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Effect of a Multi-Carbohydrase on Growth Performance, Metabolizable Energy, Nutrients Digestibility and Intestinal Morphology of Broiler Chickens

Research Article

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ABSTRACT

The effects of a multi-carbohydrase supplementation (MS, 1250 Xylanase, 750, β-glucanase, 2500 β-mananase and 1500 α-galactosidase U/kg of basal feed) at a level of 0.05% diet on growth performance, metabolizable energy**,** nutrients utilization and intestinal morphology in broiler chickens were investigated. A total of 160 day-old male Ross 308 (10 birds/pen; n=4) were fed 4 diets (corn or wheat without or with MS) from 0-42 d. Performance was monitored at 21 and 42 d and excreta were collected from d 17 to 20 for neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP) and fat digestibility and apparent metabolizable energy corrected for nitrogen (AMEn) measurements. On d 21, jejunal segment were used for histological analysis and its contents were collected for viscosity determination. Birds fed corn diets had better (P<0.01) body weight gain (BWG) and feed conversion ratio (FCR) than birds fed wheat-based diet, while birds fed MS had better BWG and FCR and lower feed intake (FI) than birds fed non-MS diet. Birds fed diets containing MS had lower digesta viscosity (–28%) compared to birds fed diets without MS (P<0.01) and a greater reduction of viscosity due to MS was observed in birds fed wheat diets (–36%) than in corn diets (–13%). Birds receiving diets with MS derived more dietary AMEn (2.15%) compared to those fed diets without MS (P<0.01). Digestibility of NDF and CP was higher (16% and 6.4%, respectively) in birds fed corn-based diet compared to birds fed wheat-based diet and in this context, digestibility of NDF in birds fed MS was higher (10%) than birds fed non-MS diet (P<0.05). Moreover, birds fed MS-diets had higher villus height (5.4%) and villus height: crypt depth ratio (6.5%) compared to birds fed non-MS diet (P<0.01). These results indicated that, although diet type impacted growth performance but MS was efficacious across diet types, implying that degradation of dietary fibrous components by feed enzymes may stimulate performance in broilers.

KEY WORDS broiler, digestibility, metabolizable energy, multi-carbohydrase.

INTRODUCTION

It is well known that the presence of soluble non-starch polysaccharides (NSP), such as arabinoxylans and β-glucans, in cereal grains can affect nutrient utilization adversely and decrease growth performance of broiler chickens. The high content of NSP in viscous cereal grains limit their use in poultry feeds. The anti-nutritive effect of NSP attributed to an increased digesta viscosity, a status associated with detrimental effects on digestion and absorption processes as well as changes in the structural morphology of the intestinal wall (Bach, 2011; Choct, 2015; Wickramasuriya *et al*. 2019). In wheat-based diets, the detrimental effect of the predominant NSP fraction, arabinoxylans, on production performance and nutrient utilization can be reduced by addition of xylanases in the diet (Wu *et al*. 2004; Choct *et al*. 2006). Studies indicated that, addition of appropriate exogenous enzymes (xylanases and βglucanases) to broiler diets based on wheat or barley, or both, has been shown to improve nutrient digestibility and performance (Ouhida *et al.* 2000; Meng *et al.* 2005). The efficacy of NSP degrading enzymes in enhancing nutrients and energy utilization in poultry is well documented and has been largely associated with reduction of intestinal viscosity through breakdown of NSP (Slominski, 2011; Kiarie *et al.* 2014; Liu and Kim, 2017).

Wheat is a major component of most animal diets in many countries and also could be considered as a suitable replacement for corn in poultry diets (Smeets *et al.* 2018). Although some nutrients and energy contents of wheat are lower than those for corn, the contents of protein, calcium, phosphorus, and some amino acids are greater in wheat (NRC, 1994). However, the inclusion level of wheat is limited often because of its variable chemical composition, as well as its high NSP content (Choct and Annison, 1992). Exogenous enzyme supplementation of wheat based diets is a common practice in commercial feeding of poultry to decrease digesta viscosity and extract a greater amount of the available nutrients from the feed consumed, thereby improving feed efficiency and reducing feed costs (Kiarie *et al.* 2014; Taylor *et al.* 2018).

On the other hand, corn is by far the most commonly used cereal grain in the diets of poultry. One reason for the widespread use of corn is the perception of consistent and high nutritional value (Slominski, 2011). It has been demonstrated that the chemical composition and nutritional value of corn is variable (Cowieson, 2005), and could be improved by supplemental enzymes (Cowieson *et al.* 2010; Gehring *et al.* 2012). However, the supplemental enzymes response in corn-based diets is generally lower than could be expected for diets based on viscous grains such as wheat, rye, and barley (Adeola and Cowieson, 2011; Slominski, 2011). Presumably, the reason for the lower magnitude of supplemental enzymes in corn-based diets is the lower concentration of antinutritive high molecular weight soluble NSP (Bedford and Schulze, 1998). Therefore, feed supplementation with NSP-degrading enzymes (NSPases) has become a common practice in poultry corn or wheat–based diets (Silversides and Bedford, 1999), and has been shown to decrease intestinal viscosity and enhance nutrient digestibility and performance, thus demonstrating potential in improving the utilization of fibrous feed ingredients in poultry diets (Jozefiak *et al.* 2010; Mendes *et al.* 2013; Kiarie *et al.* 2014).

