



Research Article

Effect of Using Corn DDGs Fortified with Xylanase and Mannanase Enzymes in Broiler Ration on Growth Performance, Nutrients Digestibility and Economics

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Abstract | Poultry feed often includes Distiller's Dried Grain with solubles (DDGS) as a source of protein and energy. However, DDGS is high in non-starch polysaccharides (NSPs), which can harm broiler performance and digestibility. This study aimed to counteract these negative effects by adding Xylanase and Mannanase enzymes to DDGS-based broiler feed, aiming to minimize the impact of NSPs and improve overall performance. The experiment involved 300 one-day-old chicks. After a one-week acclimation period, they were divided into five equally sized groups (D1 to D5). D1 served as the control group, receiving only the basal diet. The remaining groups (D2-D5) received feed containing 10% DDGS. Group D3 was supplemented with 1g/kg Xylanase, group D4 received 1g/kg Mannanase, and group D5 got a 2g/kg blend of both enzymes. All groups had access to feed and water freely throughout the 42-day trial. Results indicated that chicks fed with exogenous enzymes showed significant improvements ($P < 0.05$) in growth and nutrient digestion compared to the control D1 and other treatment group D2. Groups receiving both enzymes (D5) showed the best growth and nutrient digestibility compared to individual enzyme groups (D3 and D4) and the control group. This translated to significantly improved ($P < 0.05$) economic returns, with D5 achieving the highest gross margin. It was concluded that dietary supplementation of Xylanase and Mannanase enzymes at the rate of 1g/kg each having 10% DDGS in ration not only significantly mitigated the negative impact of DDGS in broiler feed but also improved the bird's performance and profitability.

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Keywords | Distiller's dried grain with soluble, Xylanase, Mannanase, Nutrients digestibility, Economic return



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Introduction

The thriving global poultry industry demands a steady supply of nutrients, energy, proteins,

essential amino acids, minerals, and vitamins to optimize bird performance and ensure smooth operation. Feed alone accounts for roughly 70% of production costs, posing a significant challenge in

developing countries due to the high prices of key ingredients like soybean and corn (Sihag *et al.*, 2018; Abd El-Hack *et al.*, 2015; Alagawany and Attia, 2015). Therefore, seeking cost-effective alternative ingredients and byproducts to replace these expensive staples fully or partially has become crucial. Recognizing this need, researchers in developing countries have increasingly turned to the exploration of unconventional feed ingredients for poultry diets (Alagawany and Attia, 2015; Alagawany *et al.*, 2017).

Distiller Dried Grains with soluble (DDGS) is the by-product of ethanol industry that can be utilized as protein source in poultry feedstuff. It is produced after fermentation of starch during ethanol production. In this process, the non-fermentable portion which is rich in nutrients such as fats, proteins, fiber, minerals, and vitamins is retained in high concentration as distiller dried grains with soluble (Sihag *et al.*, 2018). (Depending upon the technology used for production dried distillers are obtained in many forms such as dried distiller grain (DDG), dried distiller grain with soluble (DDGS) and high protein dried distiller grain (HPDDG) as well as wet distiller grains (WDG) and wet distiller grains with soluble (WDGS) and high protein wet distiller grains (HPWDG) (Pecka-Kiełb *et al.*, 2017).

Non-conventional feed sources with low prices like DDGS are partially replacing the main ingredients of the diet but these cheaper feed ingredients have high percentage of NSPs along with the starch. They are polymeric carbohydrates and vary from starch in composition and structure (Morgan and Bedford, 1995) and hence cannot be completely digested by birds. (Adams and Pough, 1993, Alagawany *et al.*, 2017) A part of this NSPs is water soluble which forms viscous texture in the small intestine of bird and ultimately decrease the gut performance (Ward, 1995). Use of commercial enzymes in poultry diet has a great importance. Poultry does not produce enzymes that could hydrolyze the NSPs of cell wall and thus remain unbroken. This results in reduction in the efficiency of feed conversion. (Choct *et al.*, 1995) In order to overcome this problem, supplementation of exogenous enzymes in poultry diet helps in the breakdown of NSPs. This causes reduction in intestinal viscosity and better nutrients digestibility. (Amerah, 2015). These commercial enzymes disrupt the cell wall integrity and consequently release nutrients enclosed by cell wall. (Ravindran, 2013). Supplementation of

exogenous enzymes in DDGS based diets not only improve the production in poultry (Campasino *et al.* (2015), but it also improves broiler performance, protein, and other nutrients digestibility (Ludke *et al.*, 2018).

This study focuses on the potential of NSPases (Xylanase and Mannanase) in mitigating the negative effects of non-starch polysaccharides (NSPs) present in diets containing 10% DDGS. By adding these enzymes in both individual and mixed forms, we aim to break down DDGS-based NSPs, enhance nutrient utilization, and optimize broiler performance.

Materials and Methods

Location

This study was conducted at an open shed poultry farm maintained by the Poultry Science department of The University of Agriculture Peshawar.

Poultry house management

Prior to chick arrival, the poultry shed underwent thorough sanitation. Feeders and drinkers were washed with detergent and air-dried. Sawdust bedding was laid and covered with paper for optimal hygiene. A digital thermometer monitored temperature, maintaining it close to the recommended range. Regular cleaning practices ensured a favourable and hygienic environment for the chicks.

Source of birds and feed

Three hundred 300 one-day-old chicks were acquired from a commercial hatchery. Corn DDGS was sourced from Orminic, and exogenous enzymes were imported from China to formulate rations for the experimental groups.

