Nitrogen retention, energy, and amino acid digestibility of wheat bran, without or with multicarbohydase and phytase supplementation, fed to broiler chickens¹

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ABSTRACT: The study was conducted to determine the effects of multicarbohydase (MC) preparation (700 U α -galactosidase, 2,200 U galactomannanase, 3,000 U xylanase, and 22,000 U β -glucanase per kg of diet) and phytase (Phy, 500 FTU per kg of diet) supplementation on the nutritive value of wheat bran (WB) in broiler chicks. Trial 1 determined retention of nutrients and apparent metabolizable energy corrected by nitrogen (AME_n). One reference diet (RD) protein-free (85% corn based) was fortified to determine the WB nutrient retention coefficient. Trial 2 determined standardized ileal digestibility (SID) of AA, when pancreas and liver were weighed. An additional group of bird was fed with an RD with 5% casein–corn starch diet, fortified with vitamins and minerals to quantify the endogenous fraction and determine SID of AA. For each trial, the test diets were made by mixing RD and WB 7:3 (wt/wt) and fed without or with MC or Phy or combination. Male

broilers (Cobb 500), 245 d old, were allocated to five treatments to give seven replicates (seven birds/cage). The birds were fed a commercial diet from day 0 to 10 followed by Trial 1 diets from day 11 to 18 and finally Trial 2 diets from day 19 to 21. Excreta samples were collected on days 15–18 and all birds were slaughtered on day 21 for ileal digesta. There was an interaction ($P < 0.05$) between MC and Phy on retention of DM, N, P, and AME_n. An interaction ($P < 0.05$) was also observed on SID of Arg, His, Leu, Lys, Phe, Thr, Val, Asp, Cys, Glu, and Ser. Responses of MC plus Phy supplementation were higher ($P < 0.05$) on overall SID of AA by 6.05% (75.18 to 94.26%), compared with responses for MC (2.35%; 72.04 to 88.97) or Phy (3.46%; 73.27 to 92.13). Liver and pancreas weights were affected ($P < 0.05$) by the single MC supplementation. The MC and Phy combination may be an effective strategy to improve AA utilization of WB in broiler chickens.

Key words: amino acid digestibility, broiler chickens, enzyme, nitrogen retention, wheat bran

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INTRODUCTION

Wheat bran (WB) is a by-product after dry milling of wheat to obtain flour. WB contains around 16% of CP and starch (Maes et al., 2004),

which can be partially used as corn replacement to minimize the feed cost. However, its high non-starch polysaccharides (NSP; 46%) content limits its use in feeding (Ralet et al., 1990). NSP are sugars not digested by endogenous poultry enzymes (Olukosi and Adeola, 2008). The high NSP consumption increase intestinal viscosity, reduce gut transit time and nutrient utilization (Adeola and Cowieson, 2011), and modify the structure and function of the digestive organs, reflecting on weight and secretory activity, as observed in pancreas, liver, and intestine by Wu et al. (2004). On the other hand, most phosphorus (P) in vegetable

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feedstuffs is in phytate form, which can only be degraded to a limited extent in the poultry gastrointestinal tract (Slominski, 2011). In wheat, phytate molecules are encapsulated with proteins within cells of the aleurone layer and are surrounded by carbohydrates that affect nutrient accessibility (Heard et al., 2002). Previous studies have demonstrated that the carbohydrases improve nutrient retention in broilers (Kiarie et al., 2016), reduce digesta viscosity (Amerah, 2015), as well as improve the endogenous enzymes access to nutrients and phytase (Phy) to phytate (Woyengo and Nyachoti, 2011). In addition, it improves metabolism in the digestive organs by decreasing of endogenous enzymes secretions (Mahagna et al., 1995), which results on decrease of intestine, pancreas, and liver weights (Brenes et al., 1993). The objective was to evaluate the effects of MC and Phy on retention of nutrients, apparent metabolizable energy corrected by nitrogen (AME_n), and standardized ileal digestibility (SID) of AA content of WB in broiler chicks and on liver and pancreas weights.

MATERIALS AND METHODS

All research methods and experimental procedures were approved by the ethics and animal experimentation committee at the University of Sao Paulo (project 2843/2012), Brazil.

