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Supplementation of enzyme cocktail in chickens diet is an effective approach to increase the utilization of nutrient in wheat-based diets

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Abstract

This experiment was conducted to evaluate the effect of supplementing enzyme cocktail on growth performance, digestibility of nutrients, and monosaccharide concentration in ileum and ceca of broiler chickens fed wheat-based diets. A total of 600 male broilers (42.26 ± 1.76 g, 0 day old) were used for 35 days of feeding trial consisting of 2 phases (starter phase from d 0 to 21 and finisher phase from d 21 to 35). Four dietary treatments were prepared based on wheat diets containing four levels of enzyme cocktail supplementation at 0, 0.2, 0.3, and 20 g/kg. Overall, dietary enzyme cocktail supplementation decreased feed conversion ratio (linear $p = 0.007$; quadratic $p = 0.013$) and improved (linear $p < 0.05$) the apparent ileal digestibility of dry matter (DM), crude protein, and soluble and insoluble non−starch polysaccharides. The apparent total tract digestibility of DM and gross energy were increased (linear $p < 0.01$) with increasing supplementation levels of the dietary enzyme cocktail. The concentrations of arabinose, xylose, mannose, and glucose in ileal digesta were linearly increased ($p < 0.01$) with increasing enzyme cocktail supplementation levels. In addition, the quadratic effect was observed (quadratic $p = 0.046$) in mannose concentration of ileal digesta. The concentration of arabinose, xylose, mannose, and galactose in cecal digesta was increased (linear $p < 0.05$) with increasing dietary enzyme cocktail supplementation levels. The supplementation of enzyme cocktail efficiently increased the utilization of nutrients in broiler and there was no adverse effects of high dosage supplementation level.

Keywords: Xylanase, Mannanase, β-Glucanase, Wheat, Xylose, Arabinose

INTRODUCTION

Wheat is one of the most common feed ingredients in the poultry diet which contains greater protein content than corn. However, the high non-starch polysaccharides (NSP) in the grain would adversely affect growth performance in poultry. Non-starch polysaccharides are known as anti-nutritional factors

Competing interests
No potential conflict of interest relevant to this article was reported.

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Availability of data and material
Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authors' contributions
Conceptualization: Ingale SL, Kim J.
Data curation: Ko H.
Formal analysis: Ko H.
Methodology: Ko H.
Software: Ko H, Kang HK.
Validation: Kim J.
Investigation: Ko H, Moturi J.
Writing - original draft: Kang HK, Kim J.
Writing - review & editing: Kang HK, Ingale SL.

Ethics approval and consent to participate
The project underwent proper ethical standards and the experiments (KW-170519-1) were approved by the Institutional Animal Care and Use Committee of Kangwon National University, Chuncheon, Korea.

that create viscous status within the intestinal tract and inhibit the diffusion rate of nutrients and digestive enzymes [1–3]. Arabinoxylan is the major form of NSP in grain [4,5]. In recent decades, various techniques such as enzyme supplementation have been used to decrease the adverse effects of NSP in feedstuffs [6,7].

Poultry is unable to depolymerize the arabinoxylan due to the lack of endogenous arabinoxylan degrading enzyme secretions [8,9]. Therefore, arabinoxylan degrading enzymes such as endo-β-1,4-xylanases are commonly used for broiler diets as enzyme supplements [9]. Endoxylanases hydrolyze arabinoxylan into oligosaccharide, which increase the population of beneficial bacteria, leading to a reduction of pathogenic bacteria and improvement of gut health [10–13]. The examination of the concentration of free monosaccharides comprising of NSP in the intestinal tract of broiler chickens would be suggested to evaluate the effects of endoxylanase in broiler chickens and the ability of chickens to degrade NSP in the feed. Kim et al. [6] evaluated the digestibility and concentration of monosaccharides in the gut of broiler for measuring the NSP degradation according to enzyme supplement and feed processing and suggested that the release of monosaccharides in the intestine suggested as a breakdown product of NSP. The use of exogenous enzymes would be one of the ways to utilize the NSPs. Therefore, the present study was developed based on a novel enzyme cocktail for improving the nutrients digestiblity. The aim of this study was to evaluate the effect of dietary enzyme cocktail supplementation in wheat-based diet on growth performance, apparent total tract digestibility (ATTD), apparent ileal digestibility (AID), and monosaccharides concentration in the ileum and ceca of chickens.

