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Original Research Article

Growth performance, gastrointestinal weight, microbial metabolites and apparent retention of components in broiler chickens fed up to 11% rice bran in a corn-soybean meal diet without or with a multi-enzyme supplement☆

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ABSTRACT

We investigated the effects of adding up to 11% rice bran (RB) in corn-soybean meal diets fed to broiler chickens without or with a multi-enzyme supplement (MES). The MES supplied xylanase, β-glucanase, invertase, protease, cellulase, α-amylase and mannanase with targeted activity of 2,500, 300, 700, 10,000, 1,200, 24,000, and 20 U/kg of feed, respectively. The study used a two-phase feeding program (starter, d 0 to 24; finisher, d 25 to 35) with RB added at 5% and 11%, respectively creating 4 diets in each phase. Diets were iso-caloric and iso-nitrogenous and contained phytase (500 FTU/kg) and TiO_2 as a digestibility marker. Three hundred and sixty d-old male Ross 708 broiler chicks were placed in cages based on BW (15 birds/cage) and allocated to 4 diets ($n = 6$). Birds had free access to feed and water. Body weight and feed intake were recorded. Excreta samples were collected 3 d prior to the end of each phase for apparent retention (AR) of components. Samples of birds were sacrificed on d 24 and 35 for gut weight and ceca digesta for organic acid content. There was no interaction ($P > 0.10$) between RB and MES on BWG and FCR in starter or finisher phase. In finisher phase, birds fed MES had better BWG (961 versus 858 g) and FCR (1.69 versus 1.86) than birds fed non-MES diets ($P < 0.01$). Feeding RB reduced ($P = 0.02$) BWG in finisher phase resulting in lower d 35 BW. Birds fed RB had higher ($P \leq 0.01$) gizzard weight on d 24 and 35 than non-RB birds. An interaction ($P \leq 0.01$) between RB and MES on concentrations of propionic and iso-butyric acids in ceca digesta showed that MES reduced these acids in non-RB diet. The AR of gross energy was higher ($P < 0.02$) for MES versus non-MES birds in starter and finisher phases. In conclusion, independently, RB increased gizzard weight and reduced final BW whereas MES improved growth and energy utilization.

1. Introduction

Rice (*Oriza sativa*) is cultivated in every continent of the world except Antarctica. The annual global production of paddy rice exceeds 700 million metric tons and is the most produced cereal grain after corn and wheat (Muthayya et al., 2014). After de-hulling paddy rice, subsequent mechanical processing removes the brown layer and endosperm to yield white rice and rice bran (RB) as a co-product (Saunders, 1990). Rice bran constitutes 10% of the paddy rice and thus on a global basis 70 million metric tons of RB is produced annually (Stein et al., 2015). Rice bran is a valuable feed ingredient rich in amino acids, starch, fat, vitamins and some trace

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minerals (Ravindran and Blair, 2007; Stein et al., 2015). In rice growing regions, RB can be a cost-effective feed ingredient for poultry (Ravindran and Blair, 2007, 2009). However, RB is prone to rancidity, has a high phytate content, contains trypsin inhibitor, and is high in fiber (Gallinger et al., 2004; Ravindran and Blair, 2007, 2009). These characteristics have limited the use of RB in poultry feeding programs. A maximum of 10% to 20% has been recommended for inclusion in broiler diets, depending on the geographical origin of the rice production (Martin and Farrell, 1998). Gallinger et al. (2004) reported that inclusion of 20% RB in broiler diets resulted in reduced growth performance. Just 10% RB reduced feed efficiency and tibia ash content (Gallinger et al., 2004). Other studies have recommended that RB not to be include in diets of broiler chickens of less than 21 d of age (Martin and Farrell, 1998).

Some of the challenges of using RB in practical monogastric feeding programs have been addressed through technological advancement. For example, the concentration of oil in rice bran is between 14% and 24% depending on rice variety and processing (Prakash and Ramaswamy, 1996; Kaufmann et al., 2005). Endogenous lipases are activated during milling leading to rapid hydrolysis and rancidification of the oil (Saunders, 1990). However, technologies such as extrusion, addition of stabilizers and defatting have been successful in eliminating rancidity problem in RB (Saunders, 1990; Prakash and Ramaswamy, 1996). Rice bran has a high concentration of P relative to other plant-based feed ingredients. Values of between 1.6% and 2.2% phosphorus (P) have been reported (Stein et al., 2015). Approximately, 70% to 90% of the P is in phytate form and unavailable to non-ruminants because they lack significant endogenous and microbial phytase activity in the foregut (Selle and Ravindran, 2007; Kiarie and Nyachoti, 2010). However, the advent and global feed industry acceptance of microbial phytase technology has significantly increased phytate P utilization in plant feedstuffs including RB in swine and poultry (Ravindran et al., 2006; Kiarie et al., 2015; Almeida et al., 2017).