Experimental design

Following a one-week adaptation period on a commercial ration, 300 chicks were randomly allocated to five experimental groups (D1-D5) with three replicates per group, housed in individual pens. Group D1 served as the control, receiving a basal diet. The remaining groups (D2-D5) were fed diets containing 10% DDGS supplemented with exogenous enzymes: D3 with 1 g/kg Xylanase, D4 with 1 g/kg Mannanase, and D5 with a 2 g/kg blend of both enzymes. Ad libitum access to feed and water was provided, and optimal environmental conditions were maintained throughout the 42-day trial to maximize bird performance.

Table 1: *Experimental layout of study*

Groups	DDGS in feed	Xylanase supplementation (g/kg)	Mannanase supplementation (g/kg)	Replicates			No. of chicks
				R1	R2	R3	
D1	0	0	0	20	20	20	60
D2	10%	0	0	20	20	20	60
D3	10%	1	0	20	20	20	60
D4	10%	0	1	20	20	20	60
D5	10%	1	1	20	20	20	60

Table 2: *Experimental feed composition and nutrients calculation.*

Ingredients Percentage	Ration with % of DDGS in Starter Phase		Ration with % of DDGS in Finisher Phase	
	Ration 1	Ration 2	Ration 1	Ration 2
Corn 12 % Moisture	59.30	50.82	66.29	57.81
SBM Hi Pro	35.06	32.87	27.58	25.40
Vegetable Oil	1.40	2.22	2.51	3.34
DDGS Corn	---	10.00	---	10.00
DCP	0.78	0.56	0.52	0.30
Salt	0.43	0.38	0.43	0.38
Sodium Bicarbonate	0.01	0.01	0.01	0.01
Limestone/Chips	1.85	1.98	1.65	1.78
Lysine Sulphate	0.40	0.40	0.35	0.35
DL-Methionine	0.33	0.33	0.30	0.30
L-Threonine	0.17	0.15	0.09	0.07
L-Tryptophan	0.01	0.01	0.01	0.01
L-Valine	0.02	0.02	0.02	0.02
Vit. Mix	0.10	0.10	0.10	0.10
Min. Mix	0.10	0.10	0.10	0.10
Enramycin 4%	0.01	0.01	0.01	0.01
Diclazuril 0.5 %	0.02	0.02	0.02	0.02
Choline Cl 70 %	0.08	0.08	0.07	0.07
Nutrients Calculation				
Dry Matter %	85.47	85.80	85.55	85.87
Crude Protein %	21.46	21.48	18.39	18.42
Crude Fat %	4.34	5.19	5.55	6.39
Crude Fibre %	2.85	3.20	2.68	3.02
Ash %	5.50	5.61	4.76	4.87
M.E (Kcal/Kg)	2950	2950	3100	3100

For each kg ration: vit. A=7.5 MIU, Vit. D3=1.5MIU, vit. E= 2500mg, vit. K3=750mg vit. B1=1000mg, vit. B2=1500mg, vit. B6=1000mg, vit. B12= 6000mcg, Nicotinic Acid= 7500mg, Pantothenic Acid= 4000mg, folic acid= 300mg, biotin= 5000mcg, Cu=1500mg, Se= 50mg, K= 1050mg, Fe= 30,000mg, Zn= 25000, Mn= 30,000mg, SBM= soybean meal, DDGS= distiller dried grains with solubles, DCP= Di Calcium Phosphate, Choline Cl= Choline Chloride, M.E= Metabolizable Energy, Diet 1 provided to control group (D1) without DDGS inclusion. Diet 2 provided to group D2 with 10% DDGS inclusion. Diet 2 provided to group D3 with dietary supplementation of Xylanase enzyme @ 1g/kg, to group D4 with dietary supplementation of Mannanase enzyme @ 1g/kg and to group D5 with dietary supplementation of Xylanase and Mannanase enzymes @ 1g/kg each.

Experimental layout is shown in following [Table 1.](#) groups are shown below.

Experimental feed composition

Composition of starter and finisher ration of all
March 2026 | Volume 42 | Issue 1 | Page 292

Performance parameters

Growth performance was determined by the following

parameters.

Feed intake

Feed intake was determined by subtracting the amount of feed refused from the amount of feed initially offered, expressed in grams per day.

$$\text{Feed Intake} = \text{Feed offered} - \text{Feed refused}$$

Body weight gain

Body weight was quantified on a weekly basis using a digital balance by using following formula:

$$\text{Weight Gain} = \text{Final Weight} - \text{Initial Weight}$$

Feed conversion ratio

Feed conversion ratio was calculated by using following formula:

$$\text{FCR} = \frac{\text{feed intake (g)}}{\text{Total weight gain (g)}}$$

Dressing percentage

Following established ethical protocols, two birds per replicate were selected at the trial's conclusion and euthanized. Their carcass weight was subsequently recorded using a precise digital balance. Dressing percentage was calculated by using following formula.

$$\text{DP \%} = \frac{\text{carcass weight (kg)} \times 100}{\text{Live body weight (kg)}}$$

Nutrients digestibility

Chemical composition of feed and excreta was assessed using AOAC 2000 protocols for crude protein (984.13), fibre (978.10), crude fat (920.39), moisture (930.15), and ash (942.05). Employing the total collection method as detailed by [Swiatkiewicz et al. \(2014\)](#), nutrient digestibility coefficients were calculated.

Determination of apparent metabolizable energy (AME)

Apparent metabolizable energy (AME) of the diets was determined using an adiabatic bomb calorimeter standardized with benzoic acid according to the methodology of [Swiatkiewicz et al. \(2014\)](#).

Economics

After the 42-day trial, an economic analysis was conducted to determine the return on investment for each dietary treatment. Total production costs were calculated, including the outlay for feed, day-

old chicks, enzymes, and miscellaneous/operational charges, utilizing prevailing market rates. At the end, gross margin percentage was calculated by using following formula.

$$\text{Gross margin \%} = \frac{\text{Revenue} - \text{cost of production} \times 100}{\text{Revenue}}$$

Statistical analysis

Data were compiled in an Excel spreadsheet and subjected to statistical analysis of variance (ANOVA), mean comparison, and least significant difference (LSD) tests within a completely randomized design using Statistix 10.0 software.