MATERIALS AND METHODS

The protocol for the current study was approved by the Institutional Animal Care and Use Committee of Kangwon National University, Chuncheon, Korea.

Experimental design and animal care

A total of 600 male broilers (Ross 308, 42.26 ± 1.76 g, 0 day old) were provided form commercial hatchery (JOIN, Pyeongtaek, Korea) and used for 35 days of feeding trial consisting of 2 phases (starter phase from d 0 to 21 and finisher phase from d 21 to 35). Four treatments were prepared based on wheat and contained four levels of enzyme cocktail supplementation at 0, 0.2, 0.3, and 2 g/kg. Each treatment has ten replications (floor pens) with 15 birds per pen (2,300 × 800 × 1,500 mm^3), where the rice hull was covered as litter. Temperature and humidity were controlled by an automatic ventilation system according to the Ross Broiler Management Handbook [14]. Constant light (15 Lux) was offered during the whole experimental period. Birds had *ad libitum* access to feed and water during the trial.

Experimental diets and enzyme cocktail

Basal diets were prepared according to the nutrient recommendations of Aviagen [14] for broiler chickens (Table 1). Enzyme cocktail used in the current study was a powder type of enzyme, comprised of mannanase, β-glucanase, and xylanase (Advanced Enzyme Technologies, Thane, India). The enzyme cocktail was generated through the fermentation of *Trichoderma citrinoviride* and the final supplement included 7,030,150 mannanase U/kg, 1,890,230 β-glucanase U/kg, and 39,923,180 xylanase U/kg. To supplement enzyme cocktail, 200, 300, and 2,000 g of enzyme cocktail were premixed with portions of dietary treatments, respectively, and then the respective premix was mixed to make 1 ton of the whole diets. After the mixing process, the mash diets were conditioned in the barrel covered by heating blocks to a temperature of 75℃ and then pelleted by a

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Table 1. The composition of basal diets (%)

	Starter (0–21 d)	Finisher (21–35 d)
Ingredient (%)		
Corn	44.56	46.90
Wheat	10.00	15.00
SBM (45%)	37.00	28.80
Vegetable oil	5.47	6.37
Choline-Liquid (50%)	0.03	0.05
L-Lys HCl 78%	0.17	0.22
DL-Met 100%	0.23	0.25
L-Thr 100%	0.06	0.09
Limestone	1.40	1.40
Mono calcium phosphate	0.39	0.31
Salt	0.31	0.30
$NaHCO_3$	0.18	0.11
Vitamin supplement[1)]	0.10	0.10
Mineral supplement[2)]	0.10	0.10
Sum	100	100
Nutritional values		
ME (kcal/kg)	3,060	3,200
Crude protein (%)	22.00	19.00
SID Lys (g/kg)	1.33	1.15
SID Met + Cys (g/kg)	0.91	0.85
SID Thr (g/kg)	0.88	0.79
SID Trp (g/kg)	0.26	0.22
Calcium (%)	0.80	0.76
Digestible phosphorus (%)	0.57	0.52

[1)]The vitamin premix contains the followings per kg of diet: vit.A, 18.000 IU; vit.D3, 4.500 IU; vit.E, 31.5 IU; menadione (K_3), 3.6 mg; thiamin (B_1), 1.8 mg riboflavin (B_2), 4.8 mg; pyridoxine (B_6), 3.6 mg; cobalamin (B_{12}), 0.03 mg; niacin (B_3), 22.5 mg; panthothenic acid (B_5), 15 mg; folic acid (B_9), 0.45 mg.

[2)]The mineral premix contains the followings per kg of diet: Mn, 86.4 mg; Zn, 72 mg; Fe, 74.6 mg; Cu, 6 mg; I, 1.5 mg; Co, 0.288 mg; Se, 0.216 mg. Enzyme cocktail 0.2 g/kg supplementation treatment included 1,398 mannanase U/kg, 340 β-glucanase U/kg, and 8,024 xylanase U/kg; Enzyme cocktail 0.3 g/kg supplementation treatment included 2,158 mannanase U/kg, 542 β-glucanase U/kg, and 12,084 xylanase U/kg; Enzyme cocktail 20 g/kg supplementation treatment included 13,726 mannanase U/kg, 3,870 β-glucanase U/kg, and 79,840 xylanase U/kg.

