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Original Article

Effect of Exogenous Protease, Mannanase, and Xylanase Supplementation in Corn and High Protein Corn DDGS Based Diets on Growth Performance, Intestinal Morphology and Nutrient Digestibility in Broiler Chickens

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Broiler, Exogenous enzymes, Hi-Pro DDGS, Intestinal integrity.

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ABSTRACT

A study was conducted to evaluate the effects of exogenous enzymes supplementation in high protein (Hi-Pro) corn DDGS-based diets on broiler growth performance, intestinal health, and nutrient digestibility. A total of 200 one-day-old broiler chicks were randomly allocated to four dietary treatments with five replicates of 10 birds each. A basal diet (CON) was formulated (CP: 20%; ME: 2900 Kcal/kg) containing 15% of dietary crude protein share from Hi-Pro corn DDGS. Three experimental diets were developed using basal diets; diet one was supplemented with protease (CON-P), diet two with an enzyme mix of mannanase and xylanase (CON-MX) and diet three with a protease and enzyme mix of mannanase and xylanase. A digestibility assay was carried out using Celite (insoluble acid ash), from day 29 to day 35. On day 35, four birds from each replicate were picked randomly and killed by cervical dislocation to collect ileal digesta. In addition, duodenum, jejunum and ileum lengths were measured, and tissue samples were collected from midpoints of jejunum to note villus height and crypt depths. Feed intake, body weight gain and feed conversion ratio were not affected by dietary treatments during the entire study period (p>0.05). Likewise, no difference in carcass characteristics, soft organ weight, intestinal morphology, apparent amino acids digestibility and visible ileal digestible energy was observed by the dietary treatments (p>0.05).Supplementation of exogenous protease and enzyme mix (mannanase and xylanase) had no effect on growth performance, intestinal integrity and nutrient availability of broiler chickens fed Hi-Pro DDGS-based diets.

INTRODUCTION

Distiller dried grains with soluble (DDGS) is a corn by-product that is produced by dry milling and fermentation process. The recent trend of biofuel production has provided nutritionists an opportunity to use DDGS as an alternative protein ingredient in poultry diets. Ethanol producers are continuously improving on fractioning technologies to enhance the production of ethanol and the quality of DDGS (Jung & Batal 2009). Mainly two fractioning techniques are employed by the ethanol industry, *i.e.,* front end and back end fraction technology. Only endosperm fraction of corn is subjected to fermentation in front end fractionation technology that eliminates non-fermentable fraction (germ and bran) of corn and results in (High-Pro) corn DDGS (Robinson *et al.* 2008). High protein (Hi-Pro) corn DDGS is higher in crude protein (CP), amino acids and energy than conventional corn DDGS (Singh *et al.* 2005). The inclusion of corn DDGS in broiler diet is well documented, however, higher level of corn DDGS has been reported to reduce growth performance (Lumpkins *et al.* 2004; Wang *et al.* 2007; Choi *et al.* 2008; Youssef *et al.* 2008; Loar *et al.* 2010; Zhang *et al.* 2013).

The total content and availability of nutrients change after fermentation and drying process. During DDGS production, the concentration of non-phytate increased from 0.08% to 0.34% (NRC 1994). Matsui (2002) also observed that phytate phosphorus content reduced, when soybean meal (SBM) was subjected to fermentation. Lopez *et al.* (1983) also found similar results; fermentation reduced the phytate content of corn and increased non-phytate content. During ethanol production, process alteration in the structural components of corn grain takes place, and the availability of nutrients is also modified. The application of heat during the drying process has been reported to increase phosphorus bioavailability and reduce amino acids digestibility (Amezcua & Parsons 2007).

Exogenous protease supplementation in poultry diets as a mono-component or admixture of other enzymes has become quite common (Cowieson & Adeola 2005). The supplementation of exogenous protease not only improves the CP and amino acid digestibility (Cowieson & Roos 2014) but also tends to enhance ileal digestibility of energy (Kalmendal & Tauson 2012). Cowieson *et al.* (2017 a,b) also noted the valuable effect of exogenous protease on mucin secretion, intestinal integrity, and immunity in broiler studies.