Results

Effect of using corn DDGS fortified with Xylanase and Mannanase enzymes in broiler feed on growth performance and nutrients digestibility were investigated. Data was recorded for growth performance, nutrients digestibility and economics. The results of these parameters are presented below.

Performance parameters

Results of different performance parameters are presented as follows.

Feed intake

[Table 3](#) reveals that feed intake significantly differed ($p < 0.05$) between control and enzyme-treated groups in both starter and finisher phases, as well as overall. Birds fed 10% corn distillers' dried grains with solubles (DDGS) supplemented with either individual enzymes or a mixture consumed significantly less than the control group and the group fed only 10% DDGS without enzymes. The lowest feed intake was observed in group D5, which received the mixture of enzymes in their DDGS feed.

Weight gain

[Table 4](#) shows that birds fed exogenous enzymes, either individually or as a mixture in their 10% DDGS feed, gained significantly more weight ($p < 0.05$) compared to the control group and the group fed only 10% DDGS without enzymes. This increased weight gain was observed throughout the starter, finisher phases, and overall. The highest weight gain was achieved by group D5, which received the enzyme mixture, while group D2 fed DDGS without enzymes had the lowest.

Table 3: Effect of using corn based DDGS Fortified with xylanase and mannanase enzymes in broiler feed on feed intake (in grams)

Phase	D1	D2	D3	D4	D5	P-value
Week 2	390.12 ^a ±1.23	388.46 ^a ±1.65	370.25 ^{bc} ±1.84	372.21 ^b ±1.74	360.31 ^c ±1.25	0.000
Week 3	540.23 ^a ±2.31	546.24 ^a ±2.55	534.66 ^{ab} ±2.87	538.87 ^a ±2.54	522.06 ^b ±2.36	0.025
Starter phase	930.35 ^a ±4.05	934.70 ^a ±4.19	904.91 ^b ±4.51	911.08 ^b ±4.59	882.37 ^c ±4.08	0.000
Week 4	645.16 ^a ±3.87	639.55 ^a ±3.73	625.47 ^b ±3.98	622.38 ^{bc} ±3.34	610.22 ^c ±3.25	0.001
Week 5	1030.74 ^a ±4.25	1028.81 ^a ±4.59	1014.11 ^{bc} ±4.21	1020.72 ^{ab} ±4.87	1005.41 ^c ±4.59	0.002
Week 6	1190.25 ^a ±5.06	1186.33 ^{ab} ±5.24	1172.06 ^b ±5.48	1176.55 ^b ±5.23	1160.87 ^c ±5.04	0.001
Finisher phase	2866.15 ^a ±6.03	2854.69 ^b ±6.23	2811.64 ^c ±6.58	2819.65 ^c ±6.87	2776.5 ^d ±6.15	0.000
Overall	3796.5 ^a ±9.21	3789.39 ^a ±9.54	3716.55 ^c ±9.89	3730.73 ^b ±9.14	3658.87 ^d ±9.25	0.000

D1=Control Group, D2= 10% DDGS inclusion in Feed, D3= 10% DDGS inclusion in Feed with Xyl. supplementation, D4= 10% DDGS inclusion in Feed with Man. supplementation, D5= 10% DDGS inclusion in Feed with Xyl+Man supplementation. week 2 (day 8-14) week 3 (day 15-21) starter phase (day 8-21) week 4 (day 22-28) week 5 (day 29-35) week 6 (day 36-42) Means bearing dissimilar superscripts within same row differ significantly ($P < 0.05$).

Table 4: Effect of using corn DDGS Fortified with xylanase and mannanase enzymes in broiler feed on weight gain (in grams)

Phase	D1	D2	D3	D4	D5	P-value
Week 2	258.59±2.11	251.29±2.06	260.83±3.78	256.58±3.54	263.12±2.82	0.126
Week 3	349.08 ^b ±3.18	339.83 ^c ±3.13	357.37 ^{ab} ±3.41	351.4 ^b ±3.59	362.35 ^a ±3.54	0.002
Starter phase	607.67 ^c ±4.56	591.12 ^d ±4.07	618.2 ^{ab} ±4.29	607.98 ^{bc} ±4.49	625.47 ^a ±4.17	0.000
Week 4	388.29±3.22	381.46±3.53	392.54±3.48	388.10±3.54	399.86±3.33	0.052
Week 5	541.95 ^b ±4.44	520.50 ^c ±4.12	547.54 ^{ab} ±4.20	552.08 ^{ab} ±4.11	554.8 ^a ±4.56	0.000
Week 6	588.76 ^b ±5.41	562.66 ^c ±5.83	587.2 ^b ±5.14	590.0 ^{ab} ±5.54	600.77 ^a ±5.03	0.000
Finisher phase	1519.0 ^c ±5.03	1464.62 ^d ±5.21	1526.78 ^{bc} ±5.24	1530.18 ^b ±5.56	1555.43 ^a ±5.7	0.000
Overall	2126.67 ^c ±8.53	2055.7 ^d ±8.53	2144.98 ^b ±8.71	2138.4 ^b ±8.87	2180.9 ^a ±8.33	0.000

D1=Control Group, D2= 10% DDGS inclusion in Feed, D3= 10% DDGS inclusion in Feed with Xyl. supplementation, D4= 10% DDGS inclusion in Feed with Man. supplementation, D5= 10% DDGS inclusion in Feed with Xyl+Man supplementation. week 2 (day 8-14) week 3 (day 15-21) starter phase (day 8-21) week 4 (day 22-28) week 5 (day 29-35) week 6 (day 36-42) Means bearing dissimilar superscripts within same row differ significantly ($P < 0.05$).