WB and Enzymes

In this study, the WB was a hard red spring wheat variety that was obtained from the feed mill of Sao Paulo University. The chemical composition (as-fed basis) of WB is shown in Table 1.

The enzymes from Brazilian trade representative were a multicarbohydrase (MC) blend of α -galactosidase, galactomannanase, β -xylanase and β -glucanase (ENDOPOWER BETA, Uniquimica Ltda, Sao Paulo, Brazil), and Phy (GENOPHOS, Uniquimica Ltda). The MC consisted of 40% dehydrated fermentation product from *Aspergillus niger* (PRL 2351) and *Aspergillus oryzae* (ATCC66222) by weight and 60% dehydrated malted barley that provided per kg diet 700, 2,200, 30,000, and 22,000 units of α -galactosidase, galactomannanase, xylanase, and β -glucanase, respectively. The Phy derived from *Escherichia coli* and *Citrobacter braakii* and provided 500 FTU/kg diet (Uniquimica Ltda). One unit of α -galactosidase was defined as the quantity of the enzyme that liberates 1 μ mol *p*-nitrophenol per minute. One unit of galactomannanase was defined as the quantity of the enzyme that decreases to half the initial viscosity of galactomannan per

minute. One unit of xylanase was defined as the amount of the enzyme that generated 1 μ mol of the xylose from xylan per minute at pH 5.5 and 50°C, and one β -glucanase unit was defined as the amount of the enzyme that generated 1 μ mol of the reducing sugar glucose from β -glucan per minute at pH 4.8 and 50°C. One Phy unit (FTU) was defined as the quantity of enzyme required to liberate 1 mmol inorganic phosphate per minute, at pH range 1.5–6.5, from an excess of 15 mM sodium phytate at 37°C. The maximum activity was defined at pH 4.5.

Animals and Management

Two hundred and forty-five d old male broiler chicks (Cobb 500) were procured from a commercial hatchery and allocated to five treatments in a randomized complete design; each treatment had seven replicate cages of seven birds per cage. Birds were kept in metabolic cages (1.0 $\times$ 0.4 m) in a controlled environment for 20 d. The cages were housed in an environmentally controlled room with 24 h fluorescent lighting. Room temperature was maintained at 32°C during the first week, with a weekly

Table 1. Analyzed chemical composition (as-fed basis) of WB used in the study

Nutrient component	
DM (%)	89.49
CP (%)	16.02
Ash (%)	5.71
GE (MJ/kg)	16.26
Fat (%)	3.15
Total P (%)	0.99
Ca (%)	0.16
NDF (%)	40.85
Essential amino acids (%)	
Arg	1.01
His	0.40
Ile	0.49
Leu	0.90
Lys	0.69
Met	0.24
Phe	0.52
Thr	0.64
Trp	0.27
Val	0.70
Nonessential amino acids (%)	
Ala	0.81
Asp	1.14
Cys	0.34
Glu	4.01
Gly	0.81
Pro	0.65
Ser	1.32
Tyr	0.49

reduction of 3°C until reaching a temperature of 26°C. Each metabolic cage was equipped with a feeder and a nipple drinker for ad libitum access to feed and water. Birds were vaccinated in the hatchery against Avian infectious bronchitis, Newcastle disease, and infectious bursal disease, and mortality was recorded on a daily basis throughout the study.

Diets, Experimentation, and Samples Collection

Two trials were conducted in a completely randomized design in a 2 × 2 factorial arrangement of treatments to evaluate the effects of two levels of MC (0 or 700 U α -galactosidase, 2,200 U galactomannanase, 30,000 U xylanase, and 22,000 U β -glucanase/kg diet) and two levels of Phy (0 or 500 FTU/kg diet) on WB digestibility in broiler chickens. In addition, each trial was formulated a reference diet (RD, Table 2). The test diets were made by mixing RD and WB 7:3 wt/wt basis. Based on this, in each trial, five experimental diets were developed.

Broilers were fed a commercial starter corn-soybean meal-based diet for 10 days and subsequently were introduced to experimental diets. The objective of trial 1 was to measure the retention of nutrients and AME_n in WB, using a corn-based diet from day 11 to day 18, considering 4-d adaptation and grab samples of excreta collected two times daily (0830 and 1730 h) for three consecutive days (days 15 to 18). At the end of excreta collection, the samples were thawed at ambient temperature, homogenized, and subsamples packed in plastic containers and stored in freezer until analysis.