However, considerable published data has shown that the NSPases used have specificity for specific NSP or feed ingredients, many studies were used a single or double NSP-degrading enzyme in poultry diets (Chesson, 2001; Selle *et al.* 2003; Jozefiak *et al.* 2010; Smeets *et al.* 2014, Munyaka *et al.* 2016).

Furthermore, some studies reported cases in which no effect of added NSPases on nutrient digestibility or growth performance of poultry were observed (Rebol´e *et al.* 2010; Adeola and Cowieson, 2011), whereas Rebol´e *et al.* (2010) observed no effect of addition of enzyme to diets on the performance and ileal or cecal bacterial populations. Therefore, due to the presence of a large array of chemical characteristics among NSP fractions in feed ingredients, the use of NSPase blend with specificities for a range of substrates would be more effective. This is of a particular importance for viscous grains, as the NSP fraction of these feed ingredients contain high levels of arabinoxylans, β-glucans, β-mannans and cellulose.

Thus, the aim of present study was to evaluate the effect of supplementing a blend of NSPase (β-glucanase, β-mannanase, α-galactosidase and xylanase) on growth performance, nutrient digestibility, feed AMEn, intestinal morphology and contents viscosity of broiler chickens fed diets containing corn or wheat-based diets. Moreover, due to the wide range of NSP in poultry feed ingredients, it was hypothesized that the blend of NSPase would improve performance and nutrient digestibility compared to when the enzyme is used alone.

MATERIALS AND METHODS

Birds and management

A total of 160 day-old male Ross 308 broiler chicks were obtained from a local commercial hatchery and were housed in thermostatically controlled starter battery brooders, exposed to fluorescent light for 23 h/d, and temperature-controlled room (32, 28, 25, 22, 19 and 18 ˚C during 1, 2, 3, 4, 5 and 6 weeks, respectively). On arrival, chicks were individually weighed and were randomly assigned to pens (10 birds/pen) based on body weight.

Diets and experimental design

Two different basal diets were formulated to meet NRC (1994) nutrient specifications for broiler chicks (Table 1). The diets were based on either corn or wheat grains without or with supplemental multi-carbohydrase. The multi-enzyme blend used was ZYMPEX® 008, a product of Impextraco NV, Belgium. The blend was included in the appropriate diets at a level of 0.05% according to the manufacturer's recommendation. It provided 1250, 750, 2500 and 1500 units of xylanase, β -glucanase, β-mananase and α-galactosidase, respectively, per kg of basal feed.

All diets of 0-21 d contained 0.3% chromium oxide as indigestible marker and were fed as mash. In a completely randomized design, the chicks were allocated into the 4 treatments with 4 replicate pens per treatment and 10 chicks per pen.

Table 1 Composition of the basal diets as fed

Item	Starter, 0-21 d		Finisher, 22-42 d	
	Corn	Wheat	Corn	Wheat
Ingredient %				
Corn	53.8	-	60.22	-
Wheat (14.2 % CP)	-	62.74	-	70.19
Soybean meal (44 % CP)	38.26	28.36	32.27	21.09
Soybean oil	3.72	4.54	4.10	5.00
Dicalcium phosphate	1.52	1.56	1.19	1.24
Limestone	1.23	1.22	1.29	1.27
Salt	0.45	0.38	0.34	0.27
L-lysine HCl	-	0.13	-	0.22
DL-methionine	0.22	0.23	0.09	0.10
L-threonine	-	0.04	-	0.12
Vit/Min. premix[1]	0.50	0.50	0.50	0.50
chromium oxide	0.30	0.30	-	-
Calculated composition				
Apparent metabolizable energy (AME), kcal/kg	3000	3000	3100	3100
Crude protein (CP), %	21.56	21.56	19.37	19.37
Crude fibre (CF), %	3.86	4.50	3.58	4.28
Lys, %	1.10	1.10	1.02	1.00
Met, %	0.55	0.56	0.39	0.41
Met + Cys, %	0.90	0.90	0.72	0.72
Thr, %	0.79	0.75	0.73	0.72
Tryp, %	0.31	0.29	0.27	0.25
Calcium, %	0.94	0.94	0.87	0.87
Available phosphorus, %	0.42	0.42	0.35	0.35
Sodium, %	0.19	0.19	0.15	0.15

[1] Provided per kilogram of diet: vitamin A: 9000 IU; vitamin D: 2000 IU; vitamin E: 18 IU; vitamin B_{12}: 0.015 mg; Riboflavin: 6.6 mg; D-pantothenic acid: 10 mg; Niacin: 30 mg; Menadione sodium bisulfite: 3 mg; Manganese: 100 from manganese oxide; Iron: 50 from iron sulfate; Zinc: 100 from zinc oxide; Copper: 10 from copper sulfate; Iodine: 0.99 from ethylene diamine dihydroiodide and Selenium: 0.2 from sodium selenite.

The experiment lasted for 42 d, during which feed and water were offered *ad libitum*. Birds were weighed at 21 and 42 d of age following a feed deprivation period of 2 to 4 h to determine body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR). Mortality was recorded daily and required adjustments in BWG and FI were made.

