Effects of a combination of xylanase, amylase and protease, and probiotics on major nutrients including amino acids and non-starch polysaccharides utilization in broilers fed different level of fibers

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ABSTRACT This study evaluated the effects of a combination of xylanase, amylase, and protease (XAP), with probiotics (3 *Bacillus spp.*) supplementation on apparent ileal digestibility (AID) and apparent total tract digestibility (ATTD) of nutrients in Cobb 500 broilers from 0 to 21 d. A completely randomized 2 × 4 factorial design (2 levels of fiber; 4 types of supplements) with 8 replicate cages (6 birds/cage) was used. Each low and high-fiber diet contained 500 FTU/kg *Buttiauxella sp.* phytase and was supplemented with: (a) none (control), (b) XAP (2,000 U xylanase + 200 U amylase + 4,000 U protease/kg diet), (c) probiotics (75,000 CFU/g of *Bacillus spp.)*, or (d) XAP + probiotics. High fiber decreased ($P < 0.05$) nitrogen-corrected apparent metabolizable energy (AME_n), AID of all amino acids (AA), AID and ATTD of dry matter (DM), crude protein (CP), starch, and gross energy (GE). High fiber increased ($P < 0.01$) the flow of total non-starch polysaccharides (NSP) in both ileum and total tract. The XAP + probiotics increased ($P < 0.01$) AME_n as well as AID and ATTD of DM, CP, GE, starch, while alone, XAP yielded similar improvement except for DM compared with control. The supplemental XAP alone improved ($P < 0.01$) the digestibility of most of the AAs compared with control. Moreover, XAP + probiotics increased ($P < 0.05$) AID of all AA except arginine and serine compared with control. A fiber × supplements interaction ($P < 0.05$) was found for AID of histidine and threonine, and their digestibility in high-fiber diet was improved to a level comparable to low-fiber diet by XAP + probiotics. The flow of NSP in XAP group was 5 to 6% lower than in control while NSP flow in XAP + probiotic group was further 4% lower than that of XAP group ($P < 0.01$). The results infer that the combination of XAP and probiotics can effectively optimize the nutrient digestibility in broilers fed both low and high-fiber diets.

Key words: broiler, digestibility, fiber, enzyme, probiotic

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INTRODUCTION

The prices of conventional feed ingredients such as corn and soybean meal (**SBM**) keep fluctuating with seasons, level of production, and as a result of the growing competition between animal feed, human food, and biofuel production. Nowadays, byproducts from biofuel industries and other agro-industries are being used as alternative feedstuffs to reduce the cost of production of broiler feed. However, these feed ingredients are inherently high in non-starch polysaccharides (**NSP**). These NSP produce antinutritive effect by increasing viscosity and entrapping nutrients in digesta (Tiwari et al., 2018). Broilers lack endogenous enzymes required for NSP digestion and thus exhibit a reduced feed efficiency when fiber content is increased even in a nutritionally complete diet (Singh et al., 2017). Supplemental xylanase can disrupt the plant cell wall matrix by hydrolyzing inaccessible carbohydrates and can simultaneously allow other exogenous or endogenous enzymes to gain access to proteins, starch, and phosphorus (Oryschak et al., 2002).

Exogenous protease and amylase are often supplemented along with xylanase, which not only improve the digestion of protein in the upper gastrointestinal tract (**GIT**) of broilers but also increase the digestion of starch in the encapsulated endosperm (Zanella et al., 1999). A combination of xylanase, amylase, and protease (**XAP**) can increase the nutrient digestibility of feed (Romero et al., 2013), and can improve BW gain and feed efficiency in broilers (Cowieson and Ravindran, 2008; Singh et al., 2017). Several studies have reported that the XAP combination is effective in improving nutrient digestibility and utilization of corn-SBM-based diet fed to broilers (Cowieson and Ravindran, 2008; Amerah et al., 2017). However, due to the variability in the composition of corn and SBM from

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different sources, their response can vary to exogenous enzymes and thus a greater benefit may not always be produced (Olukosi et al., 2010; Yegani and Korver, 2013). Also, because of the influence of a changing microbiome on gut health and digestion (Yadav and Jha, 2019), the optimal utilization of nutrients by broilers cannot only be ensured by providing them with a nutritionally balanced and complete diet. Therefore, to optimize the utilization of nutrients, the gut microbiome can be balanced by incorporating probiotics in the diet. The efficient strains of probiotics can competitively exclude pathogenic bacteria and can confer health benefits in the host by stimulating immunity and increasing epithelial integrity for better utilization of nutrients (Salim et al., 2013).

In the current scenario of growing concern about antibiotic resistance, it has been demonstrated that the inclusion of probiotics containing strains of *Bacillus spp.* and its combination with XAP can improve feed efficiency and growth performance similar to antibiotic growth promoters (Flores et al., 2016). In the present study, three strains of *Bacillus spp.* were used because *Bacillus spp.* have displayed better performance in terms of shelf-life, durability during processing and viability throughout distribution and use (Cartman et al., 2008). The combination of XAP and probiotics shows synergistic effects, reduces the inflammatory response of acute phase proteins, and improves gut health and nutrient's utilization, which is even more crucial in disease challenged flocks (Momtazan et al., 2011; Dersjant-Li et al., 2016). Compared with other high-fiber feed ingredients, corn-SBM-based diet has an excellent digestibility, and whether this multi-enzyme and probiotics combination could provide a considerable benefit is still intriguing. However, corn and SBM are the major ingredients being used in commercial broiler production, and there has been extensive use of exogenous enzymes and probiotics in the corn-SBM-based diet. Thus, it becomes particularly relevant to access the impact of XAP and *Bacillus spp.*-based probiotics on broilers fed diets containing variable levels of fiber added through the inclusion of high or low-fiber ingredients. We hypothesized that the supplementation of XAP and probiotics in nutritionally balanced diets would be complementary to each other and their combination would produce a more pronounced effect in high-fiber diets than in low-fiber diets. Therefore, this study aimed to investigate the effects of fiber, XAP, probiotics, and the combination of XAP and probiotics on the apparent ileal digestibility (**AID**) of amino acids (**AA**), and AID and apparent total tract digestibility (**ATTD**) of other nutrients in broiler chickens.

MATERIALS AND METHODS

The animal study protocol was approved by the Institutional Animal Care and Use Committee of University of Hawaii at Manoa, Honolulu, HI, and was in compliance with the guidelines for the care and use of agricultural animals in research and teaching (FASS, 2010).