Table 5: Effect of using corn based DDGS Fortified with xylanase and mannanase enzymes in broiler feed on feed conversion ratio

Phase	D1	D2	D3	D4	D5	P-value
Week 2	1.50 ^{ab} ±0.03	1.54 ^a ±0.05	1.41 ^{cd} ±0.02	1.45 ^{bc} ±0.08	1.36 ^d ±0.08	0.001
Week 3	1.54 ^{ab} ±0.02	1.60 ^a ±0.09	1.49 ^{bc} ±0.69	1.53 ^{abc} ±0.04	1.44 ^c ±0.06	0.030
Starter phase	1.53 ^{ab} ±0.06	1.58 ^a ±0.03	1.46 ^{bc} ±0.05	1.49 ^{abc} ±0.04	1.41 ^c ±0.09	0.021
Week 4	1.66 ^a ±0.02	1.67 ^a ±0.05	1.59 ^{ab} ±0.09	1.60 ^{ab} ±0.04	1.52 ^b ±0.02	0.049
Week 5	1.90 ^{ab} ±0.09	1.97 ^a ±0.01	1.85 ^b ±0.03	1.84 ^b ±0.04	1.81 ^b ±0.06	0.028
Week 6	2.02 ^{ab} ±0.08	2.10 ^a ±0.04	1.99 ^{bc} ±0.07	1.99 ^{bc} ±0.01	1.93 ^c ±0.02	0.017
Finisher phase	1.88 ^{ab} ±0.02	1.94 ^a ±0.05	1.84 ^{bc} ±0.04	1.84 ^{bc} ±0.09	1.78 ^c ±0.04	0.020
Overall	1.78 ^{ab} ±0.08	1.84 ^a ±0.05	1.73 ^{bc} ±0.08	1.74 ^{bc} ±0.07	1.67 ^c ±0.09	0.016

D1=Control Group, D2= 10% DDGS inclusion in Feed, D3= 10% DDGS inclusion in Feed with Xyl. supplementation, D4= 10% DDGS inclusion in Feed with Man. supplementation, D5= 10% DDGS inclusion in Feed with Xyl+Man supplementation. week 2 (day 8-14) week 3 (day 15-21) starter phase (day 8-21) week 4 (day 22-28) week 5 (day 29-35) week 6 (day 36-42) Means bearing dissimilar superscripts within same row differ significantly ($P < 0.05$).

Feed conversion ratio

Table 5 reveals a significant difference ($P < 0.05$) in feed conversion ratio (FCR) among the groups throughout the experiment. Group D5, fed the mixture of enzymes in corn DDGS feed, achieved the lowest FCR across all weeks, demonstrating the most efficient feed utilization. Groups D3, D4, and D1 exhibited intermediate FCR values, suggesting moderate feed conversion efficiency. Group D2, fed DDGS without enzymes, consistently had the highest FCR, indicating the least efficient feed utilization.

Dressing percentage

Table 6 reveals a significant difference ($p < 0.05$) in dressing percentage between the control group and the enzyme-treated groups (D3-D5). While the treated groups themselves didn't show statistically significant differences. However, there were some numerical variations. Group D2, which received no enzymes, had the lowest dressing percentage. Group D1, with individual enzymes, had an intermediate dressing percentage. Notably, all enzyme-treated groups (D3-D5) had significantly higher dressing percentages ($p < 0.05$) compared to the control. Among the treated groups, D5, fed the enzyme mixture, achieved the highest dressing percentage.

Table 6: Effect of using corn DDGS Fortified with xylanase and mannanase enzymes in broiler feed on DP

Group	Dressing percentage
D1	67.44 ^{ab} ±2.05
D2	64.26 ^b ±2.54
D3	70.14 ^a ±2.13
D4	69.89 ^a ±2.57
D5	71.32 ^a ±2.47
P- Value	0.027

D1=Control Group, D2= 10% DDGS inclusion in Feed, D3= 10% DDGS inclusion in Feed with Xyl. supplementation, D4= 10% DDGS inclusion in Feed with Man. supplementation, D5= 10% DDGS inclusion in Feed with Xyl+Man supplementation. Means bearing dissimilar superscripts within same columns differ significantly ($P < 0.05$)

Nutrients digestibility

Trials were conducted to determine the nutrients digestibility in the broilers. Results of the percent digestibility of DM, CP, CF, EE, ash and apparent metabolizable energy are presented below.

Dry matter digestibility

Table 7 reveals a significant difference ($p < 0.05$) in

DM digestibility between the control and treated groups. Among the treated groups, D5 exhibited the highest percentage of DM digestibility, while D2 displayed the lowest. The average DM digestibility for groups D1 to D5 were: 78.61 ± 1.20 , 74.54 ± 1.54 , 81.0 ± 1.15 , 79.33 ± 1.26 , and 83.66 ± 0.66 , respectively.

Ash digestibility

Results in **Table 7** indicate that digestibility of Ash content was also significantly different ($P < 0.05$) among the groups. Highest percentage of Ash digestibility was recorded in Group D5 and the lowest in group D2. Percent digestibility of ash from group D1 to D5 was recorded as (57.33 ± 1.20), (52.45 ± 1.48), (61.54 ± 1.31), (60.13 ± 1.17) and (64.67 ± 1.20) respectively.

Crude protein digestibility

Table 7 reveals that digestibility of crude protein was also significantly different ($P < 0.05$) among the groups. Groups D3 to D5 treated with exogenous enzymes showed better performance than group D1 and D2. Percent CP digestibility from group D1 to D5 was recorded as (76.34 ± 1.33), (72.19 ± 1.45), (79.56 ± 1.15), (80.78 ± 1.06), and (82.96 ± 1.35) respectively.