Rice bran also contains a higher or comparable concentration of non-soluble polysaccharides (NSP) relative to typical cereal grain co-products, especially arabinoxylans and arabinose. Defatting inevitably increases concentration of NSP and protein, significantly reducing metabolizable energy value (Annison et al., 1995; Ravindran and Blair, 2009). Improving the nutritive value of RB with application of exogenous fiber degrading enzymes (FDE) has been reported but with variable responses. For example, Farrell and Martin (1998) did not observe benefits of supplementing xylanase and β-glucanase in broilers fed RB. However (Wang et al., 1997), reported an enzyme blend (xylanase, β-glucanase and pectinase) improved performance of chicks fed irradiated Malaysian RB, but not when fed Chinese RB. Substrates in feedstuffs exists in complex relationship with various components such as protein, fat, fiber, and other carbohydrates (Kiarie et al., 2016a). It has been suggested that preparations with multiple enzyme activities may provide a competitive strategy to improve nutrient utilization in wide range of feed ingredients (Slominski, 2011). Furthermore, phytase is a common additive in majority of monogastric diets, however, too much emphasis had been placed on interpretation of FDE responses without phytase in the background (Kiarie et al., 2014). For example, if FDE and phytase are included in the same diet, the FDE hydrolyze the NSP providing greater access for the phytase to reduce the interaction of phytate with amino acids and minerals as well as reducing binding of elemental P (Zijlstra et al., 2010). Previous studies have demonstrated synergic effects of phytase and FDE on nutrient utilization in pigs (Kiarie et al., 2010, 2016b) and broilers (Woyengo et al., 2010; Liu et al., 2011; Kiarie et al., 2014), but others indicated beneficial effects originated mainly from phytase alone (Olukosi et al., 2007). We hypothesized that a multi-enzyme supplement (MES) will improve growth performance linked to nutrient digestibility and gastrointestinal ecology in broilers fed RB in a corn soybean meal diet with phytase background. Therefore, the objective was to examine growth performance, gastrointestinal weight, ceca short chain fatty acids content and apparent retention (AR) of components responses of adding up to 11% RB in corn-soybean meal diet containing phytase fed to broilers without or with a MES supplement.

2. Materials and methods

Experimental procedures and animal use were reviewed and approved (AUP# 3521) by the University of Guelph Animal Ethics Committee. Broiler chickens were cared for in accordance with the Canadian Code of Practice for the Care and Use of Animals for Scientific Purposes (CCAC, 2009).

2.1. Rice bran sample, enzyme and experimental diets

The RB sample was procured from a feed merchant in Philippines and its chemical composition is shown in Table 1. Two basal corn-soybean meal diets were prepared without or with RB (Table 2). The supplier guaranteed analyses were: < 5% crude fat and < 10% crude fiber. Based on these parameters the energy and nutrient profiles for RB were derived from INRA-CIRAD-AFZ Feed Tables (INRA, 2018) to facilitate feed formulation. Diets were prepared for a two-feeding program (starter, d 0 to 24, 5% RB) and finisher (d 25 to 35, 11% RB) and met or exceeded specifications for Ross 708 (Aviagen, 2014). The basal diets contained phytase (Biophytase 5000) at 500 FTU/kg of final feed equivalent to 0.10% non-phytate P and TiO_2 as the digestibility marker. Each basal diet was split in two portions; one portion was the control and the other portion was top-dressed with MES effectively creating a 2 × 2 factorial arrangement of treatments. The MES supplied xylanase, β-glucanase, invertase, protease, cellulase, amylase, mannanase with

Table 1
Chemical composition of rice bran (as-fed basis, %).

Item	Amount
Dry matter	91.5
Crude protein	13.0
Crude fat	13.0
Carbohydrates	
Simple sugars[1]	0.16
Sucrose	2.69
Oligosaccharides[2]	0.06
Starch	36.6
Fiber fractions	
Acid detergent fiber	5.12
Neutral detergent fiber	13.5
Total dietary fiber	15.5
Non-starch polysaccharides	8.85
Rhamnose	nd[3]
Arabinose	1.79
Xylose	2.03
Mannose	0.19
Galactose	0.48
Glucose	3.52
Uronic Acids	0.86
Lignin and polyphenols	5.24
Glycoprotein[4]	1.25
Ash	7.68
Total phosphorus	1.67
Phytate phosphorus	1.24
Non-phytate phosphorus	0.43

[1] Includes glucose and fructose.
[2] Includes raffinose and stachyose.
[3] Not detected.
[4] Neutral detergent insoluble crude protein.