For measuring SID of AA in trial 2 from day 19 to day 21, a 5% casein-corn starch diet was used for the calculation of AA flows. Chromium oxide (Cr₂O₃, 0.3%) was added in as an indigestible marker. After 3-d adaptation (Moran, 1984), all birds were weighed and killed by cervical dislocation, and abdominal cavity opened immediately to expose the digestive tract. Ileum terminal segment of 10 cm was sectioned 2 cm proximal to the ileocecal junction to access ileal digesta. The ileal samples were frozen and stored at -20°C until processed. The pancreas and liver were surgically removed and weighed using electric balance (Model BL-3200H, Shimadzu Corporation, Tokyo, Japan) and expressed in relative weight (%) of body weight.

Chemical Analysis

Excreta and ileal samples were freeze-dried for 72 h at -40°C (LH 0401, Terroni, Sao Carlos, Brazil). Before analyses, the diets, WB, excreta, and

ileal digesta samples were finely ground by an analytic mill processing to pass through a 2 mm screen (A11 Basic, IKA, Shanghai, China). Standard Association of Official Analytical Chemists (AOAC, 2005) procedures were used for determination of DM (method 930.15), ash (method 942.05), GE, nitrogen (N, method 990.03), total phosphorus (P, method 946.06), calcium (Ca, method 978.02), and NDF (Goering and Van Soest, 1970). Trial 2 diets and ileal digesta samples were further analyzed for AA content (method 994.12). The GE content was measured by a bomb calorimeter (IKA C5000, Karnataka, India) using benzoic acid as internal standard. After processing samples in a muffle furnace for 8 h at 550°C, the samples were analyzed for ash. Before Ca and P determination in the diets and excreta and chromium concentration in the ileal digesta, samples were digested in concentrated nitric acid and perchloric acid 70% (AOAC, 2006; 935.13). The Ca and chromium concentration in the diet and ileal digesta were determined by inductively coupled plasma optical emission spectrometry (Model 710 ICP-OES, Agilent Technologies, Santa Clara, CA, United States). The Fiske-Subbarow reducer solution and acid molybdate were incorporated into the digested samples to determine P concentration in spectrophotometric reading of absorption at 620 nm (Model Cary 60 UV-Vis, Agilent Technologies). The N contents were analyzed in a nitrogen distiller (Model TE-036/1, Tecnal, Piracicaba, Brazil) using the Kjeldahl method. For AA, 100 mg sample was hydrolyzed with 6M HCl at 110°C for 24 h, followed by neutralization with 4 mL of 25% (wt/vol) NaOH and cooled to room temperature. The mixture was then equalized to 50 mL volume with sodium citrate buffer (pH 2.2) and analyzed using an amino acid analyzer (1260 Infinity LCs; Agilent Technologies). Tryptophan was determined by the colorimetric method of Spies (1967) using standard curve of pure tryptophan (Merck, Germany) and detected at 590 nm, with spectrophotometer (DU-640 UV/Vis—Beckman Coulter, United States). Cyst(e)ine and methionine were analyzed as cysteic acid and methionine sulphone by oxidation with performic acid for 16 h at 0°C and neutralization with hydrobromic acid prior to hydrolysis. Cysteine was expressed as cystine.

Calculation and Statistical Analyses

Retention of nutrients was calculated as described by Hill and Anderson (1958) using the following equations:

Table 2. Composition of RDs, as-fed basis

Ingredients (%)	RD trial 1	RD trial 2
WB*		
Corn	85.01	
Corn starch		50.27
Cellulose	5.00	5.00
Casein		5.00
Dextrose		30.00
Soybean oil	2.00	1.20
Choline chloride (60%)	0.02	0.02
Salt	0.25	0.02
Calcium carbonate	0.38	0.35
Dicalcium phosphate†	2.40	2.37
Limestone	0.81	0.69
Chromic oxide		0.30
Vitamin–mineral premix‡	0.50	0.50
Kaolin#	3.62	3.99
Calculated composition		
DM (%)	89.80	92.93
CP (%)	6.90	4.51
Ca (%)	0.92	0.86
Available P (%)	0.40	0.34
ME (MJ/kg)	12.77	12.98
Na (%)	0.22	0.22
Choline (mg/kg)	2000	1900
Linoleic acid (%)	2.68	0.63