Crude fiber digestibility

Results in **Table 7** showcased the similar pattern of digestibility for CF. Crude fibre digestibility was found significantly different ($p < 0.05$) among the control and treated groups. Highest CF digestibility was found in Group D5 and lowest in D2. CF digestibility from group D1 to D5 was recorded as (69.33 ± 1.85), (64.21 ± 1.98), (73.0 ± 1.52), (72.66 ± 1.45) and (76.66 ± 2.02) respectively.

Ether extract digestibility

Results in

Table 7 indicate percentage of EE digestibility was also recorded in similar pattern. EE digestibility was found significantly different ($p < 0.05$) among the control and treated groups. Highest EE digestibility was found in Group D5 and lowest in D2. EE digestibility from group D1 to D5 was recorded as (73.66 ± 1.15), (68.32 ± 1.39), (76.46 ± 1.45), (76.46 ± 1.45) and (80.74 ± 1.20) respectively.

Apparent metabolizable energy

AME digestibility analysis, showcased in **Table 7**, revealed statistically significant differences ($p < 0.05$) between the control group (D1) and all enzyme-supplemented groups (D2-D5). The highest AME

Table 7: Effect of using corn DDGS Fortified with xylanase and mannanase enzymes in broiler feed on nutrients digestibility and AME.

Groups	DM %	Ash %	CP %	CF %	EE %	AME (KCal/kg)
D1	78.61 ^{bc} ±1.20	57.33 ^b ±1.20	76.34 ^{bc} ±1.33	69.33 ^b ±1.85	73.66 ^b ±1.15	2634.8 ^d ±5.03
D2	74.54 ^c ±1.54	52.45 ^c ±1.48	72.19 ^c ±1.45	64.21 ^c ±1.98	68.32 ^c ±1.39	2521.4 ^c ±5.27
D3	81.0 ^{ab} ±1.15	61.54 ^{ab} ±1.31	79.56 ^{ab} ±1.15	73.0 ^{ab} ±1.52	76.46 ^{ab} ±1.45	2674.3 ^c ±5.44
D4	79.33 ^{ab} ±1.26	60.13 ^b ±1.17	80.78 ^{ab} ±1.06	72.66 ^{ab} ±1.45	77.33 ^{ab} ±0.88	2691.3 ^b ±4.09
D5	83.66 ^a ±0.66	64.67 ^a ±1.20	82.96 ^a ±1.35	76.66 ^a ±2.02	80.74 ^a ±1.20	2756.0 ^a ±5.37
P-value	0.011	0.001	0.004	0.001	0.002	0.000

D1=Control Group, D2= 10% DDGS inclusion in Feed, D3= 10% DDGS inclusion in Feed with Xyl. supplementation, D4= 10% DDGS inclusion in Feed with Man. supplementation, D5= 10% DDGS inclusion in Feed with Xyl+Man supplementation, DM= dry matter, CP= crude protein, CF= crude Fiber, EE= ether extract. Means bearing dissimilar superscripts within same columns differ significantly ($P < 0.05$)

Table 8: Effect of using corn DDGS Fortified with xylanase and mannanase enzymes in broiler feed on economics.

Groups	Cost of production (Rs)	Revenue (Rs.)	Gross margin (%)
D1	638.5 ^a ±1.45	703.6 ^b ±4.23	9.73 ^c ±3.82
D2	606.52 ^b ±2.89	676.5 ^c ±2.09	10.34 ^{bc} ±3.22
D3	602.93 ^b ±2.60	706.2 ^b ±5.12	14.62 ^a ±3.54
D4	608.10 ^b ±2.11	702.9 ^b ±3.57	13.48 ^{ab} ±3.30
D5	604.08 ^b ±3.96	719.4 ^a ±2.84	16.03 ^a ±3.25
P-value	0.000	0.000	0.007

D1=Control Group, D2= 10% DDGS inclusion in Feed, D3= 10% DDGS inclusion in Feed with Xyl. supplementation, D4= 10% DDGS inclusion in Feed with Man. supplementation, D5= 10% DDGS inclusion in Feed with Xyl+Man supplementation. Means bearing dissimilar superscripts within same columns differ significantly ($P < 0.05$), Cost of production = Feed+ Day old chick+enzyme+Misc. Feed= Rs.150/kg, DDGS= Rs.70/kg, Day old chick= Rs.60/- Broiler Rate330/kg.Rs= Pakistani Rupee/

value was recorded in Group D5 (mixed enzymes; 2756.0 ± 5.37 kcal/kg), followed by Groups D4 (Mannanase; 2691.3 ± 4.09 kcal/kg) and D3 (xylanase; 2674.3 ± 5.44 kcal/kg). While Group D2 (DDGS only) exhibited a slight improvement compared to the control (2521.4 ± 5.27 kcal/kg vs. 2634.8 ± 5.03 kcal/kg), it significantly underperformed all enzyme-supplemented groups ($p < 0.05$).

Economics

Profitability for each diet was assessed through gross margin (Table 8). Using 10% corn DDGS with enzymes significantly reduced the total cost of production compared to the control group ($p < 0.05$). However, costs among enzyme-supplemented groups were not significantly different. Group D1 (control) had the highest cost, while costs decreased in groups D2-D5 (D1 = 638.5 ± 1.45, D2 = 606.52 ± 2.89, D3

= 602.93 ± 2.60, D4 = 608.10 ± 2.11, D5 = 604.08 ± 3.96).

Revenue analysis at the trial's conclusion revealed statistically significant differences ($p < 0.05$) between enzyme-treated groups and Group D2 (DDGS only). Group D5 (mixed enzymes) achieved the highest revenue (719.4 ± 2.84), followed by Group D3 (xylanase) and Group D4 (Mannanase), with Group D1 (control) earning marginally more than Group D2. Overall, significant variance in revenue generation was observed across the groups ($p < 0.05$).