Non-starch polysaccharides interfere with the mixing of digesta with endogenous enzymes by increasing the viscosity of digesta, thus acts as anti-nutritional factors (Slominski 2011). Gao *et al.* (2008) reported that the presence of arabinoxylan in the wheat-based diet leads to hypertrophy of the digestive organ. However, supplementation of xylanase in a wheat-based diet reduces the negative effect of arabinoxylan on the relative weight of digestive organs (duodenum, jejunum, and pancreas). In another study, it was pointing out that arabinoxylan not only reduces the digestibility of nutrients but also increases gastro-intestinal secretions (Angkanaporn *et al.* 1994). Supplementation of non-starch polysaccharides (NSPase) and proteases individually or in combination are reported to improve the nutrient utilization in poultry (Olukosi *et al.* 2010). Ludke *et al.* (2018) also reported that the supplementation of exogenous enzymes blend improved production in chicken. Campasino *et al.* (2015) found that the supplementation of NSPase in DDGS-based diets improved broiler performance, protein, and energy digestibility coefficient.

The effect of exogenous enzymes supplementation in Low-Pro corn DDGS based broiler diets is well explored. However, Hi-Pro corn DDGS being relatively new feedstuffs warrants the use of exogenous enzymes, more specifically mannanase, xylanase and proteases to further evaluate and improve its nutritive value for broiler chickens. Therefore, this study was planned to evaluate the effect of exogenous enzymes (mannanase, xylanase, and proteases) alone or in combination on broiler performance, apparent amino acid digestibility and apparent ileal digestible energy in Hi-Pro corn DDGS-based diet.

MATERIALS AND METHODS

All procedures followed in the conduct of the experiment were approved by the Graduate Studies and Research Board, University of Agriculture, Faisalabad, Pakistan.

Animal Husbandry and Experimental Procedure

All the ingredients except corn DDGS used in experimental diets were provided by a commercial feed mill, Sadiq Feed Mill (Pvt. Ltd.) Rawalpindi, Pakistan. Hi-Pro corn DDGS was imported from the USA, and its nutrient profile is shown in Table 1. A basal diet (CP: 20% and ME: 2900 Kcal/ Kg) was formulated, 15% dietary protein contributed by Hi-Pro corn DDGS. Three experimental treatments were given to the birds. Experimental treatments were: Basal diet supplemented with protease (Cibenza DP 100 at 250 g/ ton of feed); Basel diet supplemented with an enzyme mix of mannanase and xylanase (CON-MX; Winzyme MX-100 g/ ton of feed) and Basel diet supplemented with a protease and enzyme mix of mannanase and xylanase (CON-MXP; Winzyme MX 100g/ ton of feed + Cibenza DP 100 at 250g/ton of feed). Diets were formulated using software WinFeed 2.8 (WinFeed Ltd., Cambridge, UK). Two hundred 1-day-old broiler chicks (Ross 308) were purchased from a local commercial hatchery, Sadiq Brother Poultry Hatcheries (Pvt. Ltd.) Rawalpindi. These chicks were randomly allotted to one of four dietary treatments. Each dietary treatment had five replicates with ten birds in each replicate. The birds were reared on the concrete floor, covered with a 2-inch layer of rice husk as bedding material. The feed was offered ad libitum while the supply of fresh and clean water was made available round the clock. The room temperature was maintained at 33°C during the first week of the trial with a reduction of 3°C every week. All the birds were reared under identical managemental conditions throughout the experiment.

Table 1 – Nutrient composition of Hi-Pro corn DDGS.