*Test diets were made by mixing RD and WB 7:3 wt/wt basis, with-out or with MC (700 U of α -galactosidase, 2,200 U of galactomannanase, 30,000 U of xylanase, and 22,000 U of β -glucanase/kg of diet); Phy (provided 500 FTU/kg of diet) and MC (700 U of α -galactosidase, 2,200 U of galactomannanase, 30,000 U of xylanase, and 22,000 U of β -glucanase/kg of diet) + Phy (provided 500 FTU/kg of diet), respectively. MC and Phy replaced kaolin.

†Contained 20% Ca, 18.5% P.

‡Vitamin–mineral premix contained per kilogram of premix: vitamin A, 1,750,000 IU; vitamin D3, 550,000 IU; vitamin E, 2,750 IU; vitamin K, 400 mg; vitamin B1, 500 mg; vitamin B2, 1,250 mg; vitamin B6, 750 mg; vitamin B12, 3,000 mcg; niacin, 8,750 mg; pantothenic acid, 3,250 mg; folic acid, 200 mg; choline, 82.01 g; Fe, 12.50 g; Mn, 17.50 g; Zn, 12.50 g; Cu, 24.95 g; I, 300 mg; Se, 50 mg; Monensin 25 g; Halquinol (S, 7-dichloroquinolin-8-ol) 7,500 mg.

#Kaolin = mineral kaolinite (used as inert to adjust the formulation).

$$\text{Retention of nutrients (\%)} = 100 \times \left[\frac{(\text{NI} - \text{NO}_{\text{excreta}})}{\text{NI}} \right],$$

where NI is the nutrient intake (g), $\text{NO}_{\text{excreta}}$ is the nutrient output in excreta (g).

The retention of nutrients in WB was determined by the difference method (Fan and Sauer, 1995), with the corn-based diet as the basal diet, using the following equation:

$$\text{DA} = (\text{DD} - (\text{DB} \times \text{DN})) / \text{DWB}$$

Where DA is the digestibility or retention of a nutrient (%) in an assay feedstuff (WB), DD is

the digestibility or retention of a nutrient (%) in an assay diet (corn- and WB-containing diet), DB is the digestibility or retention of a nutrient (%) in the basal feedstuff (corn), DN is the contribution of a nutrient (decimal percentage) from corn to the assay diet, and DWB is the contribution of a nutrient (decimal percentage) from WB to the corn- and WB-based diets.

The AME content for WB was calculated according to the following equation (Woyengo et al., 2010):

$$\text{AME of WB (kcal / kg)} = [(\text{GE retention for WB, \%}) \times (\text{GE content in WB, kcal / kg})] / 100$$

The AME_n content for WB was calculated from AME as described by Hill and Anderson (1958) using the following equation:

$$\text{AME}_n (\text{kcal / kg}) = \text{AME} - (8.22 \times \text{ANR})$$

Where ANR = apparent N retained (g/kg of feed intake)

The apparent ileal digestibility (AID) of AA was calculated using formula (Nyachoti et al., 1997):

$$\text{AID (\%)} = 100 - \left[100 \times (\text{AA}_{\text{digesta}} \times \text{Cr}_2\text{O}_{3\text{diet}}) / \text{AA}_{\text{diet}} \times \text{Cr}_2\text{O}_{3\text{digesta}} \right]$$

Where AA_{diet} and $\text{AA}_{\text{digesta}}$ are AA content (mg/kg of DM) in the diet and digesta, respectively, and $\text{Cr}_2\text{O}_{3\text{diet}}$ and $\text{Cr}_2\text{O}_{3\text{digesta}}$ are indigestible marker content (mg/kg of DM) in the diet and digesta, respectively.