Excreta and digesta sampling

From d 17 to 20 of the experiment, excreta from all the birds within a pen were pooled in a bag, immediately frozen and subsequently freeze-dried. Then, excreta samples were mixed well using a blender and freeze-dried. Dried samples were ground to pass through a 0.5mm sieve and stored in airtight plastic containers at -4 ˚C until chemical analysis. On d 21, two bird per pen were euthanized by cervical dislocation and body cavity of each bird was opened. The section between duodenum and ileo-ceco-colonic junction was clamped and separated. The markings were established for jejunum from end of duodenum to Meckel's diverticulum. Jejunal segments were used for histological analysis and contents collected in separate sample bags for estimating jejunal viscosity.

Gut histomorphology

The jejunal segments at 21 d (2 birds per pen) were opened longitudinally and washed with physiological saline solution. Then, tissue samples were taken from the middle part of the jejunum. Samples for light microscopy examination (pieces about 5 cm in length) were placed in 10% formalin in 0.1 M phosphate buffer (pH=7.0) for fixation, where they were gently shaken to remove any adherent intestinal content and were sent to the histology laboratory (Faculty of veterinary Medicine, University of Tabriz, Iran) for analysis. The processing consisted of serial dehydration in graded ethanol solutions (50, 70, 80, 96, and 100%), clearing with xylene and embedding in paraffin (Luna, 1968). Tissue samples were then sectioned using a microtome at a thickness of 5 µm (3 cross sections from each sample), placed on glass slides, and stained with hematoxylin and eosin. Histological sections were examined with an Axioplan-2 optical microscope (Carl Zeiss Jena GmbH, Jena, Germany) coupled with a refrigerated Q Imaging Retiga 4000R digital camera with a charge-coupled device detector. The images were analyzed using image software. Villus height (µm) was measured from the tip of the villus to the villus crypt junction, and crypt depth was defined as the depth of the invagination between adjacent villi.

Viscosity measurement

Viscosity of jejunal was determined according to the method described by Almirall *et al.* (1995) at animal science laboratory (Iranian Animal Resraech Institute, Karaj, Iran). Fresh digesta were obtained from two bird per pen and immediately placed in a centrifuge tube and centrifuged at 3000 g for 15 min. Supernatant (0.5 mL) was withdrawn and the viscosity was determined using a Brookfield viscometer maintained at 40 ˚C with a CP40 cone with shear rates of 5 to 500/s. The reading was taken after one minute.

Chemical analysis

Experimental diets and excreta samples were finely ground using a coffee grinder for dry matter (DM), nitrogen, chromium, fat, neutral detergent fiber (NDF) and acid detergent fiber (ADF) and gross energy (GE) determination. DM was determined by drying of samples in a forced draft oven at 105 ˚C to a constant weight. Crude protein (6.25×N) in wheat was determined by the combustion method (AOAC, 2000). Fat (as ether extract) was extracted with diethyl ether by the ether extraction method (AOAC, 2000) using a Soxtec system (Foss UK Ltd.). For analysis of NDF and ADF, ANKOM 08–16–06 method was used. Diets and excreta samples were analyzed for chromium after the samples were ashed at 600 ˚C for 12 h in a muffle furnace, using inductively coupled plasma mass spectrometry according to the method of AOAC (2005), method 985.01, whereas gross energy was determined using a Parr 6200 bomb calorimeter with benzoic acid used as the standard. Samples were sent to animal science laboratory (Iranian Animal Resraech Institute, Karaj, Iran) for chemical analysis.

The following equations were used for the calculation of the apparent total tract digestibility of CP, fat, NDF, and ADF and apparent metabolizable energy corrected for nitrogen (AMEn) content of experimental diets (Hill *et al.* 1960):

Nutrient digestibility (%)= 100 (100×(Cr diet/Cr excreta) × (nutrient excreta/nutrient diet) (Eq. 1)
AMEn (kcal/kg)= GE kcal/kg diet − [GE kcal/kg excreta × (Cr % in diet / Cr % in excreta)] (Eq. 2)
−8.22 × {N% in diet − [N% in excreta × (Cr % in diet / Cr % in excreta)]}

Where:
GE: gross energy.
N: nitrogen.
Cr: chromium.
8.22: energy equivalent of uric acid nitrogen (i.e., 8.22 kcal/kg uric acid nitrogen).

Statistical analysis

For the performance, metabolizable energy, intestinal morphology, viscosity and digestibility data, the effect of multi-enzyme supplementation was evaluated using a completely randomized design in a 2 × 2 factorial arrangement using the PROC GLM procedure of SAS (SAS, 2001). The model included the main effects of diet type, enzyme, and associated 2-way interactions. Differences among means were tested using Duncan's multiple-range tests and a significance level of 0.05 was used.

RESULTS AND DISCUSSION

The effect of multi-carbohydrase supplementation (MS) on BWG, FI and FCR in broiler chickens fed diets containing corn and wheat-based diets without or with MS is shown in Table 2. Birds fed corn diets had better (P<0.05) BWG and FCR than birds fed wheat-based diet at 0-21 d, 22-42 d and 0-42 d, while birds fed MS had better BWG and FCR than birds fed non-MS diet (P<0.05). FI improved in birds fed corn diets than birds fed wheat-based diets at 22-42 d and 0-42 d. Also, FI in birds fed MS had lower than birds fed non-MS diet at 0-21d (P<0.01) and 0-42d (P<0.05); however, MS reduced FI to a greater extent in wheat diets than in corn-based diet at 0-21d (P<0.01).