Table 2
Composition of basal diet (as-fed basis, %).[1]

Item	Starter, d 0 to 24		Finisher, d 24 to 35	
	Control	+ Rice bran	Control	+ Rice bran
Ingredients				
Corn	60.69	57.9	67.31	61.16
Soy bean meal (46%)	27.34	26.50	17.57	15.70
Rice bran (defatted)	–	5.00	–	11.0
Pork meal (58%)	3.00	3.00	6.00	6.00
Soy oil	4.33	2.97	5.62	2.63
Limestone	0.68	0.71	0.19	0.26
Mono calcium phosphate	1.43	1.38	0.70	0.59
Vitamin-trace minerals premix[2]	1.00	1.00	1.00	1.00
L-lysine HCl	0.37	0.37	0.46	0.47
DL-methionine	0.36	0.36	0.35	0.36
L-threonine	0.18	0.18	0.22	0.23
L-tryptophan	–	–	–	0.01
Salt	0.22	0.23	0.19	0.20
Sodium bicarbonate	0.14	0.14	0.13	0.13
Bio-Phytase 5000[3]	0.01	0.01	0.01	0.01
TiO_2	0.25	0.25	0.25	0.25
Calculated provisions				
AME, kcal/kg	3,100	3,100	3,200	3,200
Crude protein	20.0	20.0	18.0	18.0
Crude fat	5.56	6.75	5.88	8.51
SID Lys	1.15	1.15	1.06	1.06
SID Met	0.62	0.63	0.59	0.6
SID Met + Cys	0.87	0.87	0.81	0.81
SID Try	0.21	0.21	0.17	0.17
SID Thr	0.77	0.77	0.71	0.71
Ca	0.96	0.96	0.93	0.93
Available P	0.48	0.48	0.46	0.47
Na	0.16	0.16	0.16	0.16
Cl	0.23	0.23	0.23	0.23
Analyzed provisions				
Dry matter	89.3	89.7	89.3	89.5
Gross energy, kcal/kg	3,985	4,109	4,066	4,264
Crude protein	20.46	19.96	18.56	18.03
Crude fat	5.24	7.06	6.22	9.73
Neutral detergent fiber	8.61	9.02	9.07	9.55

SID = standardized ileal digestible.
[1] Multi-enzyme supplement (MES, Canadian Bio-Systems, Calgary, AL, Canada) was top dressed to supply xylanase, β-glucanase, invertase, protease, cellulase, amylase, mannanase with targeted activity levels 2,500, 300, 700, 10,000, 1,200, 24,000, 20 U/kg of feed, respectively.
[2] Vitamin mineral premix provided per kilogram of diet: vitamin A, 880,000 IU; vitamin D_3, 330,000 IU; vitamin E, 4,000 IU; vitamin B_{12}, 1,200 mcg; biotin, 22,000 mg; menadione, 330 mg; thiamine, 400 mg; riboflavin, 800 mg; pantothenic acid, 1,500 mg; pyridoxine, 300 mg; niacin, 5,000 mg; folic acid, 100 mg; choline, 60,000 mg; iron, 6,000 mg; copper, 1,000 mg.
[3] Bio-Phytase 5000 (Canadian Bio-Systems) supplied 500 FTU/kg of feed.

targeted activity level of 2,500, 300, 700, 10,000, 1,200, 24,000, 20 U/kg of feed, respectively. The enzymes along with the enzyme assay procedures were supplied by the Canadian Bio-Systems (Calgary, AL, Canada). Diets were fed in mash form.

2.2. Birds, housing and experimental procedures

Three hundred and sixty d-old male broiler chicks (Ross × Ross 708) were allocated to 24 identical metabolic cages (15 chicks per pen) based on body weight (BW). Each cage was equipped with a feeder trough and two nipples drinkers. The room temperature was set at 32 °C on d 0 and gradually brought down to 29 °C by d 13 then gradually reduced to 24 °C by d 21. The lighting program was 23 h of light (20 lx) from d 0 to 3 followed by 20 h of light (10 to 15 lx) from d 4 onward. The 4 diets were assigned to cages to give to 6 replicates per diet. The birds had free access to diets and water for 35 d. Body weight and feed intake were measured at the end of the phase. From d 20 to 23 post-hatching, excreta samples were collected per cage for AR of components. On d 24, 8 chicks per cage were randomly euthanized by cervical dislocation. The empty gizzard and small intestine weight was recorded and ceca digesta taken for short chain fatty acids (SCFA) analyses. The remaining chicks were switched to respective finisher diets until d 35. Excreta samples were taken on d 31 to 34, and at the end of the experiment all birds were sacrificed for similar sampling and measurements as described for starter phase. Excreta and digesta samples were immediately frozen at −20 °C until required for analyses.

2.3. Sample processing and chemical analysis

The excreta samples were thawed, pooled by cage and subsequently oven-dried at 60 °C for 72 h. Samples of the RB, diets and dried excreta samples were finely ground. All samples were analyzed for dry matter (DM), gross energy (GE), neutral detergent fiber (NDF), acid detergent fiber (ADF), nitrogen, crude fat, and titanium. Dry matter determination was carried out according to standard procedures ((AOAC, 2005), method 930.15). Gross energy was determined in a bomb calorimeter (IKA – WERKE bomb calorimeter [C7000, GMBH & CO., Staufen, Germany]) using benzoic acid as a calibration standard. The NDF and ADF contents were determined according to (Van Soest et al., 1991) using Ankom 200 Fiber Analyzer (Ankom Technology, Fairport, NY). Nitrogen was determined with a CNS-2000 carbon, N, and sulfur analyzer (Leco Corporation, St. Joseph, MI) according to the combustion method 968.06 (AOAC, 2005). The crude protein (CP) values were calculated by multiplying analyzed nitrogen values by 6.25. Crude fat content was determined using ANKOM XT 20 Extractor (Ankom Technology, Fairport, NY). Titanium content was measured on a UV spectrophotometer following the method of Myers et al. (2004).