Animals and Housing

A total of 384-day-old broiler chicks (Cobb × Cobb 500) were used in this digestibility study. The chicks were individually weighed, wing tagged, and randomly placed in cages with 6 birds per cage, and with 8 cages replications per treatment. The chicks were housed in an environmentally controlled facility, and the brooding temperature was maintained with supplemental heat. The temperature ranged from 35°C in the first week to around 25°C attained at the end of the third week by gradual reduction. The chicks were provided photoperiod of 24-h light with ad libitum and unrestricted access to feed and water at all the times. The birds were also monitored at least in the morning and evening daily, and all incurring mortalities were recorded along with the feed intake until the end of the study period.

Diets and Experimental Design

All the diets were formulated to have the same or approximately equal amount of metabolizable energy and crude protein and to meet or exceed the nutrients requirements of broiler (Table 1; NRC, 1994). The treatments contained 2 levels of fibers and 4 approaches of feed additives supplementation in a 2 × 4 factorial arrangement. The 2 fiber levels were low and high fiber, and the 4 approaches of feed additives supplementation were (a) control, without supplementation, (b) multi-enzymes (XAP), (c) probiotics, and (d) the combination of multi-enzymes and probiotics (XAP + probiotics). Each of the 8 diets was supplemented with 500 FTU/kg of *Buttiauxella sp.* phytase. The XAP was used in appropriate diets to provide 2,000 U of xylanase, 200 U of amylase, and 4,000 U of protease per kg. The probiotics contained 3 *Bacillus spp.* strains and was supplemented in the diet to provide 75,000 CFU per kg. The level of fiber was increased in high-fiber diet by the inclusion of wheat middling, canola meal, and corn distiller's dried grain with solubles (**DDGS**). The diets were fed as crumble from d 1 to d 21 of age. The XAP and probiotics were supplied by Danisco Animal Nutrition/Dupont, UK and were applied as a top dressing on the diets.

Exogenous Enzymes and Probiotics

Enzyme activity levels in final feed samples (200 g) were measured at the DuPont Nutrition Biosciences Innovation Laboratories (Brabrand, Denmark)/Eurofins Scientific, Inc. (Nutrition Analysis Center, Des Moines, IA) in duplicate, and reported as activity units. Xylanase activity was used as a marker of enzyme recovery. One FTU is defined as the quantity of enzyme that

Table 1. Ingredient composition and nutritive value of the experimental diets (g/100 g, as-fed basis unless otherwise indicated).

	Low-fiber diet				High-fiber diet			
Ingredients	Control	XAP[1]	Probiotics[2]	XAP + Probiotics	Control	XAP[1]	Probiotics[2]	XAP + Probiotics
Corn	63.92	63.92	63.92	63.92	53.05	53.05	53.05	53.05
Wheat midds	0.00	0.00	0.00	0.00	7.00	7.00	7.00	7.00
SBM	29.93	29.93	29.93	29.93	19.90	19.90	19.90	19.90
Canola meal	0.00	0.00	0.00	0.00	4.25	4.25	4.25	4.25
Corn DDGS	0.00	0.00	0.00	0.00	7.00	7.00	7.00	7.00
MBM	2.00	2.00	2.00	2.00	3.88	3.88	3.88	3.88
Soya oil	0.50	0.50	0.50	0.50	2.00	2.00	2.00	2.00
Limestone	1.09	1.09	1.09	1.09	0.92	0.92	0.92	0.92
DCP	0.65	0.65	0.65	0.65	0.00	0.00	0.00	0.00
Lysine	0.00	0.00	0.00	0.00	0.15	0.15	0.15	0.15
Methionine	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22
Threonine	0.04	0.04	0.04	0.04	0.08	0.08	0.08	0.08
Tryptophan	0.00	0.00	0.00	0.00	0.02	0.02	0.02	0.02
Nacl	0.34	0.34	0.34	0.34	0.23	0.23	0.23	0.23
Vitamin mix[3]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Mineral mix[4]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Phytase	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Chromic oxide	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Calculated nutrient content								
AME_n, (kcal/kg)	2962	2962	2962	2962	2935	2935	2935	2935
Crude protein	21.06	21.06	21.06	21.06	21.01	21.01	21.01	21.01
Crude fiber	2.64	2.64	2.64	2.64	3.42	3.42	3.42	3.42
Lysine	1.08	1.08	1.08	1.08	1.10	1.10	1.10	1.10
Methionine	0.54	0.54	0.54	0.54	0.55	0.55	0.55	0.55
Threonine	0.79	0.79	0.79	0.79	0.80	0.80	0.80	0.80
Met + Cys	0.89	0.89	0.89	0.89	0.90	0.90	0.90	0.90
Ca	0.84	0.84	0.84	0.84	0.87	0.87	0.87	0.87
Total P	0.61	0.61	0.61	0.61	0.60	0.60	0.60	0.60
Na	0.32	0.32	0.32	0.32	0.34	0.34	0.34	0.34
Cl	0.26	0.26	0.26	0.26	0.28	0.28	0.28	0.28
Choline (mg/kg)	3952	3952	3952	3952	3760	3760	3760	3760
Determined nutrient content (%)								
Dry matter	88.64	87.82	88.59	88.51	88.53	88.51	88.92	89.08
GE, kcal/kg	4009	3978	4008	4003	4090	4094	4111	4111
Crude protein	20.97	20.78	20.99	20.92	20.63	20.63	20.75	20.71
Total ash	5.01	5.05	5.21	5.03	4.99	5.07	4.93	5.07
Starch	34.02	34.56	34.72	34.97	31.32	31.25	31.05	31.65
Ether extract	3.11	3.15	3.07	3.15	5.34	5.24	5.31	5.29
Total NSP[5]	9.25	9.19	9.23	9.20	11.17	11.20	11.16	11.20

[1]XAP was supplemented (top-dressed) at 100 gm per MT of diet. XAP is a combination of xylanase, amylase, and protease providing 2,000 U of xylanase, 200 U of amylase, and 4,000 U of protease per kg diet.

[2]Probiotics was added at 100 gm per MT and contained 3 *Bacillus spp.* strains (75,000 CFU/g). SBM is soybean meal; DDGS is distiller's dried grain with solubles; DCP is dicalcium phosphate; MBM is meat and bone meal.

[3]Supplied per kilogram of diet: 66,138 IU of vitamin A, 19,841 IU of vitamin D_3, 331 IU of vitamin E, 0.2 mg of vitamin B_{12}, 1.3 mg of biotin, 19.8 mg of vitamin K_3, 19.8 mg of thiamine, 66 mg of riboflavin, 110 mg of d-pantothenic acid, 40 mg of pyridoxine, 551 mg of niacin, and 11 mg of folic acid.