SBM, soybean meal; Lys, lysine; Met, methionine, Thr, threonine; ME, metabolizable energy; SID, standardized ileal digestibility; Cys, cysteine; Trp, tryptophan.

220 hp pellet mill (Model; 12 types, Matador, Denmark) with a 2.8 mm diameter die.

Growth performance

Body weight (BW; g/bird) and amount of feed were measured at the start and the end of the feeding phases. Body weight gain (BWG; g/bird) was calculated from differences in BW between d 0 and 21 and those between d 21 and 35. The feed intake (FI; g/bird) was calculated from differences between the amount of feed provided initially and the remaining feed depending on the phase feeding (starter, 0–21 d; finisher, 21–35 d). The amount of feed consumtion for 21 to 30 d was measured and reflected in the calculation of FI during the finisher phase, as two birds (30 d) were selected from each pan for the digestibility test. The feed conversion ratio (FCR) was calculated by dividing FI by BWG of each phase.

Apparent ileal and total tract digestibility

At d 30, two birds per replicate were selected then each one bird was individually allocated into a metabolic cage (500 mm width × 400 mm depth × 520 mm height) for determination of ATTD (%) of dry matter (DM), crude protein (CP), gross energy (GE), and soluble and insoluble NSP in diets. Diets containing chromic(III) oxide (2.5 g/kg diets) and water were provided *ad libitum*. From d 33 to 35, excreta were collected after eliminating feathers and scales. From d 30 to 35, diets containing chromic(III) oxide (2.5 g/kg diets) were provided to birds in floor pens. At the end of feeding trial (d 35) three birds with similar BW per replicate were slaughtered then digesta samples were collected from ileum and ceca. Collected excreta and digesta samples were lyophilized using freeze dryer for 72 h. Lyophilized excreta and digesta samples were ground using a Wiley laboratory mill (Thomas Model 4 Wiley® Mill, Thomas scientific, Swedesboro, NJ, USA) on 1 mm screen and stored at −20℃ until analysis. The nutrient digestibility was calculated using the following equations: ATTD (%) = 100 − (100 × [chromic(III) oxide in feed % / chromic(III) oxide in excreta %] × [nutrient in excreta % / nutrient in feed %]); AID (%) = 100 − (100 × [chromic(III) oxide in feed % / chromic(III) oxide in ileum digesta %] × [nutrient in ileum digesta % / nutrient in feed %]).

Chemical analysis

Feeds, excreta, and digesta samples were analyzed for DM (Method 930.15), ether extract (EE, Method 2003.03), ash (Method 942.05), and CP (Method 990.03) [15]. Gross energy of feeds, excreta, and digesta samples were analyzed using a 6400 model bomb calorimetry (Parr Instruments, Moline, IL, USA) and calibration was performed by benzoic acid. Non-starch polysaccharide was estimated according to the method of Englyst et al. [16]. Monosaccharide concentration was determined according to Hosseindoust et al. [17] using gas-liquid chromatography (Agilent 6890 N, Agilent, Santa Clara, CA, USA).

Statistical analysis

Data were analyzed by ANOVA using the GLM procedure of SAS (SAS Institute, Cary, NC, USA) in a randomized complete block design. Dependent variables consisted of growth and digestibility of nutrient. Individual broiler chickens was experimental unit for digestibility paramenetrs and the average of a pen was experimental unit for growth performance and FI. An orthogonal polynomial contrast test was used to determine the linear or quadratic effects of enzyme cocktail supplementation levels in the diets. Significance and tendency for statistical tests were set at $p < 0.05$ and $0.05 \leq p \leq 0.10$, respectively.

RESULTS

Growth performance

During the starter phase, BWG showed a tendency to increase (linear $p = 0.079$) with increasing dietary enzyme cocktail supplementation levels (Table 2). During the finisher phase, FCR tended to be improved (linear $p = 0.084$; quadratic, $p = 0.065$) with increasing dietary enzyme supplementation levels. Overall, dietary enzyme cocktail supplementation improved FCR (linear $p < 0.007$; quadratic $p < 0.013$).