Rice bran samples were further analyzed for minerals (P and phytate P) and carbohydrates (simple sugars, sucrose, oligosaccharides, starch, fiber fractions including lignin and glycoprotein). Standard AOAC (2005) procedures were used for total P (965.17) determination. Phytate P was assayed using the procedure described by Haug and Lantzsch (1983). Non-phytate P was calculated by subtracting phytate P from the total P contents. Simple sugars (fructose and glucose), sucrose, and oligosaccharides raffinose and stachyose were determined by gas-liquid chromatography according to the procedure described by Slominski et al. (2004). Starch was analyzed using the Megazyme Total Starch Kit (Megazyme International Ireland Ltd., Co. Wicklow, Ireland). Non-starch polysaccharides were determined by gas-liquid chromatography (component neutral sugars) and by colorimetry (uronic acids) using the procedure described by Englyst and Cummings (1984, 1988) with modifications (Slominski et al., 2006). Due to the high solubility of NSP in the NDF solution and therefore losses of NSP on NDF analysis, total dietary fiber was determined by a combination of NDF and neutral detergent-soluble NSP measurements, and was calculated as the sum of NDF and NDF-soluble NSP (Slominski et al., 1994, 2006). Neutral detergent fiber-soluble NSP were calculated as total sample NSP minus NSP present in the NDF residue. Neutral detergent insoluble crude protein (NDICP, glycoprotein) represented the amount of crude protein present in the NDF residue. The value for lignin with associated polyphenols was calculated by difference between the total fiber and NDICP + NSP contents.

The concentration of short chain fatty acids (citric, lactic, formic, acetic, propionic and butyric) were assayed in thawed ceca digesta (Leung et al., 2018). Briefly, approximately 0.1 g of the digesta sample was resuspended with 1 mL of 0.0025 mol/L H_2SO_4 (1:10, wt/vol) in a microcentrifuge tube, tightly closed and vortexed vigorously until sample completely dissolved. The tubes were then centrifuged at 11,000 × *g* for 15 min and 400 μL of supernatant transferred to HPLC vial and topped with 400 μL of 0.0025 mol/L H_2SO_4 buffer. The resulting digesta fluid was then assayed for SCFA

using HPLC (Hewlett Packard 1100, made in Germany) with Rezex ROA-Organic Acid LC column, 300 mm × 7.8 mm from Phenomenex and Refractive Index detector at 400 °C (Agilent 1260 Infinity RID from Agilent Technologies, made in Germany) (De Baere et al., 2013).

Xylanase activity in diets was assayed using Xylazyme AX tablets (Megazyme International Ltd., Bray, Ireland). One unit of xylanase was defined as the quantity of the enzyme that liberated 1 μmoL of xylose equivalent per min.

2.4. Calculations and statistical analysis

The apparent retention of components was calculated as described by Kim et al. (2017). Data was analyzed using general linear model procedures of SAS (SAS Inst. Inc., Cary, NC). The model included the main effects of RB, MES and associated two-way interactions. Treatment differences were considered significant at $P < 0.05$ and trends ($0.05 < P < 0.10$) were discussed.

3. Results

Xylanase activity was determined to confirm accuracy of inclusion of MES and feed mixing. The analyzed xylanase activities in the starter diets were 376, 2,450, 218 and 2,686 U/kg of feed for the control, control + MES, RB and RB + MES, respectively. The corresponding xylanase activities for finisher phase were 134, 2,457, 88 and 1,997 U/kg of feed, respectively. The concentration of CP, crude fat, starch and total dietary fiber in RB were 14.2%, 14.0%, 40.0% and 16.8% DM, respectively (Table 1). The most dominant mono sugars in the NSP fraction was glucose and xylose. The concentration of lignin and polyphenols was 5.7% DM.

There was no interaction ($P > 0.10$) between RB and MES on BWG, feed intake (FI) and FCR in the entire experiment (Table 3). Feed intake was not affected ($P > 0.10$) by dietary treatments except in the starter phase where birds fed MES tended to eat more feed ($P = 0.07$) than non-MES birds. In the starter phase, the main effects of MES were such that, MES-fed birds had improved BWG ($P < 0.01$) and a tendency for improved FCR ($P = 0.06$) compared with non-MES birds. Birds fed RB tended to have higher BWG than birds not fed RB in the starter phase (884 versus 860 g, $P = 0.07$). Feeding RB reduced BWG in the finisher phase resulting in lower d 35 BW (1,804 versus 1,855 g, $P = 0.02$) relative to birds not fed RB. In the finisher phase, birds fed MES had better BWG (961 versus 858 g) and FCR (1.69 versus 1.86) than birds fed non-MES diets.