Nutrients	%age
Dry matter	91.8
Crude protein	46.8
Ether extract	3.40
Crude fiber	0.51
Ash	5.10
Phosphorus (total)	0.92
Calcium	0.24
Lysine	1.131
Methionine	1.048
Cysteine	0.856
Threonine	1.682
Arginine	2.071
Iso-Leucine	1.799
Leucine	5.550
Valine	2.371
Met + Cys	1.904
Aspartic Acid	3.480
Serine	2.370
Glutamic Acid	8.100
Glycine	1.650
Alanine	3.390
Phenylalanine	2.400
Histidine	1.280

[1]All analysis were run in duplication and results are reported on a DM basis except Dry matter %

Exogenous enzymes

The NSPase (Winzyme MX®@100 g/mt) used in this study was provided by Suntaq International Limited, China. Winzyme MX® was a combination of endo1, 4 D xylanase and β-mannanase made by advanced deep ventilation fermentation method and contained 5000 IU/g β-mannanase and 15000 IU/g Xylanase. One unit of β-mannanase was added to the amount of enzyme, which liberates 1 µmol of reducing sugar (mannose) from 3 mg/ml of mannan solution in 1 min, at 37°C and pH5.5 and one unit of Xylanase is defined as that amount of enzyme required to liberate 1 µmol reducing sugar from xylan solution per minute under the condition of xylan solution concentration 5 mg/ml, at 37°C and pH value 5.50,

The exogenous enzyme protease (Cibenza DP 100 @250g/MT) used in this experiment was provided by Novus International Inc. One gram of Cibenza DP 100 contained 600000 U/g. Cibenza DP 100 produced by *Bacillus licheniformis*.

Data collection

Weekly data on body weight and feed intake were recorded to calculate feed conversion ratio (FCR). On day 35, two birds per replicate were randomly picked and killed to record carcass %, relative breast, thigh and soft organ weight (liver, heart, and gizzard).

Digestibility assay and intestinal sampling

On day 29, a digestibility assay was conducted to measure the nutrient digestibility of experimental diets. Celite® (AIA) was used as an external digestibility marker. The celite® was mixed at 1% in all the experimental diets. These diets were fed from day 29 to 35 of the trial. On day 35, two birds from each replicate were randomly selected and killed by cervical dislocation for determining duodenum, jejunum and ileum lengths. The jejunum samples were collected by following Wang *et al.* (2015). Tissue samples (1.5 cm) obtained from the midpoint of jejunum were preserved in 10% buffer formalin phosphate solution until further analysis. Villi was photographed under a light microscope (Fasina *et al.* 2010).

Ileal digesta samples were collected from the ileum of 4 birds per replicate to determine the nutrient digestibility and apparent ileal digestible energy (AIDE) of experimental diets by the method described by Scott and Boldaji (1997). For this purpose, the intestinal tract was excised, and the contents of the tract from Meckel's diverticulum to 40 mm cranial to ileal-cecal junction were flushed into 200 ml plastic cups and shifted immediately to an ice container. A few drops of formalin were also added to digesta samples to stop any bacterial activity. The digest samples within the pen were pooled and dried in hot air oven at 65°C till constant weight was achieved. Dried samples were ground to pass through 0.5 mm sieve and were stored at -10°C till further analyses.

Chemical analysis

All diets and ileal digesta samples were analyzed for total nitrogen , AIA and gross energy (GE). Total N was analyzed by using micro Kjeldahl's apparatus (AOAC, 2000) as described in recent studies (Rahman *et al.* 2019). The AIA contents of the experimental diets and digested samples were determined following Vogtmann *et al.* (1975) and GE determined by Bomb calorimeter (Parr Instrument Co., Moline, IL). The total amino acids (AAs) contents of the experimental diets and ileal digesta were analyzed by using amino acid analyzer (Biochrom 30 Plus, Biochrom Ltd. Cambridge UK). All samples were oxidized with hydrogen peroxide + formic acid + phenol solution and sodium disulfide was used to decompose the excess oxidation reagent. After oxidation the samples were hydrolyze using 6*M* HCl for 24 hours. The pH of the hydrolyzed sample was adjusted to 2.20 and filtered; the amino acid profile was determined.