Gross margin analysis, presented in Table 8, revealed statistically significant differences ($p < 0.05$) between the control group (D1) and all enzyme-treated groups (D2-D5). Group D5 (mixed enzymes) achieved the highest gross margin (16.03 ± 3.25%), followed by Group D3 (xylanase; 14.62 ± 3.54%) and Group D4 (Mannanase; 13.48 ± 3.30%). While Group D2 (DDGS only) exhibited a modest increase compared to the control (10.34 ± 3.22% vs. 9.73 ± 3.82%), it significantly underperformed all enzyme-supplemented groups ($p < 0.05$). These findings suggest that enzyme supplementation, particularly the mixed blend, significantly enhances profitability in broiler production.

Discussion

Effect of using corn based DDGS fortified with Xylanase and Mannanase enzymes in broiler feed on growth performance and nutrients digestibility were investigated. Data was recorded for growth performance, nutrients digestibility, and economics. The results of these parameters are discussed below.

Performance parameters

Results of different performance parameters are presented as follows.

Feed intake

Use of exogenous enzymes in DDGS based broiler feed is helpful in hydrolysing the NSPs present in the fibre enriched feed. These enzymes disrupt the plant cell wall integrity and release the captivated nutrients which increase the nutrients utilization and gut performance (Ravindran, 2013 & Amerah, 2015). Table 3 shows the results of using exogenous enzymes i.e. Xylanase and Mannanase either individually or as mixture in broiler feed based on 10% DDGS on Feed Intake. Results indicated that supplementation of these exogenous enzymes resulted in decrease in feed intake without compromising the feed conversion ratio. This finding aligns with previous research by Samarasinghe *et al.* (2000), Richter *et al.* (1994), Ranade and Rajmane (1992), and Kadam *et al.* (1991). These studies also observed decreased feed intake with enzyme supplementation, suggesting that birds fulfilled their nutrient needs with less feed. However, some research has reported increased feed intake with enzyme supplementation in poultry feed. This discrepancy is evident in studies by Abu-Tayyeb *et al.* (2019), Anuradha and Roy (2015), Zeng *et al.* (2015) and Alam *et al.* (2003). They attributed the increase to improved nutrient digestibility due to NSPs breakdown and release of trapped nutrients. Additionally, Mathlouthi *et al.* (2003) suggested that enzymes might increase the availability of certain minerals, like Mn, Zn, and Cu, which can stimulate feed intake in broilers (Abu-Tayyeb *et al.*, 2019). Furthermore, some studies, such as those by Hussain *et al.* (2019) and Ghazalah *et al.* (2011), found no significant effect of enzymes on feed intake in broiler and layer feed, respectively. These findings contradict both our results and those reporting increased intake. Effect of exogenous enzymes on feed intake in DDGS-based broiler feed appears to be variable and dependent on several factors. Further research is needed to fully understand the mechanisms behind this variability and optimize enzyme use for improved broiler performance.

Weight gain

In poultry rations, exogenous enzymes are primarily supplied to compensate for low endogenous enzyme levels or to introduce more enzymatic systems that the birds cannot make. (Leeson, 2001). Numerous

studies have shown that adding exogenous enzymes to poultry feed enhances the birds' overall performance (Narasimha *et al.*, 2013; Hajati *et al.*, 2009). The present study demonstrated a positive impact of exogenous enzymes on broiler weight gain. All groups receiving these enzymes (xylanase, Mannanase, or their combination) experienced increased average weight gain compared to the control groups without enzymes. This improvement can be attributed to the enzymes' ability to hydrolyse non-starch polysaccharides (NSPs) in the feed. This reduces intestinal viscosity, leading to enhanced nutrient digestibility and improved gut performance (Hafeez *et al.*, 2020; Amerah, 2015). Our findings align with existing research demonstrating the effectiveness of exogenous enzymes in enhancing broiler growth, particularly when DDGS is included in the diet. Liu *et al.* (2011) observed similar improvements in growth performance with xylanase supplementation in corn DDGS-based feed. Inayah *et al.* (2022) reported increased weight gain in broilers fed xylanase-enriched rations. Kiarie *et al.* (2014) confirmed xylanase's positive impact on weight gain in DDGS diets. Ha *et al.* (2017) demonstrated enhanced growth performance in broilers fed β -Mannanase-supplemented 10% DDGS diets. This is likely because β -Mannanase helps break down mannans that would otherwise increase intestinal viscosity and hinder nutrient absorption. Similarly, Cowieson & Ravindran (2008) reported improved weight gain in broilers fed with corn-SBM diets supplemented with various exogenous enzymes. Similar improvements were observed by Ferreira *et al.* (2016), Lei *et al.* (2017), and Cowieson *et al.* (2006) who supplemented their broilers' feed with Mannanase enzymes. However, Hussain *et al.* (2019) and Zou *et al.* (2013) found no significant improvement in weight gain with NSPase supplementation. Campasino *et al.* (2015) also reported no benefit from an exogenous enzyme mixture. Adding exogenous enzymes to broiler feed can yield mixed results, as their effectiveness often varies unpredictably among birds (Hafeez *et al.*, 2020; Cowieson *et al.*, 2006; Gao *et al.*, 2007; Choct *et al.*, 1999). However, even with this uncertainty, supplementing diets containing DDGS with these enzymes can be an effective strategy. As Campasino *et al.* (2015) demonstrated, exogenous enzymes can not only mitigate the negative effects of DDGS on broiler growth but also potentially improve their performance.