Although neither interaction between RB and MES or MES affected ($P > 0.10$) gizzard weight, RB increased ($P \leq 0.01$) gizzard weight on d 24 and 35 (Table 4). The small intestine weight was not ($P > 0.10$) affected by diets. There was no ($P > 0.10$) interaction between RB and MES on ceca digesta concentration of SCFA in the starter phase (Table 5). In the starter phase, the ceca digesta of birds fed RB had higher ($P = 0.025$) concentration of propionic acid and tended ($P = 0.07$) to have a higher concentration of total SCFA (summation of lactic, acetic, propionic, iso butyric and n-butyric acids) compared with birds not fed RB. Birds fed MES tended ($P = 0.06$) to have a lower concentration of iso-butryic acid relative to birds not fed MES. In the finisher phase, an interaction ($P \leq 0.01$) between RB and MES on concentration of propionic and iso-butyric acids in ceca digesta showed that MES reduced these acids in non-RB diet. Ceca digesta of RB fed birds exhibited lower ($P = 0.001$) concentration of iso-butryic acid relative to birds not fed RB (Table 5).

On d 24, there was no interaction ($P > 0.10$) between RB and MES on AR of components (Table 6). Added MES increased ($P \leq 0.031$) AR of CP, NDF and GE. A tendency for interaction between RB and MES ($P = 0.090$) was observed for AR of GE on d 24 (Table 6). In this context, supplemental MES tended to improve AR of GE in RB diets. In the finisher phase (d 35), there was an interaction ($P < 0.01$) between RB and MES on AR of NDF such that MES reduced AR of NDF in corn diets. Birds fed RB diets retained ($P < 0.01$) more crude fat and NDF than birds not fed RB. Added MES increased AR of CP ($P < 0.01$), crude fat ($P = 0.03$) and GE ($P = 0.02$) compared with control non-MES diets.

4. Discussion

Diet composition is one of the major factors that can influence nutrient utilization and gastrointestinal physiology, mainly through

Table 3
Effects of adding rice bran in a corn-soybean meal diet fed without or with multi-enzyme supplement (MES) on growth performance in broiler chickens.

Rice bran	MES[1]	Starter, d 0 to 24[2]					Finisher, d 25 to 35[3]				
		IBW	FBW, g	BWG, g	FI, g	FCR[4]	IBW	FBW, g	BWG, g	FI, g	FCR[4]
–	–	40.6	827	787	1,219	1.550	923	1,803	883	1,635	1.861
–	+	40.0	893	853	1,256	1.473	855	1,907	987	1,631	1.642
+	–	40.4	849	808	1,219	1.510	998	1,753	833	1,551	1.862
+	+	40.4	920	879	1,269	1.442	902	1,854	935	1,633	1.747
SEM		0.49	12.8	12.8	23.1	0.02	26.11	17.65	17.64	35.10	0.043
Main effect of rice bran											
–		40.3	860	820	1,238	1.511	889[b]	1,855[a]	935[a]	1,633	1.752
+		40.4	884	844	1,244	1.476	950[a]	1,804[b]	884[b]	1,592	1.804
SEM		0.35	9.05	9.02	16.35	0.02	18.46	13.28	13.54	24.29	0.030
Main effect of MES											
	–	40.5	838[b]	797[b]	1,219	1.530	960[b]	1,778[b]	858[b]	1,593	1.861[a]
	+	40.2	907[a]	866[a]	1,263	1.457	879[a]	1,881[a]	961[a]	1,632	1.694[b]
SEM		0.35	9.05	9.02	16.35	0.02	18.46	13.90	13.90	25.42	0.031
P-value											
Rice bran		0.779	0.073	0.074	0.789	0.257	0.030	0.019	0.019	0.271	0.256
MES		0.551	<0.01	<0.01	0.073	0.059	0.005	<0.01	<0.01	0.332	0.003
Rice bran × MES		0.551	0.849	0.867	0.791	0.818	0.601	0.935	0.935	0.200	0.201

IBW = initial body weight; FBW = final body weight; BWG = body weight gain; FI = feed intake; FCR = feed conversion ratio.
[a, b] Within a factor of analyses, means in a column with different superscripts are significantly different at $P < 0.05$.
[1] Multi-enzyme supplement supplied xylanase, β-glucanase, invertase, protease, cellulase, amylase, and mannanase with targeted activity level 2,500, 300, 700, 10,000, 1,200, 24,000, 20 U/kg of feed, respectively.
[2] Birds per cage = 15.
[3] Birds per cage = 7; 8 birds per cage were sacrificed on d 24 for digesta and gastrointestinal weight.
[4] Corrected for mortality.