Table 2 – Ingredients composition and nutrient profile of basal diet

Ingredients	%age
Hi-Pro corn DDGS	6.41
Maize	60.0
Canola Meal	7.00
Rapeseed Meal	2.65
Soybean Meal 44%	17.36
Calcium carbonate	1.86
MCP	0.93
Sodium Chloride	0.15
Sodium bicarbonate	0.21
Lysine Sulphate	0.70
Dl-Methionine	0.14
L-Threonine	0.18
L- valine	0.12
L- Tryptophan	0.02
L Isoleucine	0.13
Sunflower Oil	1.50
Vitamin and Mineral premix*	0.50
Phytase 10000 FTU/kg	0.01
Betaine	0.13
Total	100
Analyzed nutrients	%
Dry Matter	88.82
Crude Protein	20.11
Ether extract	4.61
Calcium	0.87
Total Phosphorus	0.62
Sodium	0.17
Lysine	1.35
Methionine	0.50
Met + Cys	0.90
Tryptophan	0.23
Threonine	0.92
Iso-leucine	0.90
Valine	1.06
ME Kcal/kg (Calculated)	2900

*Vitamin A 10 mg/kg, vitamin D_3 9 mg/ kg vitamin E 50 mg/Kg, vitamin K_3 3.6 mg/Kg, vitamin B_1 1.7 mg/Kg, vitamin B_2 10 mg/Kg, vitamin B_3 35 mg/Kg, vitamin B_5 11.1 mg/Kg, vitamin B_6 3.1mg/Kg vitamin B_9 1.1mg/Kg, vitamin B_{12} 1.2 mg/Kg, vitamin H_5 mg/Kg, magnesium sulphate 18 mg/Kg and zinc sulphate 2 mg/Kg , [1]moncalcium phosphate.

Calculation and statistical analysis

The apparent digestibility coefficient of the nutrient was calculated by Ravindran *et al.* (1999).

$$\text{Nutrient Digestibility (\%)} = \frac{\left(\frac{N}{AIA}\right)\text{diet} \times \left(\frac{N}{AIA}\right)\text{digest}}{\left(\frac{N}{AIA}\right)\text{diet}}$$

N= Nutrient and AIA= Acid insoluble ash

The AIDE of the diets was calculated by the following formula:

$$AIDE_{(kcal/kg)} = GE_{diet} - GE_{digesta} \times \left[\left(\frac{\text{AIA in diet}}{\text{AIA in digesta}}\right)\right]$$

AIDE = Apparent ileal digestible energy

GE = Gross energy

Data were subjected to one-way ANOVA using the GLM procedure at a probability level of $p<0.05$ to determine the statistical significance and means were analyzed by Tukey test (SAS, 2000).

RESULTS

Growth performance

Data on feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) are present in Table 3. The supplementation of NSPase (combination of mannanase and xylanase) and protease alone or in combination did not improve ($p>0.05$) the FI, BWG and FCR during starter (day 1-21), finisher (day 22-35) and overall phase (day 1-35). However, a tendency of improvement in BWG was noted by the supplementation of exogenous enzymes (protease and NSPase) during the starter phase (day 1-21) over control. This effect was not observed during the finisher phase. The combination of enzymes (mannanase, xylanase, and protease) did not show any effect over control and or when supplemented alone (Table 3).

Carcass yield, breast and thigh yield and relative organs weight (gizzard, liver, and heart) are presented in Table 4. The supplementation of exogenous enzymes did not affect ($p>0.05$) these parameters.

Intestinal health

There was no difference ($p>0.05$) in the small intestine's length (duodenum, jejunum, ileum, and total length) with the supplementation of exogenous enzymes compared to the control. Duodenum length tended to increase numerically ($p=0.78$) in those birds fed the control diet compared to the diets supplemented with exogenous enzymes. Villus height, crypt depth, and crypt width were also not improved ($p>0.05$) by the supplementation of exogenous enzymes alone or in combination.

Nutrient digestibility

No difference *was* observed in protein, and apparent amino acids digestibility ($p>0.05$) in response to protease and NSPase compared to the control. Protein digestibility tended to numerically increase by 1.49 and 1.68%, respectively in the diets supplemented with protease and combination of protease and NSPase

Table 3 – Effect of protease and NSPase enzymes on growth performance in Hi-Pro corn DDGS based diet.