Feed conversion ratio

Table 5 shows the results of using exogenous enzymes i.e. Xylanase and Mannanase either individually or as mixture in broiler feed based on 10% DDGS on feed conversion ratio. Results indicate that supplementation of exogenous enzymes in corn-based feed tends to improve FCR in broilers. Numerous studies have been conducted in past which support our findings. Present results are in line with the findings of Zou *et al.* (2013) who concluded that using a mixture of exogenous enzymes improved FCR of the broilers. Our results are also in line with the findings of Cowieson and Ravindran (2008) who concluded that the supplementation of multi enzyme improved the FCR of broilers. Ferreira *et al.* (2016) also supports our results that supplementing broiler feed with Mannanase enzyme resulted in improved FCR. Our results are also supported by Coppedge *et al.* (2012) who concluded that using NSPase in low energy diets helped in improved FCR. Our results are also supported by the findings of Inayah *et al.* (2022) who supplemented the broiler feed with Xylanase enzyme and got improvement in FCR value. Our results are also in line with the findings of Kiarie *et al.* (2012) who supplemented the broiler feed with Xylanase enzyme and found similar results. Similarly, Gade *et al.* (2017) also found improved FCR with supplementation of Xylanase which supports our findings. Nikam *et al.* (2016) also reported the same results. Our results are also in line with the findings of Abu-Tayyeb *et al.* (2019) who used multi enzymes preparation as supplement in broiler feed which resulted in lower FCR. This could be attributed to the increased pancreatic secretion and digestive enzymes activities (Campasino *et al.*, 2015). Improvement in FCR can also be associated with the improvement in nutrients utilization (Olukosi *et al.*, 2010). However, there are some research conducted in past which oppose our results. Our results are opposed by the findings of Cowieson *et al.* (2006) who found that FCR was not improved in birds with the supplementation of Xylanase enzyme in feed. Our results are also not parallel with the findings of Campasino *et al.* (2015) who did not get improvement in FCR with Xylanase supplementation. Similarly, Hussain *et al.* (2019) used blend of multi exogenous enzymes in their study and did not get any improvement in FCR which also contradicts our findings.

To evaluate the growth performance of the birds, FI, BW and FCR are considered as most significant parameters. These three parameters are known as

key performance indicators (KPI) while assessing the broiler's potential for growth performance (Abu-Tayyeb *et al.*, 2019). Results of the current study showed that supplementation of exogenous enzymes either individually or as a blend improved the BW of broilers as compared to control group. This increase in weight gain is attributed to result of improved nutrients utilization which might be due to degradation of NSPs in fiber enriched feed by exogenous enzymes and improved gut health (Ahmad *et al.*, 2020).

Dressing percentage

The proportion of hot carcass to live weight (pre slaughter) is termed as dressing percentage. Table no. 1.6 shows the results of using exogenous enzymes i.e. Xylanase and Mannanase either individually or as mixture in broiler feed based on 10% DDGS inclusion on dressing percentage. Results indicate that supplementation of these exogenous enzymes had positive impact on dressing yield. Our results are in line with the findings Alam *et al.* (2003) who concluded that inclusion of exogenous enzymes in broiler feed resulted in improved dressing percentage as compared to control group. Leeson *et al.* (1996) also obtained high dressing yield with supplementation of exogenous enzymes in poultry diet. Our results are also in line with the findings of Alqhtani *et al.* (2022) who added exogenous enzymes cocktail in broiler feed and obtained improvement in carcass yield. Our results are also in line with the findings of Attia *et al.* (2022) who supplemented low density diet with multi-exogenous enzymes and got improvement in dressing percentage of chickens. However, Abu-Tayyeb *et al.* (2019) did not find any improvement in dressing yield with supplementation of exogenous enzymes which contradicts present findings.

Nutrient digestibility

Application of exogenous enzymes is the basic principle to enhance the nutritive value of feed (Bedford & Partridge 2001). Table 7 shows the results of using exogenous enzymes i.e. Xylanase and Mannanase either individually or as mixture in broiler feed based on 10% DDGS inclusion on nutrients digestibility and apparent metabolizable energy. Results indicate that supplementation of these enzymes enhanced the nutrient digestibility of Groups D3, D4 and D5 which were fed with 10% DDGS inclusion in feed along with supplementation of exogenous enzymes. Numerous studies have been conducted which support our findings. Our results are in line with the

findings of [Sherif et al. \(2009\)](#) who observed that inclusion of commercial exogenous enzymes in corn-based broiler feed helped in improvement of crude fat and crude protein digestibility. They also got better Nitrogen retention as compared to control group. Our results are also in line with the findings of [Olukosi et al. \(2008\)](#) who concluded that supplementation of Xylanase, Amylase and Protease in broiler feed resulted in improved digestibility of all nutrients. Our results are also in line with the findings of [Yaqoob et al. \(2022\)](#) who supplemented Mannanase enzyme in broiler feed and recorded improvement in nutrients digestibility. Our results are also in line with the findings of [Sundu et al. \(2006\)](#) and [Saleh et al. \(2005\)](#) who reported that DM digestibility was significantly improved with enzymes addition. Similarly, [Yu et al. \(2007\)](#) reported that inclusion of exogenous enzyme had positive effect on CP digestibility in broilers. Our results are also in line with the findings of [Inayah et al. \(2022\)](#) who concluded that dietary supplementation of Xylanase enzyme in broiler feed improved nutrient digestibility including DM, CP, fat, P and Ca. Improvement in Nutrients digestibility may be associated with the decrease in intestinal viscosity due to these NSPs degrading enzymes [Azarfar et al. \(2013\)](#). It may also be associated with the fact that β -Mannanase tends to enhance the enzymatic activities of digestive enzymes and ultimately nutrient digestibility improves due to the degradation of cell wall and release of nutrients encapsulated in it by reducing its integrity ([Bedford, 2000](#)). Furthermore, Xylanase also degrades polysaccharides of cell wall and convert xylan into simple sugar like xylose and also releases other nutrients. Broiler then utilize the readily available sugars for further metabolism ([Dornez et al., 2009](#)). Table no. 1.7 shows the results of using exogenous enzymes i.e. Xylanase and Mannanase either individually or as mixture in broiler feed based on 10% DDGS inclusion on AME. Results indicated that dietary supplementation of these enzymes both individually and as mixture improved the values for AME. Our results are in line with the findings of [Campasino et al. \(2015\)](#) who included NSPase enzymes in corn based DDGS diet of broilers and obtained better results in metabolize energy and digestible energy. Our results are also in line with the findings of [Olukosi et al. \(2015\)](#) who found high metabolize energy with the inclusion of exogenous enzymes in DDGS based broiler diet. Our results are also in line with the findings of [Hosseini et al. \(2017\)](#) who concluded that inclusion of Xylanase enzyme in