[4]Supplied per kilogram of diet: 300 mg of manganese, 300 mg of zinc, 200 mg of iron, 25 mg of copper, 6.25 mg of iodine, and 2.5 mg of cobalt.

[5]Total NSP does not contain uronic acid. Only total neutral sugar determined using gas chromatography was considered for this study. Phytase was added at 500 FTU/kg in all diets.

releases 1 μmol of inorganic P/min from 5.0 mM sodium phytate at pH 5.5 at 37°C. One xylanase unit is defined as the amount of enzyme that releases 0.48 μmol of the reducing sugar xylose from wheat arabinoxylan per min at pH 4.2 and 50°C. One amylase unit is defined as the amount of enzyme required to release, in the presence of excess α-glucosidase, 0.20 μmol of glucosidic linkages expressed as p-nitrophenol equivalents from a maltoheptaoside substrate per min at pH 8.0 and 40°C. One protease unit is defined as the amount of enzyme that releases 1.0 μg of phenolic compound, expressed as tyrosine equivalents, from a casein substrate per min at pH 7.5 and 40°C. The presence of the probiotic was confirmed by a total *Bacillus* enumeration count and identification of the 3 specific *Bacillus* strains by colony morphology.

Sample Collection

Excreta samples were collected per cage over 3 consecutive days (from d 18 to 20) for the determination of apparent metabolizable energy (**AME**) and was stored at −20°C until further analysis. The collected excreta

samples from 3 consecutive days were pooled and mixed in a blender, and later lyophilized, ground, and stored in airtight containers at 4°C until further analysis. Ileal digesta was collected according to the procedure of Kadim et al. (2002) with some modification. Briefly, on day 20 after collection of excreta samples, the birds were fasted overnight (12 h). On day 21, all the birds were allowed access to feed for 2 h. Later, after 4 h from the start of feeding, the, birds were euthanized by intracardial injection of sodium pentobarbitone (>100 mg/kg body weight) and the contents of the distal ileum (terminal 15 cm adjacent to the ileo-cecal junction) were collected by gentle milking to avoid mucosal sloughing. The ileum was defined as the portion of the small intestine extending from the Meckel's diverticulum to 1 cm above the ileocecal junction. The digesta samples from the individual birds were pooled within a cage to provide an adequate quantity for further analysis. The digesta sample was immediately frozen in dry ice to avoid microbial degradation and later stored at −20°C until further analysis. The digesta samples were subsequently lyophilized, ground, and stored in airtight container at 4°C for further analysis.

Analytical Work

Chemical Analysis The samples of feed, ileal digesta, and excreta contents were analyzed according to the Association of Official Analytical Chemists standard procedures (AOAC, 2006) with specific methods as follows: dry matter (**DM**) by placing in a hot air oven at 65°C for overnight (method 930.15). The gross energy (**GE**) was determined by using an oxygen bomb calorimeter (Parr Bomb Calorimeter 6200, Parr Instrument Co., Moline, IL) with benzoic acid as a calibration standard. The nitrogen content was ascertained using Leco method (method 990.03) and used to calculate crude protein (**CP**) content (N × 6.25). Ether extract (**EE**) was determined by Soxhlet method (method 920.39), and the ash content was determined by burning the sample at 650°C for overnight (method 942.05). The total starch content was analyzed using a commercial kit (Megazyme International, Ireland, UK). Chromic oxide (**Cr_2O_3**) was determined by the perchloric acid method (Fenton and Fenton, 1979).

Chromatographic Analysis Total NSP content of the feed, ileal digesta, and excreta were determined by quantifying their constituent neutral sugars by gas chromatography (**GC**). Total neutral sugars were quantified as described by Englyst et al. (1994). Chromatographic analysis was done using a GC system (TRACE™ 1300 gas chromatograph, Thermo Scientific, Waltham, MA) equipped with a flame ionization detector and a fused silica capillary column (DB-17HT, Agilent Technologies, Wilmington, DE), using 2-deoxy-D-glucose as an internal standard. Amino acids were determined according to the procedure described by Sedgwick et al. (1991) with some modification. Chromatographic analysis was done in an automated High-Performance Liquid Chromatography system (Alliance Separation Module 2690, Waters, Milford, MA). The stationary phase was a 5-μm C-18 reverse phase 4.6 × 150 mm Acclaim™ 120 column (Thermo Scientific, Waltham, MA). Pre-column derivatization was done by mixing 15 μL pH-adjusted sample solution with 15 μL of o-phthalaldehyde (**OPA**) derivatization reagent by the autosampler, where the mixture was allowed to react for 10 min. Subsequently, 30 μL of the derivatized sample was injected into the separation column. The OPA derivatized AAs were detected by a fluorescence detector (RF-20Axs, Shimadzu, Columbia, MD) with excitation set at 340 nm and emission set at 420 nm wavelengths.

Digestibility and AME Calculation

The AID, ATTD, and AME were determined by the marker method using the equations below as performed by Robbins and Firman (2006) on ileal digesta:

$$\mathrm{AID\%} = 1 - \left[\left(\frac{Cr_2O_3\ \mathrm{diet}}{Cr_2O_3\ \mathrm{ileal\ digesta}}\right) \times \left(\frac{\mathrm{Nut.\ Con._{ileal\ digesta}}}{\mathrm{Nut.\ Con._{diet}}}\right)\right]$$

$$\mathrm{ATTD\%} = 1 - \left[\left(\frac{Cr_2O_3\ \mathrm{diet}}{Cr_2O_3\ \mathrm{excreta}}\right) \times \left(\frac{\mathrm{Nut.\ Con._{excreta}}}{\mathrm{Nut.\ Con._{diet}}}\right)\right],$$

$$\mathrm{AME} = \mathrm{GE_{diet}} \times \left[1 - \left(\frac{Cr_2O_3\ \mathrm{diet}}{Cr_2O_3\ \mathrm{excreta}}\right) \times \left(\frac{\mathrm{GE_{excreta}}}{\mathrm{GE_{diet}}}\right)\right],$$

where

AID% = percentage apparent ileal digestibility; ATTD% = percentage apparent total tract digestibility; AME = apparent metabolizable energy; Cr_2O_3 = chromic oxide; Nut. Con. = nutrient concentration.