The AID of AA was standardized using the average values for basal endogenous AA losses calculated using formula (Nyachoti et al., 1997):

$$\text{AAEL (g / kg)} = \text{AA}_{\text{digesta}} \times (\text{Cr}_2\text{O}_{3\text{diet}} / \text{Cr}_2\text{O}_{3\text{digesta}})$$

Where AAEL = average endogenous AA loss (g/kg of DM)

The SID of AA was calculated according to equation below as described by Opapeju (2006):

$$\text{SID (\%)} = [\text{AID} + (\text{AAEL} / \text{AA}_{\text{diet}})] \times 100$$

The GLM procedures of SAS was used to determine the main effects of, and interaction between, MC and Phy (SAS, 2009, version 9.2). The homogeneity of variances was evaluated by Hartley test and the normality of residuals by the

Shapiro–Wilk test (UNIVARIATE procedure). The statistical model used was $X_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ij}$, where μ = overall mean, α_i = MC effect, β_j = Phy effect, $(\alpha\beta)_{ij}$ = interaction between MC and Phy, e_{ij} = error contribution with average 0 and variance σ^2 , $i = 1 \dots a$, and $j = 1 \dots b$. Significance was accepted at $P < 0.05$. Tukey's least significant difference was used to detect significant differences between means whenever interaction between MC and Phy was observed.

RESULTS

This study was conducted to evaluate the nutritive value of WB for broiler chickens without or with an MC and Phy supplementation. Exogenous enzymes activities were guaranteed and suggested by GNC Bioferm Inc., Saskatoon, Saskatchewan. However, detailed analytical enzyme activities were not confirmed in mixed feeds.

The effects of MC and Phy on nutrients and AME_n retention are shown in Table 3. There was an interaction effect ($P < 0.05$) between MC and Phy on retention of DM, N, P, and AME_n . The digestibility of DM and AME_n was increased when dietary supplementation of MC was combined with Phy, compared with a single Phy supplementation or not. However, Phy supplementation in the presence of MC showed an increase in N and P retention compared with diet without MC. In the other variables, no interaction between the supplemented enzymes was identified on retention of ash, Ca, and NDF. In addition, an individual effect ($P < 0.05$) was observed for Phy on ash and Ca retention and for MC on fiber retention.

SID of AA in broiler chickens fed WB without or with exogenous enzymes are shown in Table 4. The MC + Phy combination effect was observed as an interaction ($P < 0.05$) on SID for Arg, His, Leu, Lys, Phe, Thr, Val, Asp, Cys, Glu, and Ser. This combination increased ($P < 0.05$) the overall SID of AA by 6.05% (75.94 to 94.26%), while the individual effects of MC were 2.35% (72.24 to 88.97), and Phy was 3.46% (73.37 to 92.13). Compared with the control diet, the greater retention ($P < 0.05$) of Pro (+5.23%) occurred in birds fed the MC diet, while Cys (+ 6.19%) occurred in those that were fed Phy and Ala in birds fed MC + Phy (+8.45%). No interaction ($P > 0.05$) of MC and Phy was observed on SID for Ile, Met, Trp, Ala, Gly, and Tyr.

The weight of liver and pancreas of birds are shown in Table 5. There was no interaction effect ($P > 0.05$) between MC and Phy on the relative weights of the liver and pancreas. However, individual MC

supplementation ($P < 0.05$) affected the relative weights of the liver and pancreas. Birds fed MC supplementation had lower weights of liver and pancreas when compared with control birds.

DISCUSSION

Present results in this study showed that most pronounced effects in nutrients and energy retention were associated to the MC and Phy dietary combination. The level and structure of NSP are variable in wheat (Slominski, 2011), and the main components are arabinoxylans (Amerah, 2015). The AME_n increase and nutrient retention with MC supplementation could be associated with total or partial arabinoxylan cleavage (Woyengo and Nyachoti, 2011). Therefore, NPS degradation, especially the arabinoxylans, by MC could facilitate access by the endogenous enzymes and Phy. Less fiber into the gastrointestinal tract reduces the digesta viscosity, which benefits the nutrient absorption on the intestinal lumen (Slominski, 2011; Amerah, 2015). The NSP also have capacity to bind multivalent cations (Debon and Tester, 2001), which associate with phytate of the ingredient and digesta. Thus, the MC also may increase the Phy efficacy by elimination of phytate chelating effects on the NSP (Slominski, 2011, Kiarie et al., 2016).

In this study, improvements observed in DM with enzyme supplementation can be attributed to increased protein, fat, and starch utilization by chicken. Juanpere et al. (2005) reported improvements in DM, Lipid, and starch digestibility when Phy and glycosidase were added in corn, wheat, and barley diets.