Viscosity in birds fed corn-based diets had lower than (35%) birds fed wheat-based diets (P<0.01). Birds fed diets containing MS had lower digesta viscosity (–28%) compared to birds fed diets without MS (Table 3). There was an interaction (P<0.01) between diet type and MS on jejunal digesta viscosity such that greater reduction of viscosity due to MS was observed in birds fed wheat diets (–36%) than in corn diets (–13%). However, the AMEn was not different between wheat and corn-based diets, but birds receiving diets with MS derived more dietary AMEn (2.15%) compared with those fed diets without MS (P<0.01). The digestibility of NDF and CP was higher (16% and 6.4%, respectively) in birds fed corn-based diet compared to birds fed wheat-based diet (P<0.05). In this context, digestibility of NDF in birds fed MS had higher (10%) than birds fed non-MS diet (P<0.05). Other parameters (ADF and fat digestibility) measured did not differ among treatment groups.

The effect of MS on villus height, epithelial thickness and crypt depth of jejunum at 21-day-old broiler chickens fed diets containing corn and wheat-based diets is shown in Table 4. The villus height and villus height:crypt depth ratio was higher (5.4% and 6.5%, respectively) in birds fed MS-diets compared to birds fed non-MS diet (P<0.01).

Table 2 Effects of multi-carbohydrase supplementation (MS) on performance of broiler chickens fed corn- or wheat-based diets[1]

Item	MS[2]	BW gain (g/bird)[3]			Feed intake (g/bird)[3]			Feed gain (g/g)[3]		
		0-21 d	22-42 d	0-42 d	0-21 d	22-42 d	0-42 d	0-21 d	22-42 d	0-42d
Treatments										
Corn	-	747	1484	2231	959[b]	2965	3924	1.28	1.99	1.76
	+	773	1522	2295	946[c]	2957	3904	1.22	1.94	1.7
Wheat	-	708	1437	2146	977[a]	3080	4057	1.38	2.14	1.89
	+	729	1490	2219	925[d]	3030	3955	1.26	2.03	1.78
SEM		18	35	36	5.57	46	47	0.02	0.04	0.03
Main effect										
Diet										
Corn		760[a]	1503[a]	2263[a]	952	2961[b]	3914[b]	1.23[b]	1.97[b]	1.72[b]
Wheat		718[b]	1463[b]	2182[b]	951	3055[a]	4006[a]	1.32[a]	2.08[a]	1.83[a]
Enzyme										
-		728[b]	1460[b]	2189[b]	968[a]	3022	3990[a]	1.33[a]	2.07[a]	1.82[a]
+		751[a]	1506[a]	2257[a]	936[b]	2993	3929[b]	1.24[b]	1.98[b]	1.74[b]
Probability										
Diet		< 0.01	0.04	< 0.01	0.60	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Enzyme		< 0.01	0.02	< 0.01	< 0.01	0.23	0.02	< 0.01	< 0.01	< 0.01
Diet ×Enzyme		0.66	0.69	0.82	< 0.01	0.38	0.11	0.07	0.24	0.15

[1] Each mean represents values from 4 replicates (10 birds/replicate).
[2] Multi-carbohydrase supplementation (MS) blend supplied 1250, 750, 2500 and 1500 U/kg of basal feed of xylanase, β-glucanase, β-mananase and α-galactosidase, respectively.
[3] Corrected for mortality.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 Effects of Multi-carbohydrase supplementation (MS) on jejunal viscosity, apparent metabolizable energy corrected for nitrogen (AMEn) and nutrient digestibility in broiler chickens fed corn- or wheat-based diets[1]

Item	MS[2]	Viscosity[3]	AMEn, kcal/kg	NDF[4] (%)	ADF[4] (%)	Fat (%)	CP (%)
Treatments							
Corn	-	2.13[c]	3008	32.82	18.17	87.25	77.74
	+	1.84[d]	3065	35.08	17.74	86.57	78.28
Wheat	-	3.71[a]	2995	26.11	15.24	85.46	72.76
	+	2.36[b]	3070	30.51	16.26	87.13	73.26
SEM		0.12	21.37	1.42	2.34	3.41	2.020
Main effect							
Diet							
Corn		1.99[b]	3036	33.95[a]	17.96	86.87	78.00[a]
Wheat		3.07[a]	3032	28.30[b]	15.75	86.25	73.01[b]
Enzyme							
-		2.95[a]	3001[b]	29.46[b]	16.71	86.37	75.25
+		2.10[b]	3067[a]	32.78[a]	17.01	86.75	75.76
Probability							
Diet		< 0.01	0.69	0.01	0.12	0.85	0.03
Enzyme		< 0.01	< 0.01	0.03	0.83	0.91	0.81
Diet × enzyme		< 0.01	0.39	0.46	0.61	0.74	1.10

[1] Each mean represents values from 4 replicates (10 birds/replicate).
[2] Multi-carbohydrase supplementation (MS) blend supplied 1250, 750, 2500 and 1500 U/kg of basal feed of xylanase, β-glucanase, β-mananase and α-galactosidase, respectively.
[3] Viscosity was measured in Mpa.
NDF: neutral detergent fiber and ADF: acid detergent fiber.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Crypt depth and epithelial thickness did not differ among treatment groups.

Performance data from the current study showed that BWG and FI and FCR was influenced by both the diet type and the presence of the MS; in this case, birds fed corn diets had better BWG and FCR compared to the birds receiving wheat-based diets.