Nitrogen retention (**N_{ret}**) was calculated as the difference between nitrogen intake and nitrogen present in the excreta. The N_{ret} was expressed in g/kg DM intake and was calculated using the marker method as follows:

$$\mathrm{N_{ret}} = \mathrm{N_{diet}} - \left(\mathrm{N_{excreta}} \times \frac{Cr_2O_3\ \mathrm{diet}}{Cr_2O_3\ \mathrm{excreta}}\right).$$

The AME was adjusted to zero N retention, and a correction factor of 8.22 kcal/g was used for each gram of nitrogen retained (Hill and Anderson, 1958). Hence, the nitrogen-corrected AME (**AME_n**) was calculated as follows:

$$\mathrm{AME_n} = \mathrm{AME} - 8.22 \times \mathrm{N_{ret.}}$$

Table 2. Effects of XAP and probiotics on apparent ileal digestibility (AID) of nutrients in broilers fed diets with low and high level of fiber (g/100 g, DM basis; d 0 to d 21 post-hatch).

Treatments		Variables						
		Total Ash	DM	GE	CP	EE	Starch	AIDE, kcal/kg
Low fiber	Control	38.2	70.3	70.1	73.4	86.0	96.0	3,169
	XAP	41.9	72.3	71.6	75.8	87.0	97.1	3,242
	Probiotics	39.4	71.7	70.9	74.8	86.7	96.7	3,208
	XAP + Probiotics	43.3	73.4	72.4	77.1	87.6	97.4	3,276
High fiber	Control	36.8	69.0	67.7	71.1	85.2	95.8	3,129
	XAP	39.4	71.2	69.3	73.7	86.1	96.4	3,207
	Probiotics	37.2	70.3	68.5	72.5	85.8	96.0	3,167
	XAP + Probiotics	41.1	72.3	70.2	75.0	86.7	96.9	3,239
	SEM[1] (n = 8)	2.96	0.78	0.52	0.42	1.68	0.37	23.70
Main effects (Factors)								
Fiber	Low	40.7	71.9^{a}	71.2^{a}	75.3^{a}	86.8	96.8^{a}	3,224^{a}
	High	38.6	70.7^{b}	68.9^{b}	73.1^{b}	86.0	96.3^{b}	3,185^{b}
	SEM (n = 32)	2.34	0.59	0.26	0.25	0.84	0.32	11.85
Supplements	Control	37.5	69.6^{b}	68.9^{c}	72.2^{d}	85.6	95.9^{c}	3,149^{c}
	XAP	40.6	71.7^{a,b}	70.4^{a,b}	74.7^{b}	86.5	96.7^{a,b}	3,224^{a,b}
	Probiotics	38.3	71.0^{a,b}	69.7^{b,c}	73.6^{c}	86.3	96.4^{b,c}	3,188^{b,c}
	XAP + Probiotics	42.2	72.9^{a}	71.3^{a}	76.0^{a}	87.1	97.1^{a}	3,257^{a}
	SEM (n = 16)	2.57	0.66	0.37	0.32	1.19	0.34	16.76
***P* value**	Fiber	0.159	0.038	<0.001	<0.001	0.477	0.002	0.025
	Supplements	0.110	0.002	<0.001	<0.001	0.837	<0.001	<0.001
	Fiber × Supplements	0.994	0.999	0.999	0.991	1.000	0.675	1.000

$^{a–d}$Within a column, means without a common superscript differ ($P < 0.05$).

[1]Pooled SEM (8 cage of 8 birds in each treatment).

XAP was supplemented (top-dressed) at 100 gm per MT of diet. XAP is a combination of xylanase, amylase, and protease providing 2,000 U of xylanase, 200 U of amylase, and 4,000 U of protease per kg diet. Probiotics was added at 100 gm per MT and contained 3 *Bacillus spp.* strains (75,000 CFU/g). Total NSP is the sum of total neutral sugars obtained from gas chromatography.

DM = dry matter, GE = gross energy, CP = crude protein, EE = ether extract, NSP = non-starch polysaccharides, AIDE = apparent ileal digestible energy.

Calculation of the flow of NSP components in ileal and total tract level was done by considering the concentration of the respective NSP component in ileal digesta or excreta and Cr_2O_3 in diet and ileal digesta or excreta. The NSP flow was expressed in g/kg DM intake and was calculated as follows:

$$\text{NSP flow}_{\text{ileal digesta/excreta}} = \left(\text{NSP}_{\text{ileal digesta/excreta}} \times \frac{\text{Cr}_2\text{O}_{3\ \text{diet}}}{\text{Cr}_2\text{O}_{3\ \text{ileal digesta/excreta}}}\right).$$

Statistical Analysis

The data obtained were analyzed using the MIXED procedure of SAS (v9.2, SAS Institute, Cary, NC) to evaluate treatment effects and compare test variables consisting of fiber and supplements in 2 × 4 factorial arrangement. The main effects of fiber and supplements and their interaction were tested on each of the determined parameters. Significance effect was declared at $P < 0.05$ and considered a trend at $P < 0.10$. Upon a significant global effect, the treatment means were separated using Tukey's method.

RESULTS

Analyzed enzyme activity recovery in feed for xylanase (85 to 110%), amylase (80 to 94%), and protease (88 to 107%) confirmed that the enzymes were added to XAP supplemented feed in the amount declared and was within 20% of the targeted levels. The *Bacillus* enumeration count revealed that the desired concentration of the bacterial colony was maintained in the mixed feed and *Bacillus* recovery was within 1 log cfu/g of the target dose. The analyzed nutrients content and NSP in the feed were also consistent with the calculated amount in feed formulation (Table 1).

Nutrient Utilization

No interaction ($P > 0.05$) was observed between fiber and supplements for AIDE, and AID of total ash, DM, GE, CP, EE, and starch (Table 2). High fiber decreased AIDE, and AID of DM, GE, CP, and starch ($P < 0.05$). The combined activities of XAP and probiotics improved AIDE and AID of DM, GE, CP, and starch compared with the control ($P < 0.01$). The single activity of XAP or probiotics also improved ($P < 0.01$) AID of CP, while only XAP increased AIDE, and AID of GE and starch. Neither fiber level nor supplement or

Table 3. Effects of XAP and probiotics on apparent total tract digestibility (ATTD) of other nutrients in broilers fed low and high level of fibers (DM basis, g/100 g; d 0 to d 21 post-hatch).