As for improvement in N retention by carbohydrases action could be associated with lower endogenous and exogenous N losses and an increase on dietary protein hydrolysis (Adeola and Cowieson, 2011). Ravindran et al. (1999) observed similar responses when broilers were fed with wheat–casein diets supplemented with Phy and glycanase. In addition, Cowieson and Masey O'Neill (2013) reported that xylanase addition in wheat-based diets improved N coefficients by around 3% in broilers at day 28.

Phytate binds with proteins under acidic conditions to form binary protein–phytate complexes making pepsin unable to act on these complexes (Cowieson et al., 2017). However, Cowieson and Ravindran (2007) reported that N retention increased by Phy supplementation, which was associated with cleavage site of phytate–protein and reductions of

Table 3. ATTD of nutrients and AME_n content in WB fed without or with MC* and Phy[†] supplementation to broiler chickens—trial 1

Item	Phy 0		Phy 50		SEM	P value [‡]		
	MC 0	MC 200	MC 0	MC 200		MC	Phy	MC × Phy
DM (%)	74.07 ^d	78.21 ^b	76.04 ^c	80.32 ^a	0.47	0.001	0.014	0.018
N (%)	77.46 ^c	79.50 ^b	80.06 ^b	83.49 ^a	0.41	0.041	<0.001	0.032
Ash (%)	40.05	41.81	43.74	44.34	0.34	0.089	0.010	0.105
Ca (%)	49.16	50.97	53.39	53.22	0.49	0.103	0.013	0.114
P (%)	48.26 ^c	49.52 ^c	52.19 ^b	54.40 ^a	0.41	0.610	<0.001	0.033
NDF (%)	44.76	49.19	44.86	48.86	0.44	0.001	0.245	0.112
AME _n (MJ/kg)	5.84 ^c	6.41 ^b	6.16 ^b	6.80 ^a	0.07	0.004	0.072	0.047

ATTD = apparent total tract digestibility. *N* = 7.

^{a-d}Means assigned different letters within a factor of analysis (MC and Phy and their interactions) are significantly different, *P* < 0.05.

*MC, multicarbohydase (700 U α-galactosidase, 2,200 U galactomannanase, 30,000 U xylanase, and 22,000 U β-glucanase/kg of diet).

[†]Phy, Phytase (500 FTU/kg diet).

[‡]MC = multicarbohydase level (0 vs. 700 U α-galactosidase, 2,200 U galactomannanase, 30,000 U xylanase, and 22,000 U β-glucanase/kg diet); Phy = phytase level (0 vs. 500 FTU/kg diet).

endogenous N flow. Afsharmanesh et al. (2008) observed that broilers (aged 21 d) fed with wheat- and Phy-based diets had an N retention increased by the least 11%, compared with those fed non-Phy-supplemented diet. Moreover, Phy can largely prevent

the formation of binary protein–phytate complexes by prior hydrolysis of these complexes in the superior digestive tract, increasing N accessibility (Cowieson et al., 2017). Thus, in this study possibly the Phy hydrolysis of phytate also improved N retention.

Table 4. SID of amino acids in WB fed without or with MC* and Phy[†] supplementation to broiler chicks (21 d)—trial 2

