive bacterial communities, which limits bacterial interference with nutrient absorption and may reduce the potential pathogenic population (Anderson *et al.*, 2023). Microbial populations in birds can also be impacted by dietary factors. Indeed, Sadati *et al.* (2022) observed higher numbers of Escherichia coli and lactobacilli in the gut of broilers fed wheat than in corn. Variations in the gut bacteria of chickens can modify the mucosal structure and consequently impact the ability to absorb nutrients. This could account for some of the variation in body weight gain seen in birds given multienzyme supplements.

Intestinal Viscosity and pH

Viscosity of intestinal contents is the limiting factor of poultry performance. An increase in the viscosity of the solution at the end of the small intestine causes a decrease in the rate of feed passage an increase in the population of pathogenic microbes (the terminal part of the ileum and cecum) and a decrease in the absorption of nutrients and impaired growth (Ayres *et al.,* 2019). In the present study, the use of 20% WDDGS increased the viscosity of the duodenum and ileum, which increased the total population of aerobic bacteria and coliform (Table 3) and ultimately decreased the growth performance (Table 2). An increase in the viscosity of the digestive material following the consumption of wheat in poultry has been reported (Barasch & Grimes, 2021). When polysaccharides are dissolved in water, they create viscous solutions. This viscosity property of NSPs is the first mechanism by which the access of digestive enzymes to nutrients is reduced (Barasch & Grimes, 2021). It has also been reported that increased digestive viscosity minimizes the ability of intestinal contents to mix, a process critical for micelle formation and absorption of fat and fat-soluble nutrients (Michels *et al.,* 2025). According to Arczewska-Wlosek *et al.* (2019), broilers fed diets high in rye experienced a decrease in intestinal viscosity following xylanase supplementation. This decrease in intestinal viscosity was linked to an increase in intestinal digestion and absorption. According to research by Kouzounis *et al.* (2021), arabinoxylan fermentation was enhanced when xylanase was added to their wheat-based diets. According to research by Khose *et al.* (2020), adding enzyme supplements to diets that contain soluble dried sorghum grains (sDDGS) dramatically decreased the number of insoluble NSPs and raised the number of free sugars (xylose and arabinose) in the intestinal digesta. According to that study, the birds' access to these free sugars may have given them the nutrition they needed, improving their FCR. According to this study, the main way that enzyme supplementation helps is by reducing viscosity, with the release of sugars coming in second (Vandeplas *et al.,* 2010). Exogenous enzymes release monosaccharides via two different mechanisms: first, they break down NSPs, which deliberates the release of monosaccharides; second, they break down NSPs within the endosperm, exposing the starch to the endogenous amylase and releasing additional glucose (Van Hoeck *et al.,* 2021).

Previous research has demonstrated that gastrointestinal pH may be affected differently by multienzyme supplementation in wheat-based diets. According to Van Hoeck *et al.* (2021), xylanase had no effect on the pH of any digestive tract segments. In other studies, Sozcu (2019) demonstrated that the enzyme caused the pH of the duodenum, jejunum, and cecum to drop in broilers given a diet high in wheat. Our findings demonstrated that the duodenum, jejunum, and ileum, the main sites of nutrient digestion and absorption, are the locations where the pH decrease brought on by enzyme supplementation occurs. Lowering intestinal pH has been shown to enhance pancreatic secretion of digestive enzymes and bile acid sequestration for lipid emulsification, thereby improving nutrient absorption and digestion. One possible explanation for the impact of enzyme supplementation on intestinal pH is its indirect influence on intestinal microbiota. According to Simic *et al.* (2023), supplementing with xylanase increased the amount of lactic acid in the intestinal contents. The abundance of *Lactobacillus* species and other beneficial intestinal microbiota may be correlated with lactic acid production. Another study (Gao *et al.,* 2020) demonstrated that lactobacilli grew more rapidly when they were supplemented with enzymes. Thus, it is reasonable to assume that a similar mechanism was responsible for the pH drop seen in this study.

Blood and Hematology Variables

Serological analyses are a way to verify the bird's health conditions and may indicate possible changes in the physiological system besides influences on management, weather conditions, and animal nutrition (Minafra *et al.,* 2015). There was a linear increase in Albumin concentration with DDGS; however, all values for this variable are within a normal range, which, according to Schmidt *et al.* (2007), is between 0.8 to 2.0 g/dL in healthy birds. Albumin is a metabolite indicative of protein synthesis and may make up as much as 50% of the total serum protein in birds. It is responsible for transporting various nutrients such as calcium, zinc, magnesium, copper, fatty acids, and, in the case of birds, specifically, thyroid hormones (Maciel *et al.,* 2007). Gupta *et al.* (2017) reported that 10% rice DDGS significantly increased serum albumin, which is consistent with our results. Broiler chickens' lower blood serum ALT concentration suggests that there is no liver or tissue damage, as the measured values fall within the range considered normal for the majority of bird species (19 to 50 IU/L) (Schmidt *et al.,* 2007). The experimental treatments did not affect other blood parameters. A study by Dinani *et al.* (2019) found that there was no statistically significant variation in the serological parameters (globulin, serum SGOT, SGPT, and ALP enzyme activity, total protein, and triglyceride) when the diet was supplemented with or without enzymes in diets containing varying levels of rice DDGS compounds. Our findings corroborated those of Kumar *et al.* (2016), Wani *et al.* (2017), and other researchers who found no discernible impact of distilled grains on lipid metabolites. In line with our findings, researchers (Dinani *et al.,* 2019) did not find a statistically significant difference in the proportion of lymphocytes and heterophils between the control group and various DDGS concentrations with or without enzymes, as well as their interactions. Every parameter under investigation fell within the typical physiological range. Therefore, it can be concluded that the inclusion of WDDGS up to the level of 20% in the diet of broiler chickens had no adverse effect on the biochemical composition of the blood of experimental birds.

CONCLUSION

In general, the use of WDDGS up to 10% in the broiler diet did not have a negative effect on performance, and due to its reasonable price, it can be considered in the broiler diet. Increasing the level of WDDGS to 20% negatively affected the performance, and adding multienzyme to the diet containing WDDGS reduced these negative effects to a certain extent and improved the FCR of broiler chickens.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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