		Variables							
Treatments		Total ash	DM	GE	CP	EE	Starch	N_{ret}, g/kg	AME_n, kcal/kg
Low fiber	Control	29.9	76.6	79.5	68.9	86.5	96.3	26.1	3382
	XAP	32.5	77.7	80.3	70.0	87.7	97.2	26.5	3422
	Probiotics	31.4	77.2	80.1	69.6	87.1	96.8	26.4	3409
	XAP + Probiotics	34.6	78.1	81.1	70.9	88.0	97.2	26.8	3446
High fiber	Control	29.2	74.1	76.6	64.8	85.8	95.9	24.2	3340
	XAP	31.4	75.4	77.5	66.1	86.5	96.3	24.7	3381
	Probiotics	31.0	74.7	77.2	65.5	86.2	96.2	24.5	3366
	XAP + Probiotics	33.1	75.8	78.3	67.0	87.1	96.6	24.9	3407
	SEM[1] (n = 8)	2.64	0.57	0.35	0.51	1.66	0.31	0.19	15.40
Main effects (Factors)									
Fiber	Low	32.1	77.4^{a}	80.3^{a}	69.8^{a}	87.3	96.9^{a}	26.4^{a}	3415^{a}
	High	31.2	75.0^{b}	77.4^{b}	65.9^{b}	86.4	96.3^{b}	24.6^{b}	3373^{b}
	SEM (n = 32)	2.22	0.40	0.26	0.37	0.98	0.24	0.14	10.78
Supplements	Control	29.6	75.4^{b}	78.1^{c}	66.8^{c}	86.1	96.1^{b}	25.1^{c}	3361^{c}
	XAP	32.0	76.6^{a,b}	78.9^{b}	68.1^{a,b}	87.1	96.7^{a}	25.6^{a,b}	3401^{a,b}
	Probiotics	31.2	76.0^{a,b}	78.6^{b,c}	67.6^{b,c}	86.6	96.5^{a,b}	25.4^{b,c}	3387^{b,c}
	XAP + Probiotics	33.9	77.0^{a}	79.7^{a}	68.9^{a}	87.5	96.9^{a}	25.9^{a}	3427^{a}
	SEM (n = 16)	2.37	0.46	0.29	0.42	1.25	0.27	0.16	12.51
***P*-value**	Fiber	0.435	<0.001	<0.001	<0.001	0.414	<0.001	<0.001	<0.001
	Supplements	0.084	0.007	<0.001	<0.001	0.817	0.003	<0.001	<0.001
	Fiber × Supplements	0.986	0.995	0.990	0.982	0.999	0.783	0.993	0.999

[a–c]Within a column, means without a common superscript differ ($P < 0.05$)

[1]Pooled SEM (8 cage of 8 birds in each treatment).

XAP was supplemented (top-dressed) at 100 gm per MT of diet. XAP is a combination of xylanase, amylase, and protease providing 2,000 U of xylanase, 200 U of amylase, and 4,000 U of protease per kg diet. Probiotics was added at 100 gm per MT and contained 3 *Bacillus spp.* strains (75,000 CFU/g). Total NSP is the sum of total neutral sugars obtained from gas chromatography.

DM = dry matter, GE = gross energy, CP = crude protein, EE = ether extract, NSP = non-starch polysaccharides, N_{ret} = Nitrogen retention, AME_n = nitrogen-corrected apparent metabolizable energy.

their combination had any significant effect on AID of total ash and EE ($P > 0.05$).

No interaction was observed between fiber and supplements for AME_n, and ATTD, i.e., nutrient retention of total ash, DM, GE, CP, EE, and starch (Table 3). High fiber decreased ATTD of DM, GE, CP, starch, N_{ret} and reduced AME_n ($P < 0.01$). The probiotics had no significant effect on ATTD of these parameters compared with control, but XAP alone improved all of these variables except ATTD of DM. The use of XAP + probiotics improved the ATTD of DM, GE, CP, starch, N_{ret} and increased AME_n compared with the control ($P < 0.01$). Similar as the effect on AID, ATTD of total ash and EE was not affected by fiber or supplements ($P > 0.05$).

An interaction ($P < 0.05$) between fiber and supplements was observed for the AID of histidine and threonine (Table 4). Both XAP and XAP + probiotics improved the digestibility of histidine and threonine in the high-fiber diet but not in low-fiber diet. The probiotics alone was not different than control in either low or high-fiber diet. High fiber decreased ($P < 0.05$) the AID of all amino acids compared with low-fiber groups. The probiotics alone had no significant improvement in the AID of AA compared with control while the individual application of XAP increased ($P < 0.01$) AID of 8 out of 18 amino acids measured. In addition, the combined supplementation of XAP + probiotics enhanced ($P < 0.05$) AID of all measured AA except arginine and serine compared with control. The application of XAP improved ($P < 0.001$) AID of AA in average, while the effect of probiotics was not different than either control or XAP. However, AID of AA on average was higher ($P < 0.001$) in XAP + probiotics compared with either control or probiotics group.

No interaction ($P > 0.05$) was observed between fiber and supplements for ileal and total tract NSP fractions analyzed for total neutral sugars (Tables 5 and 6). High fiber increased ($P < 0.01$) the concentration of arabinose, xylose, mannose, glucose, and total NSP at ileal level while it also increased ($P < 0.05$) the flow of rhamnose at the total tract level. The high fiber did not affect the flow of fucose and galactose at either level ($P > 0.05$). The individual application of probiotics decreased the ileal flow of arabinose and mannose while XAP additionally decreased the ileal flow of total NSP ($P < 0.01$) compared with control. Both probiotics and XAP alone decreased the total tract flow of arabinose, mannose, galactose, and total NSP ($P < 0.01$). The combination of XAP + probiotics significantly ($P < 0.01$) reduced the ileal flow of arabinose, mannose, galactose, and total NSP, while it also decreased ($P < 0.05$) the flow of rhamnose at total tract level relative to control diet.

Table 4. Main effects of fiber and supplements (XAP, probiotics) on apparent ileal digestibility (AID) of amino acids in broilers fed low and high level of fibers (DM basis, g/100 g; d 0 to d 21 post-hatch).