Item	Phy 0		Phy 50		SEM	P value [‡]		
	MC 0	MC 200	MC 0	MC 200		MC	Phy	MC × Phy
Essential AA (%)								
Arg	88.06 ^c	88.97 ^c	92.13 ^b	94.26 ^a	0.37	0.079	<0.001	0.041
His	79.26 ^c	80.95 ^{bc}	81.03 ^b	83.92 ^a	0.33	0.052	0.002	0.004
Ile	82.90	84.34	85.04	86.37	0.31	0.113	0.001	0.129
Leu	79.36 ^c	80.01 ^c	81.77 ^b	84.46 ^a	0.39	0.034	<0.001	<0.001
Lys	78.30 ^d	80.48 ^c	82.19 ^b	84.59 ^a	0.39	0.054	0.007	0.038
Met	85.90	87.88	87.06	89.45	0.35	0.005	0.021	0.411
Phe	71.68 ^d	73.19 ^c	75.61 ^b	77.45 ^a	0.43	0.017	<0.001	0.033
Thr	81.16 ^c	84.92 ^b	83.46 ^b	88.03 ^a	0.42	<0.001	<0.001	0.044
Trp	78.31	80.14	80.57	80.98	0.42	0.047	0.101	0.137
Val	76.36 ^c	77.62 ^{bc}	78.15 ^b	80.46 ^a	0.38	0.034	0.004	0.045
Nonessential AA (%)								
Ala	70.02	72.24	73.37	75.94	0.42	0.016	<0.001	0.144
Asp	78.36 ^c	80.72 ^b	81.77 ^b	84.86 ^a	0.46	0.022	0.004	0.036
Cys	72.16 ^d	74.47 ^c	76.31 ^b	79.66 ^a	0.38	0.244	<0.001	0.041
Glu	84.64 ^d	88.55 ^b	86.47 ^c	70.50 ^a	0.36	<0.001	0.067	<0.001
Gly	84.90	88.05	87.54	90.19	0.37	<0.001	0.032	0.198
Pro	80.72 ^c	84.14 ^b	85.79 ^{ab}	87.20 ^a	0.42	0.012	0.007	0.030
Ser	80.30 ^c	83.19 ^b	81.48 ^c	86.29 ^a	0.37	<0.001	0.014	0.026
Tyr	76.97	76.98	78.03	77.95	0.32	0.711	0.044	0.689

The endogenous ileal AA losses (g/kg of DM) using the casein diet were as follows: Arg, 0.21; His, 0.39; Ile, 0.18; Leu, 0.25; Lys, 0.28; Met, 0.13; Phe, 0.21; Thr, 0.42; Trp, 0.27; Val, 0.36; Ala, 0.34; Asp, 0.48; Cys, 0.07; Glu, 0.6; Gly, 0.24; Pro, 0.22; Ser, 0.49; and Tyr, 0.19 g/kg. *N* = 7.

^{a-d}Means assigned different letters within a factor of analysis (MC and Phy and their interactions) are significantly different, *P* < 0.05.

*MC, multicarbohydase (700 U α-galactosidase, 2,200 U galactomannanase, 30,000 U xylanase, and 22,000 U β-glucanase/kg diet).

[†]Phy, Phytase (500 FTU/kg diet).

[‡]MC = multicarbohydase level (0 vs. 700 U α-galactosidase, 2,200 U galactomannanase, 30,000 U xylanase, and 22,000 U β-glucanase/kg diet); Phy = phytase level (0 vs. 500 FTU/kg diet).

Table 5. Relative weight of liver and pancreas (%live weight) in broiler chicks (trial 2) fed WB without or with MC* and Phy[†] supplementation

Item*	Phy 0		Phy 50		SEM	P value [‡]		
	MC 0	MC 200	MC 0	MC 200		MC	Phy	MC × Phy
IBW (g)	45	44	46	44	0.23	0.823	0.645	0.866
FBW (g)	614	647	633	663	8.13	0.101	0.183	0.104
ADG (g/d)	27.10 ^c	28.71 ^b	27.95 ^{ab}	29.48 ^a	0.17	0.037	0.091	0.029
ADIF (g/d)	38.95	37.90	38.19	37.62	0.28	0.104	0.046	0.076
G:F (g/g)	0.70 ^d	0.76 ^b	0.73 ^c	0.78 ^a	0.02	0.003	0.074	0.017
Liver weight (g)	3.94	3.03	3.51	3.42	0.06	0.032	0.121	0.112
Pancreas weight (g)	2.53	1.96	2.40	2.34	0.003	0.005	0.101	0.087

N = 7.

*IBW, initial body weight; FBW, final body weight; ADFI, average daily feed intake; AFI, average feed intake; G:F, gain to feed ratio.

^{a-d}Means assigned different letters within a factor of analysis (MC and Phy and their interactions) are significantly different, $P < 0.05$.

*MC, multicarbohydase (700 U α -galactosidase, 2,200 U galactomannanase, 30,000 U xylanase, and 22,000 U β -glucanase/kg diet).

[†]Phy, Phytase (500 FTU/kg diet).