Table 4
Effects of adding rice bran in a corn-soybean meal diet and fed without or with multi-enzyme supplement (MES) to broiler chickens on gizzard and small intestine weight (g/kg BW).

Rice bran	MES[1]	Day 24		Day 35	
		Gizzard	Small intestine	Gizzard	Small intestine
–	–	17.4	30.1	12.87	25.01
–	+	18.4	30.0	12.93	23.94
+	–	19.4	30.0	14.60	25.40
+	+	19.8	31.2	14.85	26.80
SEM		0.625	0.937	0.351	0.947
Main effects of rice bran					
–		17.9[b]	30.1	12.9[b]	24.5
+		19.6[a]	30.6	14.7[a]	26.1
SEM		0.442	0.662	0.248	0.670
Main effects of MES					
–		18.37	30.05	13.74	25.21
+		19.09	30.61	13.89	25.37
SEM		0.442	0.662	0.248	0.670
P-value					
Rice bran		0.015	0.560	<0.001	0.102
MES		0.264	0.560	0.662	0.866
Rice bran × MES		0.600	0.520	0.791	0.205

[a, b] Within a factor of analyses, means in a column with different superscripts are significantly different at $P < 0.05$.
[1] Multi-enzyme supplement supplied xylanase, β-glucanase, invertase, protease, cellulase, amylase, and mannanase with targeted activity level 2,500, 300, 700, 10,000, 1,200, 24,000, 20 U/kg of feed, respectively.

the contents of anti-nutritional factors and the nature of the substrate available (Kiarie et al., 2014, 2017). The focus of the current study was on the fiber fraction in RB and therefore the pre-trial chemical analyses focused on characterization of fiber for selection of enzyme activities. Rice bran energy and nutrients specification for diet formulation were from book values to formulate isocaloric and isonitrogenous diets. However, chemical analyses of the feed samples (Table 2) indicated that the RB diets had higher gross energy likely linked to higher fat content in RB than supplier guaranted. Poultry diet with high fat is expected to reduce feed intake and improve FCR (Slominski et al., 2006). However, perhaps the slightly higher fat in RB diets had not effects in the current study since birds fed RB diets had similar feed intake and FCR to birds fed non-RB diets. Addition of 5% RB tended to improve BWG in the starter phase, however, a reverse effect was observed when 11% RB was added in the finisher phase. It has been speculated that poultry requires a moderate amount of diet structure for proper gut development and functionality (Mateos et al., 2012). Diet structure is critical in stimulating gizzard development, influencing digesta passage rate and improving gut motility by enhancing endocrine cholecystokinin release which stimulates the secretion of pancreatic enzymes and gastroduodenal refluxes (Mateos et al., 2012; Xu et al., 2015). It is no coincidence that we observed increased gizzard weight in birds fed RB, specifically birds fed RB had 9% and 14% higher gizzard weight compared with non-RB birds in the starter and finisher the phases, respectively. Similarly, Wang et al. (1997) observed increased size of gastrointestinal tract in poultry fed RB. Extended gizzard retention time increases interaction of feed particles with gastric juices and thus improves digestion and feed efficiency (Xu et al., 2015). This may partly explain the increased retention crude fat and NDF seen in broilers fed 11% RB in the present study. However, the increased gizzard size in birds fed RB did not result in increased BWG, FCR or GE retention suggesting the presence of fiber was detrimental to the overall nutrients utilization. This could be partly linked to increased visceral maintenance energy consumption. Gut metabolism has been estimated to account for 20% to 36% of energy use in chickens (Cant et al., 1996).

Based on origin of paddy rice production, a maximum of 10% to 20% has been recommended for inclusion in broiler diets. Other studies have recommended that RB not to be include in diets of broilers less than 21 d of age (Martin and Farrell, 1998). Soluble fiber fractions are often linked to the negative effects of NSP in poultry nutrition, however, the present data suggests that concentration of insoluble NSP could also be relevant as demonstrated by poor growth observed due to higher RB in the finisher phase. Gut transit time and motility are some of the mechanisms that have been postulated to be influenced by insoluble fiber with consequences of hindering endogenous enzymes access to their respective substrates and thus impairment of nutrient utilization and growth performance (Bedford and Schulze, 1998). It is also plausible other

Table 5
Effects of adding rice bran in a corn-soybean meal diet and fed without or with multi-enzyme supplement (MES) to broiler chickens on ceca fermentation activity (μmol/L).