	Main effects (factors)										
	Fiber			Supplements					*P* value		
Var	Low	High	SEM[1] (n = 32)	Ctrl	XAP	Probiotics	XAP + Probiotics	SEM[2] (n = 16)	Fiber	Suppl	Fiber × Suppl
CP	75.3	73.1		72.2	74.7	73.6	76.0				
Indispensable amino acids											
Arg	77.4[a]	73.2[b]	0.41	74.7	75.8	74.9	75.9	0.57	<0.001	0.324	0.710
His	76.6	74.7	0.37	74.0	76.6	74.9	77.3	0.52	<0.001	<0.001	0.041
Ile	77.4[a]	75.2[b]	0.39	74.1[c]	77.3[a,b]	75.7[b,c]	78.2[a]	0.55	<0.001	<0.001	0.995
Leu	75.5[a]	72.9[b]	0.46	72.3[b]	74.5[a,b]	74.0[a,b]	76.1[a]	0.63	<0.001	<0.001	1.000
Lys	77.2[a]	75.4[b]	0.32	74.4[c]	76.9[a,b]	75.9[b,c]	77.9[a]	0.46	<0.001	<0.001	0.999
Met	79.3[a]	77.0[b]	0.42	76.8[b]	78.7[a,b]	78.1[a,b]	79.0[a]	0.59	<0.001	0.048	1.000
Phe	77.2[a]	74.7[b]	0.37	73.9[b]	76.8[a]	75.6[a,b]	77.5[a]	0.52	<0.001	<0.001	1.000
Thr	77.0	74.6	0.35	74.2	76.7	75.0	77.3	0.49	<0.001	<0.001	0.038
Trp	79.3[a]	76.5[b]	0.37	76.3[b]	78.1[a,b]	77.7[a,b]	79.5[a]	0.52	<0.001	<0.001	0.991
Val	76.5[a]	75.2[b]	0.39	74.0[b]	76.4[a]	75.5[a,b]	77.5[a]	0.55	0.025	<0.001	0.999
Dispensable amino acids											
Ala	77.3[a]	74.8[b]	0.41	74.1[b]	76.7[a]	75.8[a,b]	77.5[a]	0.58	<0.001	<0.001	1.000
Asp	75.0[a]	72.9[b]	0.37	72.2[c]	74.7[a,b]	73.5[b,c]	75.6[a]	0.52	<0.001	<0.001	0.997
Cys	76.5[a]	74.0[b]	0.38	73.6[b]	75.6[a,b]	74.9[a,b]	76.9[a]	0.53	<0.001	<0.001	0.998
Glu	77.1[a]	74.2[b]	0.36	73.6[c]	76.5[a,b]	75.2[b,c]	77.3[a]	0.51	<0.001	<0.001	0.608
Gly	76.3[a]	74.6[b]	0.36	74.2[b]	75.8[a,b]	75.2[a,b]	76.5[a]	0.51	0.002	0.018	1.000
Ser	77.3[a]	74.6[b]	0.69	74.1	76.6	75.7	77.3	0.97	0.009	0.123	0.957
Tyr	77.8[a]	73.6[b]	0.37	74.6[b]	76.3[a,b]	75.3[a,b]	76.8[a]	0.52	<0.001	0.015	1.000
Avg	77.1[a]	74.6[b]	0.31	74.2[c]	76.5[a,b]	75.5[b,c]	77.3[a]	0.44	<0.001	<0.001	0.995

[a–c]Within a column, means without a common superscript differ ($P < 0.05$).
[1]Pooled SEM (32 cage of 8 birds in each treatment).
[2]Pooled SEM (16 cage of 8 birds in each treatment).
XAP was supplemented (top-dressed) at 100 gm per MT of diet. XAP is a combination of xylanase, amylase, and protease providing 2000 U of xylanase, 200 U of amylase, and 4,000 U of protease per kg diet. Probiotics was added at 100 gm per MT and contained 3 *Bacillus spp.* strains (75,000 CFU/g). CP, crude protein; var, variables; Ctrl, control; Suppl, supplement.

Table 5. Effects of XAP and probiotics on ileal flow (g/kg DM) of components of total non-starch polysaccharides in broilers fed low and high level of fibers (DM basis; d 0 to d 21 post-hatch).

		Variables							
Treatments		rha	fuc	ara	xyl	man	glu	gal	Total NSP
Low fiber	Control	1.74	2.33	17.96	16.77	2.97	24.06	16.46	82.30
	XAP	1.70	2.29	16.35	16.19	2.15	23.56	15.72	77.95
	Probiotics	1.72	2.30	16.68	16.45	2.38	23.86	16.08	79.45
	XAP + Probiotics	1.68	2.26	15.16	16.06	1.82	23.28	14.50	74.77
High fiber	Control	1.79	2.34	21.36	22.43	3.41	30.52	16.70	98.56
	XAP	1.75	2.30	19.66	21.81	2.56	29.94	15.86	93.89
	Probiotics	1.79	2.31	20.17	22.14	2.88	30.31	16.14	95.75
	XAP + Probiotics	1.73	2.27	18.53	21.65	2.25	29.66	14.55	90.65
	SEM[1] (n = 8)	0.07	0.07	0.41	0.66	0.09	0.57	0.73	1.43
Main effects (Factors)									
Fiber	Low	1.71	2.29	16.54[b]	16.37[b]	2.33[b]	23.69[b]	15.69	78.62[b]
	High	1.77	2.31	19.93[a]	22.01[a]	2.78[a]	30.11[a]	15.81	94.71[a]
	SEM (n = 32)	0.05	0.04	0.20	0.36	0.04	0.38	0.64	0.72
Supplements	Control	1.76	2.34	19.66[a]	19.60	3.19[a]	27.29	16.58[a]	90.43[a]
	XAP	1.73	2.30	18.00[b]	19.00	2.36[c]	26.75	15.79[a]	85.92[b,c]
	Probiotics	1.75	2.30	18.42[b]	19.29	2.63[b]	27.08	16.11[a]	87.60[a,b]
	XAP + Probiotics	1.71	2.26	16.85[c]	18.86	2.04[d]	26.47	14.53[b]	82.71[c]
	SEM (n = 16)	0.06	0.05	0.29	0.48	0.06	0.45	0.67	1.01
***P* value**	Fiber	0.087	0.813	<0.001	<0.001	<0.001	<0.001	0.667	<0.001
	Supplements	0.595	0.812	<0.001	0.668	<0.001	0.352	<0.001	<0.001
	Fiber × Supplements	0.993	1.000	0.997	1.000	0.954	1.000	0.995	0.998

[a–d]Within a column, means without a common superscript differ ($P < 0.05$).
[1]Pooled SEM (8 cage of 8 birds in each treatment).
XAP was supplemented (top-dressed) at 100 gm per MT of diet. XAP is a combination of xylanase, amylase, and protease providing 2,000 U of xylanase, 200 U of amylase, and 4,000 U of protease per kg diet. Probiotics was added at 100 gm per MT and contained 3 *Bacillus spp.* strains (75,000 CFU/g). Total NSP is the sum of total neutral sugars obtained from gas chromatography. rha, rhamnose; fuc, fucose; ara, arabinose; xyl, xylose; man, mannose; glu, glucose; gal, galactose.