Age (Days)	Parameters	Treatments					
		CON[1]	CON-P[2]	CON-MX[3]	CON-PMX[4]	SEM	*p* value
1-21	Feed intake (g)	1141.8	1186.7	1165.2	1163.1	17.9	0.397
	Weight gain (g)	819.9	850.1	858.3	859.2	11.1	0.077
	FCR*	1.392	1.395	1.357	1.354	0.01	0.066
22-35	Feed intake (g)	2187.6	2171.2	2153.1	2106.2	27.9	0.232
	Weight gain (g)	1303.8	1290.5	1294.8	1261.3	22.3	0.587
	FCR	1.679	1.683	1.664	1.670	0.01	0.892
1-35	Feed intake (g)	3329.4	3357.9	3318.3	3269.3	40.9	0.503
	Weight gain (g)	2122.9	2140.6	2153.1	2120.5	29.7	0.847
	FCR	1.568	1.568	1.541	1.542	0.01	0.227

Means of 5 replicates having ten birds per replicate

*Feed conversion ratio

[1] Contain % Hi-Pro DDGS based diet, [2]Hi-Pro DDGS + Protease (Cibenza DP 100)

[3] Hi-Pro DDGS + NSPase (Wizyme MX), [34]Hi-Pro DDGS Protease (Cibenza DP100) + NSPase (Winzyme MX)

Table 4 – Effect of protease and NSPase on carcass and organ characteristics in Hi-Pro corn DDGS based diet.

Parameters (%)	CON[1]	CON-P[2]	CON-MX[3]	CON-PMX[4]	SEM	*p* value
Carcass	65.98	65.90	65.96	66.67	0.57	0.750
Breast	27.31	28.20	28.10	28.49.	0.85	0.792
Thigh	8.15	8.02	8.10	8.24	0.31	0.966
Liver	2.40	2.45	2.37	2.20	0.12	0.565
Gizzard	1.05	1.07	1.14	0.94	0.06	0.200
Heart	0.65	0.59	0.60	0.56	0.02	0.238

Means of 5 replicates having ten birds per replicate

[1] Conn 15% Hi-Pro DDGS based diet, [2]Hi-Pro DDGS + Protease (Cibenza DP 100)

[3]Hi-Pro DDGS + NSPase (Wizyme MX), [4]Hi-Pro DDGS + Protease (Cibenza DP100) + NSPase (Winzyme MX)

Table 5 – Effect of protease and non-starch polysaccharidases on intestinal morphology in Hi-Pro corn DDGS based diet.

Parameters	CON[1]	CON-P[2]	CON-MX[3]	CONPMX[4]	SEM	*p*-Value
Duodenum (cm)	32.10	30.60	31.00	30.50	1.23	0.787
Jejunum (cm)	78.30	76.10	73.70	79.60	2.30	0.320
Ileum (cm)	77.10	78.80	74.50	79.60	2.01	0.321
Small intestine (cm)	187.50	185.50	179.20	189.70	3.84	0.284
Villus Height (µm)	817.80	846.6	822.4	854.9	32.0	0.757
Crypt depth (µm)	107.59	104.32	116.92	123.60	5.90	0.125
Crypt width (µm)	40.77	39.99	46.09	50.71	2.01	0.053
VH/CD[5]	7.81	8.30	7.24	7.14	0.41	0.207

Means of 5 replicates having ten birds per replicate

[1] Contain % Hi-Pro DDGS based diet [2]Hi-Pro DDGS + Protease (Cibenza DP 100)

[3] Hi-Pro DDGS + NSPase (Wizyme MX), [34]Hi-Pro DDGS + Protease (Cibenza DP 100)+ NSPase(Winzyme MX)

[5] Villus height/ Crypt depth

compared to the control. A similar trend was observed on apparent amino acids digestibility and AIDE by the supplementation of exogenous enzymes in corn and Hi-Pro DDGS based diets.