This observation was expected because it is well recognized that feed ingredients such as wheat that are rich in viscous NSP result in poor broiler growth performance relative to nonviscous cereals such as corn (Marquardt *et al.* 1994; Jia *et al.* 2009; Rodríguez *et al.* 2012).

Table 4 Effects of multi-carbohydrase supplementation (MS) on villus height, crypt depth, and epithelial thickness in the jejunum of broiler chickens[1]

Item	MS[2]	Villus height (μm)	Crypt depth (μm)	Villus height:crypt depth	Epithelial thickness (μm)
Treatments					
Corn	-	795	390	2.04	55.48
	+	842	377	2.23	58.75
Wheat	-	777	389	2.00	60.24
	+	820	392	2.09	55.73
SEM		10.5	8.8	0.04	2.24
Main effect					
Diet					
Corn		818	383	2.13	57.12
Wheat		798	390	2.04	58.10
Enzyme					
-		786[b]	389	2.02[b]	57.87
+		831[a]	384	2.16[a]	57.25
Probability					
Diet		0.06	0.39	0.06	0.70
Enzyme		< 0.01	0.54	< 0.01	0.78
Diet × enzyme		0.78	0.32	0.31	0.11

[1] Each mean represents values from 4 replicates (10 birds/replicate).
[2] Multi-carbohydrase supplementation (MS) blend supplied 1250, 750, 2500 and 1500 U/kg of basal feed of xylanase, β-glucanase, β-mananase and α-galactosidase, respectively.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Indeed, the present data showed that wheat-based diets contained more than the concentration of dietary fiber relative to corn diets, which may be implicated in the higher jejunal digesta viscosity observed in birds fed wheat diets. An accepted paradigm is that increased intestinal digesta viscosity due to dietary fibrous is the key mechanism for poor growth and feed utilization in broilers fed wheat-based diets (Bedford and Schulze, 1998). In according with our results, other studies showed that wheat diets contained more than the concentration of NSP relative to corn diets, which may be implicated in the higher jejunal digesta viscosity observed in birds fed wheat diets (Kiarie *et al.* 2014).

However, in disagreement with our data, Munyaka *et al.* (2016) showed that birds fed corn diets had poor performance than wheat-based diets. The reason of these observation was the presence of corn distillers dried grains with solubles (corn DDGS) in corn-based diets in their experiment. Corn DDGS are known to have high concentration of NSP (Bach, 2011; Semencenko *et al.* 2013), and therefore have negatively influenced performance of the birds. Our results illustrate that, multi-carbohydrase supplementation can improve growth performance in broiler chickens. The benefits of NSP-degrading enzymes (NSPases) in wheat-based diets for poultry is well documented and has been extensively reviewed (Munyaka *et al.* 2016; Liu and Kim, 2017). NSPases are widely used to improve poultry performance and reduce feed costs. Although their mode of action is not completely understood, reducing intestinal chyme viscosity, hydrolysis of specific chemical bonds in feedstuffs, elimination of the nutrient-encapsulating effect of the cell wall, and breakdown of anti-nutritional factors that are present in many feed ingredients, are some of the modes of actions that have been proposed (Silva and Smithard, 2002; Lazaro *et al.* 2003; Kiarie *et al.* 2013; Masey O'Neill *et al.* 2014). MS have been found to be useful in both wheat and corn-based diets and it would be expected, because the use of blend NSPase for a vast range of NSP concentration in corn or wheat would be more effective. Our data was in agreement with other studies (Kiarie *et al.* 2014; Munyaka *et al.* 2016; Wickramasuriya *et al.* 2019; Ghadeer *et al.* 2021) which reported that the use of NSP-degrading enzymes improved performance of birds fed corn and wheat-based diets. In addition, supplementation of corn-soybean based diets with xylanase and glucanase resulted in improved FCR in a 42 d study (Cowieson *et al.* 2010), which is in accordance with the improvement in FCR that was observed in this study.

Intestinal viscosity in birds fed wheat-based diets had higher than (35%) birds fed corn-based diets. These results were expected because the presence of high fiber in wheat-based diets would increase intestinal viscosity in broilers chicks. Birds fed diets containing MS had lower (–28) intestinal viscosity compared to birds fed non-MS diet and greater reduction of viscosity due to MS was observed in birds fed wheat diets (–36) than in corn diets (–13). It has been suggested historically that the mechanism of action of NSPases is viscosity reduction (Shakouri *et al.* 2009). As expected, NSPases treatment mitigated the digesta viscosity, this is in accordance with Engberg *et al.* (2004), Esmaeilipour *et al.* (2012) and Liu and Kim (2017). Also, this is in agreement with the report of Munyaka *et al.* (2016) in which the efficacy of xylanase and glucanase in reducing jejunal viscosity was much higher in the wheat-based diet compared to the corn-based diet. It seems, the improved performance of broilers by adding MS can be attributed to offset the negative effects of NSP on digesta viscosity in the gut. Indeed, there is evidence that diets based on corn still respond well to enzyme addition, suggesting there is a mechanism other than viscosity reduction. Previous evaluation of NSPases in corn diets fed to broilers suggested the mechanism may be a result of an increase in feed use efficiency driven primarily through a significant increase in BWG with a marginal effect on feed intake (Masey O'Neill *et al.* 2011; Masey O'Neill *et al.* 2012).