Rice bran	MES[1]	Day 24						Day 35					
		Lactic	Acetic	Propionic	Iso-butyric	n-butyric	TSCFA[2]	Lactic	Acetic	Propionic	Iso-butyric	n-butyric	TSCFA[2]
–	–	25.8	73.5	4.51	6.42	19.8	130.0	13.0	75.3	9.59[a]	8.61[a]	12.1	118.6
–	+	28.5	66.2	4.13	5.24	18.1	122.2	20.1	78.6	4.42[b]	4.40[b]	14.7	122.1
+	–	33.9	79.9	5.70	6.51	19.4	145.4	21.0	71.9	6.44[ab]	2.51[b]	11.6	113.5
+	+	30.8	76.6	5.61	5.62	18.7	137.4	29.0	73.4	8.12[a]	2.65[b]	13.3	126.5
SEM		4.95	4.86	0.55	0.53	1.82	7.83	5.84	4.24	1.15	0.88	1.55	9.31
Main effect of rice bran													
–		27.2	69.9	4.32[b]	5.83	19.0	126.1	16.5	76.9	7.00	6.51[a]	13.4	120.4
+		32.3	78.2	5.66[a]	6.06	19.1	141.4	25.0	72.7	7.28	2.59[b]	12.5	120.0
SEM		3.50	3.43	0.39	0.37	1.29	5.53	4.13	3.00	0.82	0.62	1.09	6.58
Main effect of MES													
–		29.8	76.7	5.11	6.47	19.6	137.7	17.0	73.6	8.02	5.57[a]	11.9	116.0
+		29.6	71.4	4.87	5.43	18.4	129.8	24.5	76.0	6.27	3.53[b]	14.0	124.3
SEM		3.50	3.43	0.39	0.37	1.29	5.53	4.13	3.00	0.82	0.62	1.09	6.58
P-value													
Rice bran		0.310	0.100	0.025	0.661	0.947	0.066	0.162	0.323	0.813	0.001	0.544	0.964
MES		0.969	0.294	0.670	0.064	0.519	0.324	0.211	0.584	0.144	0.031	0.181	0.386
Rice bran × MES		0.564	0.685	0.796	0.800	0.785	0.989	0.945	0.839	0.008	0.023	0.811	0.615

TSCFA = total short chain fatty acids.
[a, b] Within a factor of analyses, means in a column with different superscripts are significantly different at $P < 0.05$.
[1] Multi-enzyme supplement supplied xylanase, β-glucanase, invertase, protease, cellulase, amylase, and mannanase with targeted activity level 2,500, 300, 700, 10,000, 1,200, 24,000, 20 U/kg of feed, respectively.
[2] TSCFA is the summation of lactic acid, acetic, propionic, iso-butyric and butyric acid.

Table 6
Effects of adding rice bran in a corn-soybean meal diet and fed without or with multi-enzyme supplement (MES) on apparent retention of components in broiler chickens.

Rice bran	MES[1]	Day 24					Day 35				
		Dry matter	Crude protein	Crude fat	Neutral detergent fiber	Gross energy	Dry matter	Crude protein	Crude fat	Neutral detergent fiber	Gross energy
–	–	73.0	68.5	82.5	10.4	74.3	74.8	64.7	85.6	20.1[b]	75.8
–	+	73.4	65.6	86.4	21.3	75.7	75.6	68.9	88.8	12.1[c]	76.3
+	–	71.3	66.4	81.7	10.7	72.4	74.3	65.3	89.9	22.1[ab]	75.6
+	+	73.1	65.9	85.0	24.3	75.7	75.8	67.6	92.5	26.8[a]	78.0
SEM		0.90	2.15	1.53	1.97	0.53	0.61	1.01	1.29	1.82	0.56
Main effect of rice bran											
–		73.2	67.1	84.4	15.8	75.0	75.2	66.8	87.2[b]	16.1[b]	76.1
+		72.2	66.1	83.3	17.5	74.0	75.0	66.5	91.2[a]	24.5[a]	76.8
SEM		0.64	1.52	1.08	1.39	0.375	0.43	0.72	0.91	1.29	0.40
Main effect of MES											
–		72.1	67.5	82.1[b]	10.6[b]	73.3[b]	74.5	65.0[b]	87.7[b]	21.1	75.7[b]
+		73.3	65.8	85.7[a]	22.8[a]	75.7[a]	75.7	68.3[a]	90.7[a]	19.5	77.2[a]
SEM		0.64	1.52	1.08	1.39	0.375	0.43	0.72	0.91	1.29	0.40
P-value											
Rice bran		0.278	0.672	0.473	0.407	0.086	0.804	0.730	<0.01	<0.01	0.194
MES		0.227	0.440	0.031	<0.01	<0.01	0.073	<0.01	0.034	0.369	0.017
Rice bran × MES		0.444	0.591	0.836	0.500	0.090	0.571	0.364	0.791	<0.01	0.106

[a, b] Within a factor of analyses, means in a column with different superscripts are significantly different at $P < 0.05$.
[1] Multi-enzyme supplement supplied xylanase, β-glucanase, invertase, protease, cellulase, amylase, and mannanase with targeted activity level 2,500, 300, 700, 10,000, 1,200, 24,000, 20 U/kg of feed, respectively.

factors other than NSP may have contributed to observed poor growth in RB fed birds in finisher phase.