Apparent ileal and total tract digestibility

Dietary enzyme cocktail supplementation improved (linear $p < 0.05$) the AID of DM, CP, soluble NSP, and insoluble NSP (Table 3). In addition, the quadratic tendency was observed (quadratic $p = 0.074$) in AID of insoluble NSP. The ATTD of DM and GE were improved (linear $p < 0.01$) with

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Table 2. Effects of broilers fed diets containing graded levels of enzyme cocktail on growth performance[1)]

Item	Enzyme cocktail (g/kg)[2)]				SEM	*p*-value	
	0	0.2	0.3	20		Linear	Quadratic
1–21 d							
BWG (g/bird)	654	696	689	693	13.8	0.079	0.186
FI (g/bird)	1,035	1,078	1,042	1,060	13.2	0.522	0.370
FCR	1.59	1.55	1.52	1.54	0.03	0.207	0.449
22–35 d							
BWG (g/bird)	1,362	1,372	1,363	1,369	41.6	0.409	0.530
FI (g/bird)	2,473	2,427	2,353	2,429	67.1	0.492	0.368
FCR	1.81	1.77	1.74	1.78	0.04	0.084	0.065
Overall							
BWG (g/bird)	2,015	2,068	2,051	2,062	43.8	0.181	0.311
FI (g/bird)	3,509	3,505	3,394	3,489	67.3	0.575	0.469
FCR	1.74	1.70	1.66	1.70	0.02	0.007	0.013

[1)]Each mean represents values from 10 replicates (15 birds/replicate).

[2)]Mannanase + β-glucanase + xylanase.

BWG, bodyweight gain; FI, feed intake; FCR, feed conversion ratio.

Table 3. Effects of broilers fed diets containing graded levels of enzyme cocktail on digestibility of nutrients[1)]

Item	Enzyme cocktail (g/kg)[2)]				SEM	*p*-value	
	0	0.2	0.3	20		Linear	Quadratic
Apparent ileal digestibility (%)							
DM	65.37	66.14	69.09	68.50	0.59	< 0.001	0.255
CP	61.94	62.44	64.22	63.91	0.73	0.024	0.585
GE	64.78	65.19	67.09	65.61	0.68	0.158	0.175
Soluble NSP	19.22	21.10	22.49	23.41	0.82	< 0.001	0.558
Insoluble NSP	17.83	19.94	21.13	20.17	0.84	0.035	0.074
Apparent total tract digestibility (%)							
DM	70.23	71.06	72.17	72.36	0.42	< 0.001	0.453
CP	66.04	67.05	67.30	67.45	0.64	0.125	0.507
GE	69.52	70.67	72.17	72.09	0.50	< 0.001	0.227
Soluble NSP	28.72	29.51	30.40	29.76	1.19	0.454	0.552
Insoluble NSP	20.48	23.95	22.04	22.74	1.02	0.293	0.183

[1)]Each mean represents values from 10 replicates (15 birds/replicate).

[2)]Mannanase + β-glucanase + xylanase.

DM, dry matter; CP, crude protein; GE, gross energy; NSP, non-starch polysaccharides.

increasing supplementation levels of a dietary enzyme cocktail.

Monosaccharides concentration in ileum and ceca

The concentrations of xylose, arabinose, mannose, and glucose in ileal digesta were increased (linear $p < 0.001$) with increasing dietary enzyme cocktail supplementation levels (Table 4). In addition, the quadratic effect was observed ($p = 0.046$) in mannose concentration of ileal digesta. The concentration of arabinose, xylose, mannose, and galactose in cecal digesta increased (linear $p < 0.05$) with increasing dietary enzyme cocktail supplementation levels.