The use of MS may have beneficial effects in corn or wheat diets for broilers, perhaps through improvements in nutrient digestibility and AMEn. Our results indicated that, regardless of the type of cereal grain, dietary AMEn (2.15%) and digestibility of NDF (10%) improved in birds fed MS than birds fed non-MS diet. This effect is probably mediated through changes in the cell wall architecture achieved by hydrolysis of chemical bonds in dietary NPS, which may release encapsulated nutrients. Finally, degradation of NSPs with subsequent disruption of the cell wall, allows endogenous digestive enzymes (pancreatic- and epithelial-enzymes) to access cell-bound nutrients. Therefore, by increasing the endogenous enzyme's access to entrapped nutrients, the digestibility of nutrients, as well as AMEn would be increased. This might explain why MS improved AMEn and NDF utilization in both wheat and corn-based diets. In agreement with our study, Torok *et al.* (2008) showed that chickens fed barley-plus-enzyme diet had a significantly higher AMEn than chickens fed a control barley diet, as well as, Pirgozliev *et al.* (2015) and Wickramasuriya *et al.* (2019) reported that NSPase supplementation improved dietary AMEn and nutrient digestibility in broiler chickens fed corn or wheat-based diets. In contrast, the findings of Munyaka *et al.* (2016) showed that no improvement in AMEn was observed due to the addition of β-glucanase and xylanase to the diets of broiler chicks.

The villus height is associated with the absorption capacity of the enterocytes, and the presence of short villi will decrease the surface area for nutrient absorption (Parsaie *et al.* 2007). In the present study, in birds fed MS-diet, there was an improvement in villus height (5.4%) and villus height to crypt depth ratio (6.5%) in the jejunum. However, inclusion of MS had no significant effect on crypt depth or epithelial thickness. Most positive effects of NSPase in birds seemed to be related to decreased gut contents viscosity. Probably, the atrophy villus height was resulted from the increased digesta viscosity when broilers fed wheat-based diets, because the viscous environment can encapsulate nutrients thus preventing effective contact between villi and nutrients, leading to a weakened intestinal structure and function (Spector, 1976). These findings agreed with the previous study completed by Luo *et al.* (2009). Also, Karimi and Zhandi (2015) reported significant improvement in the intestinal morphological parameters of broiler chickens due to β-glucanase and / or β-mannanase supplementations. Therefore, it can be concluded that NSPase may counter the negative effects of NSP on intestinal morphology through reducing digesta viscosity.

CONCLUSION

In summary, MS was efficacious across different diet types on growth performance. Based on our results, inclusion of 0.05% of a blend of xylanase, β -glucanase, β-mananase and α- galactosidase in both corn and wheat-based diet improved growth performance (0-42 d), NDF digestibility, AMEn, jejunal morphology and digesta viscosity of Ross 308 broiler chickens, but had no effect on ADF, CP and fat digestibility. In conclusion, our data shows that diet influenced growth performance whilst enzyme supplementation was more efficacious across diet types suggesting that degradation of dietary fibrous components by feed enzymes may stimulate growth performance in broiler chickens.

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REFERENCES

Adeola O. and Cowieson A.J. (2011). Opportunities and challenges in using exogenous enzymes to improve non-ruminant animal production. *J. Anim. Sci.* **89,** 3189-3218.

Almirall M., Francesch M., P´erez-Vendrell A.M., Brufau J. and Esteve-Garcia E. (1995). The differences in intestinal viscosity produced by barley and β-glucanase alter digesta enzyme activities and ileal nutrient digestibilities more in broiler chicks than in cocks. *J. Nutr.* **125,** 947-945.

AOAC. (2000). Official Methods of Analysis. 17th Ed. Association of Official Analytical Chemists, Arlington, Washington, DC., USA.

AOAC. (2005). Official Methods of Analysis. 18th Ed. Association of Official Analytical Chemists, Gaithersburg, MD, USA.

Bach K.E. (2011). Effects of polymeric carbohydrates on growth and development in pigs. *J. Anim. Sci.* **89,** 1965-1980.

Bedford M.R. and Schulze H. (1998). Exogenous enzymes for pigs and poultry. *Nutr. Res. Rev.* **11,** 91-114.

Chesson A. (2001). Non-starch polysaccharide degrading enzymes in poultry diets: Influence of ingredients on the selection of activities. *World's Poult. Sci. J.* **57,** 260-263.

Choct M. (2015). Feed non-starch polysaccharides for monogastric animals: Classification and function. *Anim. Prod. Sci.* **55,** 1360-1366.

Choct M. and Annison G. (1992). Anti-nutritive effect of wheat pentosans in broiler chickens: Roles of viscosity and gut microflora. *British Poult. Sci*. **33,** 821-834.

Choct M., Sinlae M., Al-Jassim R.A.M. and Pettersson D. (2006). Effects of xylanase supplementation on between-bird variation in energy metabolism and the number of Clostridium perfringens in broilers fed a wheat-based diet. *Australian J. Agric. Res.* **57,** 1017-1021.

Cowieson A.J. (2005). Factors that affect the nutritional value of maize for broilers. *Anim. Feed Sci. Technol.* **119,** 293-305.

Cowieson A.J., Bedford M.R. and Ravindran V. (2010). Interactions between xylanase and glucanase in maize-soy-based diets for broilers. *British Poult. Sci*. **51,** 246-257.