[‡]MC = multicarbohydase level (0 vs. 700 U α -galactosidase, 2,200 U galactomannanase, 30,000 U xylanase, and 22,000 U β -glucanase/kg diet); Phy = phytase level (0 vs. 500 FTU/kg diet).

The MC and Phy increased ($P < 0.05$) the AME_n of WB by 9.76% and 5.14%, respectively, compared with WB without enzymes. Present observations are in agreement with [Gallardo et al. \(2017\)](#) who reported that birds fed with canola meal plus Phy–MC combination showed higher energy retention compared with control diet and diets with alone enzymes. [Selle et al. \(2009\)](#) reported that broilers fed a Phy–xylanase wheat-based diet showed an increase AME_n of 0.63 MJ/kg on a DM basis.

The increase of the ash, Ca, and P retention by the Phy in this study suggests a clear and large hydrolysis of mineral–phytate complexes. Present results are in agreement with [Ravindran et al. \(2000\)](#) and [Selle et al. \(2009\)](#), who reported beneficial effects of the Phy in Ca and P retention in some feedstuffs. In barley-based diets, [Juanpere et al. \(2005\)](#) observed that supplementation of Phy and b-glucanase improved P retention in broilers by 15% when compared with control diet. Based on diets with low-to-high P levels, [Ravindran et al. \(2000\)](#) observed that Phy supplementation improved P retention in wheat-sorghum-soy. [Afsharmanesh et al. \(2008\)](#) also related that addition of Phy in wheat-based diets increased P retention by 11.8%. In addition, improvements in Ca retention by Phy supplementation were expected because Phy liberates Ca from the Ca–phytate complex. This may be associated with phytate reduction, allowing greater Ca release and bird use ([Selle et al., 2009](#)), for example, in bone tissue, improving mineralization and dietary P and Ca use ([Karimi et al., 2013](#)).

Significant effects ($P < 0.05$) from MC were observed on NDF retention. Thus, a greater NSP

concentration in the diet may result in a higher efficacy for carbohydrase supplementation.

The present results indicate that AA digestibility was improved in the broilers when fed with WB diets supplemented with exogenous enzymes. Thus, there were beneficial effects of enzymes on N retention increasing the absorption of AA ([Dadalt et al., 2017](#); [Gallardo et al., 2017](#)). The MC and Phy combination showed a higher effect on AA digestibility in the SID of Arg, His, Leu, Lys, Phe, Thr, Val, Asp, Cys, Glu, and Ser. [Selle et al. \(2003\)](#) observed positive response in AA digestibility when supplied Phy plus xylanase in wheat-based diets. On the other hand, negative effect of phytate on AA digestibility was largely associated with an increase in endogenous AA loss from the intestine rather than a direct impact on dietary protein retention ([Cowieson et al., 2017](#)). This is in agreement with [Selle et al. \(2006\)](#), who observed greater responses of Phy supplementation on the digestibility of Cys, Thr, Pro, and Gly and reported that a great part of Phy effects on AA was associated with reduction of endogenous AA losses and no increase in dietary AA digestibility.

Decreasing the reabsorption of endogenous AA also promotes N losses, and NSP possibly increases secretion and decreases reabsorption of endogenous AA ([Nyachoti et al., 2000](#)). Therefore, phytate may decrease digestion in the diet, and NSP may increase losses of endogenous AA ([Cowieson et al., 2017](#)).

The present study did not aim to evaluate broiler performance, especially under high-WB diet. However, the positive results for energy and N retention showed a significant interaction of MC

× Phy in average daily feed intake and gained to feed ratio.

The increase in fiber consumption in birds produce changes in the size and weight of digestive organs that are associated with the increase of intestinal viscosity, decrease gut transit time (Gracia et al., 2009), reduction of nutrient utilization (Adeola and Cowieson, 2011), and decrease in the secretory endogenous enzymes activity of the pancreas, liver, and intestine (Wu et al., 2004). Thus, the lower weight of liver and pancreas in birds fed with MC compared with control birds may be attributed to positive effects of enzymes of the MC complex in nutrient absorption by the fiber degradation, decreased the secretory activity of these organs (Veldman and Vahl, 1994), and consequently decreased their weights. Brenes et al. (1993) observed that birds fed diet low level of fiber showed lower weight of liver and pancreas compared with birds fed high-fiber diets.

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