broiler feed resulted in improved AME. Our results are parallel with the results of [Esmaeilipour et al. \(2012\)](#) who also concluded that supplementation of Xylanase in poultry feed resulted in improved AME. The positive impact of Xylanase in improvement of AME could be attributed to the fact that arabinoxylans, which are the major NSPs in broiler feed are responsible for increased digesta viscosity. This increased viscosity has negative impact on feed digestion and absorption of nutrients. However dietary Xylanase hydrolyzes this NSPs and releases nutrients which increases nutrients absorption and AME ([Riddell and Kong, 1992](#), [Choct et al., 1999](#)). Similar findings were noted by [Cho et al. \(2013\)](#) who found that supplementation of Mannanase enzyme resulted in improved AME. [Daskiren et al. \(2004\)](#) also noted that the dietary supplementation of endo-beta-D-Mannanase improved ME in broilers. Our results are also supported by [Mussini et al., 2011](#) who concluded that supplementation of β -Mannanase in corn-soybean meal diet resulted in improved AME. Similar results are also reported by [Schulze et al. \(2006\)](#). Our results are also in line with the findings of [Alqhtani et al. \(2022\)](#) who added exogenous enzymes cocktail in broiler feed and obtained improvement in AME retention. Our results are also parallel with the findings of [Amerah et al. \(2017\)](#) who supplemented corn-SBM based broiler diet with Xylanase with some other exogenous enzymes and obtained improved results for AME. Similarly, [Ao et al. \(2009\)](#) also reported better AME values for broilers whose diet was supplemented with NSPs degrading enzymes. [Cowieson et al. \(2010\)](#) and [Aftab \(2012\)](#) also reported improvement in AME retention with supplementation of exogenous enzymes in broiler feed which support our results.

Economics

Economic benefits with supplementation of exogenous enzymes are associated with reduction in feed production cost. Dietary supplementation of exogenous enzymes like Xylanase and Mannanase provide some flexibilities in feed formulation with unconventional low-cost ingredients [Costa et al. \(2008\)](#). Table 8 illustrates the data for economics of broiler production in experimental trial. It was obvious from group D2 that inclusion of 10%DDGS in broiler feed reduced its cost of production. However, due to less weight gain net profit was not reasonable. We supplemented exogenous enzymes in feed so that the negative impact of high level DDGS on

weight gain could be minimized. Results indicated that dietary supplementation of both Xylanase and Mannanase not only countered the negative impact on weight gain, but it also further improved the bird's performance and nutrients digestibility. These factors collectively improved the economics of broiler production. Dietary supplementation of both enzymes either individually or as mixture in broiler feed increased the gross margin and resulted in better economic returns as compared to control group and D2. In alignment with the present study, [Kaushal et al. \(2019\)](#) in their study concluded that using enzymes and probiotics as feed additive in broiler diet result in high profitability which is in line with the present findings. Results of the present study are also parallel with the findings of [Narasimha et al. \(2013\)](#) who concluded that supplementation of enzymes in broiler diet reduced feeding cost and cost per kg live weight. Similarly, [Alabi et al. \(2019\)](#) concluded in their review that supplementation of exogenous enzymes in broiler feed formulated from cheap and alternative feed ingredients is highly beneficial in least cost economic production which strongly supports our results. Our results are also parallel with the findings of [Marchiori et al. \(2022\)](#) who concluded that using a blend of exogenous enzymes in low nutritional diet resulted in high profit. Results of the present study are also parallel with the findings of [Habib, 2017](#) who supplemented the poultry feed with Xylanase along with other exogenous enzymes as mixture and recorded more economic benefits as compared to control group. Similarly, [Khose et al., 2017](#) also concluded that incorporating DDGS in broiler feed along with enzymes supplementation is highly cost effective and profitable which is also in line with the present findings. Similarly, [Ghazala et al., 2011](#) concluded that replacing 50% soya bean with DDGS along with supplementation of exogenous enzymes proved to be highly economical which also supports present findings that supplementing exogenous enzymes in high level DDGS based diet is highly economical.

Conclusions and Recommendations

It was concluded that inclusion of 10% DDGS in broiler feed fortified with Xylanase and Mannanase enzymes either individually or as mixture not only reduces cost of production but has positive impact on broiler's performance, nutrients digestibility and profitability.

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Novelty Statement

Unlike previous studies, the present work elucidates the combined impact of substrate-specific enzymes and corn DDGS on broiler health and feed economics. It establishes enzyme-assisted DDGS utilization as a viable strategy for lowering production costs without adverse health effects.

Author's Contribution

Muhammad Waqas: Carried out the research work and prepared the original draft of the manuscript.

Sarzamin Khan: Supervised the research work

Rafiullah: Provided resources for the research and contributed to manuscript review

Umer Saddique Khattak: Reviewed and edited the final manuscript to improve language and grammar

Generative AI or AI assisted technology statement

The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors have no conflict of interest.

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