Engberg R.M., Hedemann M.S., Steenfeldt S. and Jensen B.B. (2004). Influence of whole wheat and xylanase on broiler performance and microbial composition and activity in the digestive tract. *Poult. Sci.* **83,** 925-938.

Esmaeilipour O., Moravej H., Shivazad M., Rezaian M., Aminzadeh S. and Van Krimpen M.M. (2012). Effects of diet acidification and xylanase supplementation on performance, nutrient digestibility, duodenal histology and gut microflora of broilers fed wheat based diet. *British Poult. Sci*. **53,** 235-244.

Gehring C.K., Bedford M.R., Cowieson A.J. and Dozier W.A. (2012). Effects of corn source on the relationship between *in vitro* assays and ileal nutrient digestibility. *Poult. Sci.* **91,** 1908-1914.

Ghadeer A.A., Abdallah E.M., Rasha R.B. and Mahmoud H.F. (2021). Effect of a multicarbohydrase supplementation to diets varying in metabolisable energy level on the performance, carcase traits, caecal microbiota, intestinal morphology, and nutrient digestibility in broiler chickens. *Italian J. Anim. Sci.* **20,** 215-225.

Hill F.W., Anderson D.L., Renner R. and Carew Jr L.B. (1960). Studies on the metabolizable energy of grain and grain products for chickens. *Poult. Sci.* **39,** 573-579.

Jia W., Slominski B.A., Bruce H.L., Blank G., Crow G. and Jones O. (2009). Effects of diet type and enzyme addition on growth performance and gut health of broiler chickens during subclinical *Clostridium perfringens* challenge. *Poult. Sci.* **88,** 132-140.

Jozefiak D., Rutkowski A., Kaczmarek S., Jensen B.B., Engberg R.M. and Hojbjerg O. (2010). Effect of β-glucanase and xylanase supplementation of barley- and rye-based diets on caecal microbiota of broiler chickens. *British Poult. Sci.* **51,** 546-557.

Karimi K. and Zhandi M. (2015). The effect of β -mannanase and β-glucanase on small intestine morphology in male broilers fed diets containing various levels of metabolizable energy. *J. Appl. Anim. Res*. **43,** 324-329.

Kiarie E., Romero L.F. and Nyachoti C.M. (2013). The role of added feed enzymes in promoting gut health in swine and poultry. *Nutr. Res. Rev.* **26,** 71-88.

Kiarie E., Romero L.F. and Ravindran V. (2014). Growth performance, nutrient utilization, and digesta characteristics in broiler chickens fed corn or wheat diets without or with supplemental xylanase. *Poult. Sci.* **93,** 1186-1196.

Lazaro R., Garcia M., Aranibar M.J. and Mateos G.G. (2003). Effect of enzyme addition to wheat-, barley-, and rye-based diets on nutrient digestibility and performance of laying hens. *British Poult. Sci.* **44,** 256-265.

Liu W.C. and Kim I.H. (2017). Effects of dietary xylanase supplementation on performance and functional digestive parameters in broilers fed wheat-based diets. *Poult. Sci*. **96,** 566-573.

Luna L.G. (1968). Manual of Histologic staining Methods of the Armed Forces Institute of Pathology. McGraw-Hill, New York.

Luo D., Yang F., Yang X., Yao J., Shi B. and Zhou Z. (2009). Effects of xylanase on performance, blood parameters, intestinal morphology, microflora and digestive enzyme activities of broilers fed wheat-based diets. *Asian-Australasian J. Anim. Sci.* **22,** 1288-1295.

Marquardt R.R., Boros D., Guenter W. and Crow G. (1994). The nutritive value of barley, rye, wheat and corn for young chicks as affected by use of a *Trichoderma reesei* enzyme preparation. *Anim. Feed Sci. Technol.* **45,** 363-378.

Masey O'Neill H.V., Lui N., Wang J.P., Diallo A. and Hill S. (2011). Effect of xylanase on performance and apparent metabolisable energy in starter broilers fed diets containing one maize variety harvested in different regions of china. *Asian-australasian J. Anim. Sci.* **25,** 515-523.

Masey O'Neill H.V., Mathis G., Lumpkins B.S. and Bedford M.R. (2012). The effect of reduced calorie diets, with and without fat, and the use of xylanase on performance characteristics of broilers between 0 and 42 days. *Poult. Sci.* **91,** 1356-1360.

Masey O'Neill H.V., Smith J.A. and Bedford M.R. (2014). Multicarbohydrase for non-ruminants. *Asian-Australasian J. Anim. Sci.* **27,** 290-301.

Mendes A.R., Ribeiro T., Correia B.A., Bule P., Macas B., Falcao L., Freire J.P.B., Ferreira L.M.A., Fontes C.M.G.A. and Lordelo M.M. (2013). Low doses exogenous xylanase improve the nutritive value of triticale-based diets for broilers*. J. Appl. Poult. Res.* **22,** 92-99.

Meng X., Slominski B.A., Ngachati C.M., Campbell L.D. and Guenter W. (2005). Degradation of cell wall polysaccharide by combinations of carbohydrate enzymes and their effect on nutrient utilization and broiler chicken performance. *Poult. Sci*. **84,** 37-43.

Munyaka P.M., Nandha N.K., Kiarie E., Nyachoti C.M. and Khafipour E. (2016). Impact of combined β-glucanase and xylanase enzymes on growth performance, nutrients utilization and gut microbiota in broiler chickens fed corn or wheat-based diets. *Poult. Sci.* **95,** 528-540.