DISCUSSION

It has been reported that starch content of corn during dry milling process is utilized for ethanol production and non-starch polysaccharide content of the remaining proportion increased 2 to 3 folds over intact grain (Spiehs *et al.* 2002). Kim *et al.* (2008) analyzed the polymeric sugars content of DDGS and reported that corn DDGS contained a high content of glucan, xylan, and arabinan. In another study Pedersen *et al.* (2014) reported that NSP content of corn DDGS ranged 250-337 g/kg on DM basis and the distribution of sugar constituent was in the order of xylose >

arabinose > NCP-glucose > mannose > galactose. It is also well documented that during drying process amino acids digestibility were compromised due to heat treatments (Amezcua and Parsons 2007; Bandegan *et al.* 2009; Olukosi *et al.* 2010).

Previous studies that evaluated the supplemental effect of exogenous enzymes reported inconsistent findings (Choct *et al.* 1999; Cowieson *et al.* 2006; Gao *et al.* 2007). In the present study, supplementation of protease, NSPase and the combination of protease and NSPase did not improve weight gain and feed conversion ratio_ENREF_24_ENREF_48. The reason for the lack of improvement due to exogenous enzymes supplementation on growth performance in the present study could be because Hi-Pro DDGS has less content of non-starch polysaccharide and less exposure to heat. Another reason for the lack of improvement might be due to the negative effect of exogenous protease supplementation on the secretion of the endogenous enzyme as reported by Kaczmarek *et al.* (2014). Olukosi *et al.* (2007, 2010) reported similar results. Olukosi *et al.* (2007, 2010) reported that the blend of xylanase, amylase and protease had no effect on growth performance and feed efficiency. Campasino *et al.* (2015) reported that the supplementation of NSPase (xylanase, glucanase, and galactosidase) in corn DDGS-based diet did not improve weight gain and FCR in the control diet. However, the enzyme supplementation in low energy diet (132 Kcal/kg less energy than control) improved weight gain. Zou *et al.* (2013) reported that the enzyme supplementation (mannanase, xylanase, and glucanase) in broiler diet did not improve weight gain. But FCR was improved. This improvement in FCR was attributed to the increased pancreatic secretions and activities of digestive enzymes.

Cowieson & Ravindran (2008) reported that the supplementation of enzymes (xylanase, protease, and amylase) in corn -SBM-based diet improved weight gain and feed efficiency, but feed intake remained unaffected. In another study, Cowieson *et al*. (2006) reported that the supplementation of xylanase, amylase, and protease improved the weight gain but did not improve the FCR.

O'Neil *et al*. (2012) reported that the supplementation of xylanase in corn-SBM based diets cause reduction of digesta viscosity and convert arabinoxylans to Xylo-oligomers which may act as prebiotic for beneficial bacteria. A similar finding was also observed by Ferreira *et al*. (2016), and they reported that the supplementation of mannanase alone in corn-SBM based diet improve weight gain and FCR. In the present study, the supplementation of exogenous enzymes did not improve growth performance.

Carcass and soft organ weights were not affected by different dietary treatments. Mahmood *et al.* (2018) also reported that the supplementation of protease enzyme did not improve carcass yield, breast meat yield, thigh meat yield, gizzard, liver, and heart. Similar findings were also reported by various scientists (Gao *et al.* 2007; Hajati 2010; Opoku *et al.* 2015; Dalólio *et al.* 2016).

The small intestine is a major component of the digestive tract for digestion and nutrient absorption. Pluripotent columnar cells of the small intestine can

Table 6 – Effect of protease and non-starch polysaccharidases on crude protein, amino acids and apparent ileal digestible energy in Hi-Pro corn DDGS based diet.