Table 4. Effects of broilers fed diets containing graded levels of enzyme cocktail on concentration of monosaccharides[1)]

Item	Enzyme cocktail (g/kg)[2)]				SEM	*p*-value	
	0	0.2	0.3	20		Linear	Quadratic
Ileum (g/kg, DM)							
Arabinose	3.38	3.50	3.63	3.69	0.06	< 0.001	0.602
Xylose	4.65	4.69	4.72	4.79	0.02	< 0.001	0.637
Mannose	0.76	0.90	0.88	0.93	0.02	< 0.001	0.046
Galatose	6.32	6.71	6.78	6.70	0.03	0.431	0.265
Glucose	3.58	3.87	3.97	4.06	0.03	< 0.001	0.359
Ceca (g/kg, DM)							
Arabinose	2.38	2.42	2.54	2.56	0.05	0.006	0.912
Xylose	3.03	3.15	3.53	3.51	0.09	< 0.001	0.455
Mannose	0.60	0.69	0.75	0.73	0.04	0.011	0.110
Galatose	5.45	5.94	6.65	6.57	0.40	0.030	0.483
Glucose	2.39	2.54	2.70	2.60	0.11	0.106	0.246

[1)]Each mean represents values from 10 replicates (15 birds/replicate).

[2)]Mannanase + β-glucanase + xylanase.

DM, dry matter.

DISCUSSION

In the present study, dietary enzyme cocktail improved FCR of broiler chickens. Similar studies reported that dietary xylanase and β-glucanase supplementation in wheat-based diets led to improved performance of broilers, which is associated with the viscosity of digesta in the intestine of broiler chickens [7,8,13]. Enzyme supplemented to wheat-rye-barley-based diets reduced the viscosity of gut digesta in laying hens, resulting a greater digestibility of DM, fat, and NSP [11]. The FI in the present study was not affected by dietary treatments. These results might relate to the nutrient digestibility of diets. It may be associated with the point that the increase of nutrient digestibility caused by the effect of the enzyme cocktail probably improved the FCR.

Dietary enzyme cocktail supplementation improved AID of DM, CP, and both soluble and insoluble NSP in the present study. As young broiler chickens fed wheat-based diets containing NSP-degrading enzymes such as xylanase and β-mannanase, digestibility of nitrogen, NSP, fat, and starch in the small intestine increased effectively [17,18]. Digestibility of nutrients in diets containing corn distillers dried grains with solubles, which contains a high concentration of NSP, also increased as xylanase was supplemented in diets for broiler chickens [3]. The ATTD of DM and GE were significantly improved by dietary enzyme cocktail supplementation in this study. Significant increases of monosaccharides concentration in intestinal digesta of birds fed diets containing dietary enzyme cocktail were observed in the present study. A similar study reported that the increasing dietary levels of β-mannanase improved the nitrogen and GE retention, which is associated with the usage of the released NSP monomers such as arabinose, mannose, galactose, and glucose as nutrients [17].

The NSP-degrading enzymes can improve nutrient digestibility by reducing the nutrient encapsulating effects in plant cell walls [1,7]. Monosaccharides such as arabinose, xylose, mannose, glucose, and galactose could be released from the enzymatic reaction on NSP, and high concentration of monosaccharides in intestinal digesta would indicate a high hydrolyzing potential to degrade NSP [2,6]. Concentrations of arabinose and xylose were increased in the ileal digesta of broiler fed the diets containing xylanase supplementation [5,7]. In the present study, concertation

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of arabinose, xylose, and mannose in both ileal and cecal digesta were increased with increasing dietary enzyme cocktail supplementation levels, glucose, and galactose concentrations also were high in ileal and ceca digesta, respectively. These results supported that the enzyme cocktail supplementation increased the degradation of NSP to increase the release of main NSP monomers including arabinose, xylose, and mannose in the ileum and ceca. In the current study, the linear effect of enzyme cocktail supplementation levels was shown remarkable in the digestibility test, but no quadratic effects was found. It would support that a high dose of enzyme cocktail had not any adverse effects in growth performance and nutrients digestibility. Therefore, the increase of some monosaccharides of intestinal digesta in the current study would be implied that the dietary supplementation of the enzyme cocktail effectively degraded the NSP of the broiler diets.

CONCLUSION

In summary, the supplementation of enzyme cocktail increased the utilization of nutrients without any advers effects of high supplementary dose. Therefore, the supplementation of the current enzyme cocktail would be recommended for broiler chickens fed wheat-based diets.

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