NRC. (1994). Nutrient Requirements of Poultry, 9th Rev. Ed. National Academy Press, Washington, DC., USA.

Ouhida I., Perez J.F., Gasa J. and Puchal F. (2000). Enzymes (β-glucanase and arabinoxylanase) and/or sepiolite supplementation on the nutritive value of maize-barley-wheat based diets for broiler chickens. *British Poult. Sci.* **41,** 617-624.

Parsaie S.J., Shariatmadari F., Zamiri M.J. and Khajeh K. (2007). Influence of wheat-based diets supplemented with xylanase, bile acid and antibiotics on performance, digestive tract measurements and gut morphology of broilers compared with a maizebased diet. *British Poult. Sci.* **48,** 594-600.

Pirgozliev V., Rose S.P., Pellny T., Amerah A.M., Wickramasinghe M., Ulker M., Rakszegi M., Bedo Z., Shewry P.R. and Lovegrove A. (2015). Energy utilization and growth performance of chickens fed novel wheat inbred lines selected for different pentosan levels with and without xylanase supplementation. *Poult. Sci.* **94,** 232-239.

Rebolé A., Ortiz L.T., Rodríguez M.L., Alzueta C., Trevino J. and Velasco S. (2010). Effects of inulin and enzyme complex, individually or in combination, on growth performance, intestinal microflora, cecal fermentation characteristics, and jejunal histomorphology in broiler chickens fed a wheat- and barley-based diet. *Poult. Sci.* **89,** 276-286.

Rodríguez M.L., Rebolé A., Velasco S., Ortiz L.T., Trevino J. and Alzueta C. (2012). Wheat- and barley-based diets with or without additives influence broiler chicken performance, nutrient digestibility and intestinal microflora. *J. Sci. Food Agric.* **92,** 184-190.

SAS Institute. (2001). SAS®/STAT Software, Release 8. SAS Institute, Inc., Cary, NC. USA.

Selle P.H., Huang K.H. and Muir W.I. (2003). Effects of nutrient specifications and xylanase plus phytase supplementation of wheat-based diets on growth performance and carcass traits of broiler chicks. *Asian. J. Anim. Sci.* **16,** 1501-1509.

Semencenko V.V., Mojovic L.V., Radosavljevic M.M., Terzic D.R., Milasinovic-Seremesic M.S. and Jankovic M.Z. (2013). Possibilities of utilization of co-products from corn grain ethanol and starch production (Review). *Hem. Ind*. **67,** 385-397.

Shakouri M.D., Iji P.A., Mikkelsen L.L. and Cowieson A.J. (2009). Intestinal function and gut microflora of broiler chickens as influenced by cereal grains and microbial enzyme supplementation. *J. Anim. Physiol. Anim. Nutr.* **93,** 647-658.

Silva S.S. and Smithard R.R. (2002). Effect of enzyme supplementation of a rye-based diet on xylanase activity in the small intestine of broilers, on intestinal crypt cell proliferation and on nutrient digestibility and growth performance of the birds. *British Poult. Sci.* **43,** 274-282.

Silversides F.G. and Bedford M.R. (1999). Effect of pelleting temperature on the recovery and efficacy of a xylanase enzyme in wheat-based diets. *Poult. Sci.* **78,** 1184-1190.

Slominski B.A. (2011). Recent advances in research on enzymes for poultry diets. *Poult. Sci.* **90,** 2013-2023.

Smeets N., Nuyens F., Van Campenhout L. and Niewold T. (2014). Variability in the *in vitro* degradation of non-starch polysaccharides from wheat by feed enzymes. *Anim. Feed Sci. Technol.* **187,** 110-114.

Smeets N., Nuyens F., Van Campenhout L., Delezie E. and Niewold T.A. (2018). Interactions between the concentration of non-starch polysaccharides in wheat and the addition of an enzyme mixture in a broiler digestibility and performance trial. *Poult. Sci*. **97,** 2064-2070.

Spector M.D. (1976). Small intestinal mass of the rat is partially determined by indirect effects of intraluminal nutrition. *Gastroenterol.* **52,** 3-5.

Taylor A.E., Bedford M.R., Pace S.C. and Miller H.M. (2018). The effects of phytase and xylanase supplementation on performance and egg quality in laying hens. *British Poult. Sci*. **59,** 554-561.

Torok V.A., Ophel-Keller K., Loo M. and Hughes R.J. (2008). Application of methods for identifying broiler chicken gut bacterial species linked with increased energy metabolism. *Appl. Environ. Microbiol*. **74,** 783-791.

Wickramasuriya S.S., Kim E., Shin T.K., Cho H.M., Kim B., Patterson R., Yi YJ., Park S., Balasubramanian B. and Heo J. (2019). Multi-carbohydrase addition into a corn-soybean meal diet containing wheat and wheat by-products to improve growth performance and nutrient digestibility of broiler chickens. *J. Appl. Poult. Res.* **28,** 399-409.

Wu Y.B., Ravindran V., Thomas D.G., Birtles M.J. and Hendriks W.H. (2004). Influence of phytase and xylanase, individually or in combination, on performance, apparent metabolisable energy, digestive tract measurements and gut morphology in broilers fed wheat-based diets containing adequate level of phosphorous. *British Poult. Sci*. **45,** 76-84.