Nutrients (%)	CON[1]	CON-P[2]	CON-MX[3]	CON-PMX[4]	SEM	*p*-Value
Protein	81.20	82.41	81.39	82.57	0.46	0.167
Cysteine	80.29	81.02	80.68	80.76	0.60	0.860
Methionine	88.87	90.05	89.41	90.64	0.62	0.244
Met + Cys	85.35	86.39	85.80	86.54	0.41	0.189
Threonine	80.99	81.65	81.59	82.05	0.48	0.508
Valine	80.61	81.59	80.91	81.89	0.85	0.699
Isoleucine	81.90	82.75	82.21	83.03	0.66	0.632
Leucine	87.76	87.98	87.80	88.48	0.59	0.822
Phenylalanine	82.35	83.40	82.95	83.94	0.55	0.258
Histidine	80.64	82.12	81.69	82.28	0.64	0.301
Lysine	80.75	81.87	81.12	82.09	0.70	0.509
Arginine	88.62	89.17	89.47	89.21	0.52	0.680
[5]AIDE Kcal/kg	2941.9	2957.9	2992.2	2975.7	30.1	0.686

Means of 5 replicates having 10 birds per replicate

[1] Contain % Hi-Pro DDGS based diet, [2]Hi-Pro DDGS + Protease (Cibenza DP 100)

[3] Hi-Pro DDGS + NSPase (Wizyme MX) [4] Hi Pro DDGS+ Protease (Cibenza DP 100) + NSPase (Winzyme MX)[5] apparent ileal digestible energy

differentiate into digestive, absorptive, or mucin-producing roles (Moog 1950; Cheng and Leblond 1974). Intestinal integrity (villus height, crypt depth, and goblet cell) are influenced by the dietary manipulation especially the supplementation of enzymes (Baurhoo *et al.* 2007; Salim *et al.* 2013). Mehri *et al.* (2010) reported that supplementation of 0.09% β- mannanase in corn-SBM basal diet improved villus height. Zou *et al.* (2013), also reported improved intestinal integrity by the supplementation of xylanase, glucanase, and mannanase in broilers. In the current study, no improvements in intestinal integrity were observed by the supplementation of exogenous enzymes (mix of mannanase, xylanase, and protease). Duodenum, jejunum and ileum weight and length remained the same by the enzyme (proteases and a mix of mannanase and xylanase) when supplemented alone or in combination. Opoku *et al.* (2015) also found that protease and mannase alone in 30% wheat DDGS based broiler diet did not affect duodenum, jejunum and ileum weight and length. In the present study, the supplementation of protease and mix of mannanase and xylanase did not improve the apparent ileal digestibility of CP and amino acids. Opoku *et al.* (2015) found similar findings when they supplemented mannanase in 30% wheat DDGS based diet; however, with the supplementation of protease nitrogen digestibility was improved. Improvement in nitrogen digestibility by protease supplementation in Opoku *et al.* (2015) study might be due to high inclusion level of DDGS that provided more undigested nitrogen fraction for the action of protease compared to the present study.

Campasino *et al.* (2015), also reported that the enzyme supplementation did not affect protein digestibility up to 10% inclusion level of corn DDGS, however, the improvement was observed at15% DDGS inclusion. Olukosi *et al.* (2015), reported that the supplementation of protease alone or the combination with xylanase and amylase improved nitrogen digestibility.

Apparent ileal digestible energy did not vary in response to protease and the mix of mannanase and xylanase. Similar results were reported by Olukosi *et al.* (2010). Olukosi *et al.* (2010) supplemented the blend of enzymes (Xylanase, amylase, and protease) and observed no improvement in AIDE in corn-SBM meal and corn DDGS based diet. However, Campasino *et al.*(2015) reported contradictory results and found that the supplementation of NSPase improved ME and digestible energy in corn DDGS based broiler diets. The improvement by enzyme supplementation was more pronounced when higher levels of corn DDGS were used. (Olukosi *et al.* (2015))reported an improvement in metabolizable energy with NSPase and protease. They used Low Pro corn DDGS which has high fiber content and might have led to more pronounced improvement.

In conclusion, the supplementation of NSPase and protease in Hi-Pro corn DDGS based diet did not improve the growth performance, intestinal morphology and nutrient digestibility in broiler chicken.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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