Table 6. Effects of XAP and probiotics on total tract flow (g/kg DM) of components of total non-starch polysaccharides in broilers fed low and high level of fibers (DM basis; d 0 to d 21 post-hatch).

		Variables							
Treatments		rha	fuc	ara	xyl	man	glu	gal	Total NSP
Low fiber	Control	1.54	1.62	15.20	16.80	2.27	23.80	8.55	69.78
	XAP	1.48	1.58	13.52	16.17	1.60	23.27	7.46	65.09
	Probiotics	1.51	1.59	13.97	16.58	1.82	23.51	7.63	66.62
	XAP + Probiotics	1.45	1.55	12.30	15.99	1.32	23.05	6.69	62.35
High fiber	Control	1.59	1.61	17.57	22.67	2.37	30.03	9.06	84.90
	XAP	1.53	1.56	15.82	22.00	1.69	29.41	7.87	79.88
	Probiotics	1.56	1.57	16.33	22.41	1.98	29.76	8.15	81.77
	XAP + Probiotics	1.49	1.53	14.52	21.80	1.35	29.07	7.00	76.77
	SEM[1] (n = 8)	0.05	0.03	0.35	0.45	0.06	0.63	0.37	1.10
Main effects (Factors)									
Fiber	Low	1.50[b]	1.58	13.75[b]	16.38[b]	1.75[b]	23.41[b]	7.58	65.96[b]
	High	1.54[a]	1.57	16.06[a]	22.22[a]	1.85[a]	29.57[a]	8.02	80.83[a]
	SEM (n = 32)	0.04	0.02	0.18	0.27	0.04	0.48	0.37	0.62
Supplements	Control	1.56[a]	1.62	16.39[a]	19.73	2.32[a]	26.92	8.80[a]	77.34[a]
	XAP	1.51[a,b]	1.57	14.67[b]	19.09	1.64[c]	26.34	7.67[b,c]	72.49[b]
	Probiotics	1.54[a,b]	1.58	15.15[b]	19.50	1.90[b]	26.64	7.89[b]	74.20[b]
	XAP + Probiotics	1.47[b]	1.54	13.41[c]	18.90	1.34[d]	26.06	6.85[c]	69.56[c]
	SEM (n = 16)	0.04	0.02	0.25	0.34	0.05	0.53	0.28	0.81
***P* value**	Fiber	0.027	0.402	<0.001	<0.001	0.007	<0.001	0.064	<0.001
	Supplements	0.018	0.080	<0.001	0.172	<0.001	0.305	<0.001	<0.001
	Fiber × Supplements	0.995	1.000	0.996	1.000	0.614	0.995	0.988	0.984

[a–c]Within a column, means without a common superscript differ ($P < 0.05$).

[1]Pooled SEM (8 cage of 8 birds in each treatment).

XAP was supplemented (top-dressed) at 100 gm per MT of diet. XAP is a combination of xylanase, amylase, and protease providing 2,000 U of xylanase, 200 U of amylase, and 4,000 U of protease per kg diet. Probiotics was added at 100 gm per MT and contained 3 *Bacillus spp.* strains (75,000 CFU/g). Total NSP is the sum of total neutral sugars obtained from gas chromatography. rha, rhamnose; fuc, fucose; ara, arabinose; xyl, xylose; man, mannose; glu, glucose; gal, galactose.

DISCUSSION

The purpose of this study was to investigate the impact of supplementing exogenous XAP and probiotics either individually or in combination on the improvement of nutrient digestibility in young broilers fed nutritionally adequate diets containing a varying level of fiber. It is a fact that the endogenous enzymes secreted by broilers do not adequately degrade all fiber present in feed and there are undegraded substrates available for exogenous enzymes. The high-fiber diets have a higher amount of undigestible NSP which are degraded marginally in the GIT. The soluble NSP added through high fiber thus increases the viscosity of digesta and causes a decline in the overall digestibility (Annison and Choct, 1991; Bedford, 2000). The high fiber increased the ileal and total tract flow of total NSP, while it decreased the digestibility all AA in ileum and that of DM, GE, CP, and starch in both ileum and total tract. The reduction in the digestibility of nutrients in both ileum and total tract of broilers fed high fiber-containing diets in the present study suggests that the access of enzymes to their appropriate substrates might have been hindered due to the encapsulation of nutrients and increased viscosity of digesta. High level of fiber inclusion in the diet achieved through incorporation of wheat-DDGS in wheat-SBM diet can also reduce DM and energy retention in broilers (Bolarinwa and Adeola, 2012). Similarly, the addition of fiber through corn-DDGS in a corn-SBM diet has been reported to reduce energy retention and AME in comparison to the basal corn-SBM diet (Adeola and Ileleji, 2009; Adeola et al., 2010).

The digestibility of several nutrients including GE, CP, and starch was improved by the addition of XAP in both high- and low-fiber diets. The improvement in digestibility of CP and energy by the addition of XAP in the present study is in agreement with the improvement in nutrient digestibility observed by Romero et al. (2013) and Cowieson and Adeola (2005). Similar to the improvements in the AID of CP, the XAP increased the digestibility of several AA and the overall improvement in average AA by XAP was by 3.1% compared with control. In addition to the improvements observed for XAP supplementation, probiotics also produced an increment in AID of CP while the AIDE and ATTD of CP and energy were not significantly different compared with control. This agrees with the effects observed by Wealleans et al. (2017) where probiotics similarly yielded only numerically higher CP and energy digestibility. The probiotics improve gut health and provide a better environment for the action of enzymes and their impact on digestibility could be more profound when compared in disease challenged flocks (Dersjant-Li et al., 2016). The combination of XAP and probiotics in the present study resulted in an additional increase in

the ileal digestibility of CP and total tract digestibility of GE than that delivered by supplements individually. The magnitude of improvement in the digestibility of ileal AA by the XAP + probiotics were higher than that of XAP alone (4.2 vs. 3.1%) when compared with control. The relative improvement in the digestible energy was higher in the ileum compared to the total tract (3.4 vs. 2.0% for XAP + probiotics), which can be attributed to the lesser availability of substrate in the hind-gut. This difference in relative improvement at 2 levels of GIT is similar to the findings of Amerah et al. (2017) and Cowieson and Ravindran (2008). This additive activity of XAP and probiotics was also reported by Wealleans et al. (2017) for GE and AME_n, but the effect was not significant for CP. Likewise, Murugesan et al. (2014) observed improvements in nitrogen retention, AME_n, and nutrient utilization of starch and AA in response to the combined application of protease, phytase, and *Bacillus* probiotics. The digestibility of CP of control treatment in both low- and high-fiber groups in the present study was lower than that of the digestibility of CP of negative control diet used in the study of Wealleans et al. (2017), which may be responsible for the difference in the response. The additional improvement attained in the digestibility of energy and other nutrients by the combination of supplements is indicative of a complementary effect of XAP + probiotics over their usage alone. The exact mechanism of this additive effect due to the combined use of XAP and probiotics is still unclear to our understanding. However, several studies have suggested that carbohydrase enzyme can depolymerize NSP and starch while phytase and protease can degrade phytate and protein, respectively (Cowieson and Adeola, 2005; Cowieson and Ravindran, 2008; Bedford and Cowieson, 2012). This would ensure prebiotic substrate for beneficial bacteria and could restrict the flow of undigested protein used by pathogenic bacteria (Bedford, 2000). The negative impact of pathogenic bacteria on gut health and nutrient utilization can also be abated by supplying probiotics in the diet (Yadav and Jha, 2019). The probiotics can competitively exclude the pathogenic bacteria, produce bacteriocins to reduce pathogenic burden, enhance the development of intestinal villi, and thus improve the gut mucosal integrity and enhance absorption of nutrients (Samanya and Yamauchi, 2002). Probiotics improve gut health and provide a conducive environment for better enzyme activity to improve digestibility (Salim et al., 2013). Thus, it can be believed that the combined inclusion of XAP and probiotics in diet can result in a complementary improvement of nutrient digestibility than those delivered by either of the supplements alone (Momtazan et al., 2011). However, the improvement in the digestibility of total ash and fat did not correspond to the improvements achieved for other nutrients by the supplements. The effect of phytase added in the background of all diets may have yielded adequate improvement in the digestibility of minerals, and that might have limited the scope for further enhancement (Olukosi et al., 2010). The digestibility of fat in either low- or high-fiber diet was not affected by the supplements. This could be due to the presence of a different level of soy oil used in both low- and high-fiber diets to balance AME of feed. This lack of improvement in the digestibility of fat agrees with Amerah et al. (2017) and Romero et al. (2014) as these authors did not observe any increment in fat digestibility in response to the addition of XA and XAP in the corn-SBM-based diet. The impact of XAP and probiotics on the digestibility of nutrients was not significantly affected by fiber levels, except for the digestibility of histidine and threonine, where interaction was found between fiber and supplements for these amino acids. It is also rational to note that the difference in the content of NSP in 2 levels of fiber may not have been large enough to yield a significant improvement in the digestibility of all nutrients. High-fiber diet understandably has a higher level of antinutrient factor (Yadav et al., 2019) which is responsible for the greater depression of the coefficient of digestibility. The combination of XAP and probiotics has a potential to bring improvement in such diet because of the availability of additional substrate for hydrolysis, thus releasing more nutrients entrapped in the matrix (Bedford, 1996; Cowieson et al., 2010; Wealleans et al., 2017).

The ileal and total tract concentration of total NSP was lower in XAP-supplemented diets compared with control and it was further reduced in XAP + probiotics group. This result agrees with that of Amerah et al. (2017) where XAP reduced the flow of ileal NSP compared to the negative control diet. The reduction in NSP flow by the combination of XAP and probiotics resonates well with a similar study conducted by Wealleans et al. (2017), where XAP + DFM (containing multi-strain of *Bacillus spp.*) caused more disappearance of NSP compared with control than XAP or DFM alone. It shows that the combined activity of XAP and probiotics are favorably effective in relieving antinutrient effects of NSP. The reduction in the concentration of total NSP in the total tract compared with that in the ileum of control diet (9 vs. 7.7%) is indicative of further degradation of NSP that takes place in the large intestine, mostly due to the action of microbes. The decrease in the ileal flow of total NSP by probiotics was not significantly different than control but it significantly improved at the total tract level. This suggests that probiotics can influence fermentation and hence it can efficiently utilize NSP in the hind-gut (Liu et al., 2017). It is expected that the proper combination of enzymes and probiotics can increase the digestibility of energy, protein, starch, NSP, and AA, and decrease the activity of pathogenic bacteria by competitive exclusion and by increasing the availability of readily fermentable substrates to the beneficial bacteria (Bedford and Cowieson, 2012). Probiotics containing *Bacillus sp.* can interact with other gut bacteria to stabilize the gut microbial colony

and improve gut health (Hong et al., 2005). Moreover, due to their ability to produce enzymes, probiotics containing *Bacillus and Lactobacillus sp.* can also contribute in increasing digestibility in the host leading to improved growth performance (Jin et al., 2000; Hmani et al., 2017). Many enzymes like xylanase, amylase, glucanase, phytase, protease, pectinase, and mannanase, and some additives like probiotics and organic acids are generally used to enhance the digestibility and utilization of nutrients in broilers. However, their mechanism of action and efficacy may depend on the types of substrates, and the combination of enzymes and additives used in the diet (Cowieson, 2010).

In conclusion, the combination of XAP and probiotics improved the nutrient digestibility in broilers fed low- or high-fiber diet to a greater degree than by the individual use of the supplements. Moreover, it was confirmed that XAP and probiotics could improve nutrient digestibility in either corn-SBM-based diet or corn-SBM with added high-fiber ingredients even when the formulated diets are nutritionally adequate. The combination of probiotics and XAP in this study led to significant additional improvement in the digestibility of several nutrients in the ileal and the total tract beyond the improvement contributed by their individual application. This provides evidence to confirm our hypothesis that the combination of XAP and probiotics has a complementary effect and can further improve the digestibility of nutrients beyond their separate application in feed. However, the combination produced significantly more improvement in high-fiber diet compared with low-fiber diet only for the AA histidine and threonine. Furthermore, this additive activity of XAP and probiotics is highly effective in improving nutrients utilization in broilers which can ultimately allow for reducing excretion of essential nutrients and thus lead to an increase in profitability in broiler production. However, further research on synergism, antagonism and passage rate of multi-enzymes, and the characteristic changes in microbiota and gut mucosa of the host is warranted to determine the precise mechanism and optimal combination of enzymes and probiotics for maximum utilization of nutrients.

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