The efficacy of exogenous feed enzymes in poultry nutrition is well documented and quite often linked with decreasing intestinal viscosity through degradation of soluble NSP (Bedford and Schulze, 1998; Adeola and Cowieson, 2011; Slominski, 2011). The multi-enzyme supplement (MES) used in the present study contained fiber degrading enzymes, protease and α-amylase. Addition of MES improved growth performance and nutrient retention independent of RB. Similarly, El-Full et al. (2000) showed that feed enzyme mixture containing α-amylase, β-glucanase, protease, lipase and cellulase improved growth, FCR, protein and energy efficiency of RB-containing-diets fed to broilers. Contrasting observations have also been made in broilers fed RB with supplemental enzymes. For example, broilers fed diets containing either 20% or 40% RB supplemented with an enzyme mixture containing xylanase, α-amylase, β-glucanase and proteases, and without or with 170 U/g phytase had no beneficial effects on growth performance (Aboosadi et al., 1996; Farrell and Martin, 1998). Differences associated with the nature of the enzyme used individually or in combination, the inclusion rates of the enzymes, the extent of reduction in nutrient density in the control diet, as well as the microbial sources of enzymes could influence the responses seen in animals (Ravindran, 2013; Kiarie et al., 2016a). Moreover, the source of RB and processing conditions may influence supplemental enzyme responses. For example, Wang et al. (1997) reported an enzyme mixture (xylanase, β-glucanase and pectinase) improved performance of chicks fed irradiated Malaysian rice bran, but not when fed Chinese rice bran.

The ceca anaerobic fermentation mainly produces volatile fatty acids in a largely conservative molar proportion of acetic acid > butyric acid > propionic acid (Svihus et al., 2013). The concentration of SCFA in the hindgut is indicative of microbial diversity and activity as influenced by available substrates (Kiarie et al., 2013). The feed composition in particular fiber has significant impact on gut microbial ecology (Apajalahti et al., 2004). Rice bran tended to increase SCFA in the starter phase mainly due to increase in propionic acid. However, RB had modest effect on ceca digesta concentration of SCFA in the finisher phase and surprisingly reduced concentration of iso-butyric despite increased retention of NDF. The SCFA are highly volatile, and it may be that concentration in the digesta at one-point sampling may not be a quantitative indication of amount produced (Kiarie et al., 2013). Nonetheless, as birds do not possess enzymes to hydrolyze NDF, the increased retention of NDF in RB fed birds in finisher phase was most likely a result of microbial degradation. Increased NDF retention in finisher phase might suggest that longer exposure resulted in microbial adaptation in fiber degradation as has been demonstrated elsewhere (Batal and Parsons, 2002; Kiarie et al., 2017).

It has been suggested that enzymes release fermentable oligosaccharides in the process of NSP depolymerization which are fermented to SCFA (Kiarie et al., 2013). However, in the present study, MES reduced concentration of propionic and iso-butyric in non-RB diets in the finisher phase. Furthermore, this correlated with reduced retention of NDF in response to MES in non-RB diets. Carbon and energy from luminal compounds (dietary, endogenous, or both) that are either resistant to attack by digestive fluids or absorbed so slowly by the host promote bacteria growth (Kiarie et al., 2013). Thus, a feed additive that improves nutrients digestibility will impact bacteria ecology and consequently efficiency of nutrients utilization by the host (Bedford and Cowieson, 2012; Kiarie et al., 2013). It has been demonstrated that exogenous feed enzymes can influence composition and metabolic potential of gut microflora in poultry (Choct et al., 1996; Kiarie et al., 2014; Munyaka et al., 2016). This may be achieved by improving the absorption of nutrients in the proximal gut, which results in a reduction in the quantity of nutrients in the terminal ileum and ceca that are available as substrates for bacteria fermentation (Bedford and Cowieson, 2012; Kiarie et al., 2013). Reduced iso-butyric acid in birds fed non-RB diets with MES indicated reduced nitrogen metabolism in the ceca perhaps as a result of increased amino acids absorption in the small intestine. Indeed, whereas we did not observe an interaction between RB and MES on AR of CP, numerically MES improved AR of CP in non-RB diet by 6.5% and that of RB diet by 3.5%. The magnitude of MES effects on AR of GE was higher in the starter (+3.3%) than finisher phase (+2.2%) perhaps indicating the response of the enzyme reduces with age (Bedford and Schulze, 1998). Feeding MES improved NDF retention in the

starter phase and not the finisher phase. Considering the relation between substrate and enzyme, it is rather difficult to explain the aforementioned observation, but it could be indicative of microbial adaptation as bird ages (Batal and Parsons, 2002; Kiarie et al., 2017).

5. Conclusion

Independently, RB reduced final BW whereas MES improved growth and energy utilization. Increased gizzard weight in birds fed RB was not accompanied by increased nutrient digestibility suggesting the negative effect of fiber was more significant. Reduction of iso-butyric acid due to MES in non-RB diet suggested reduced formation of protein fermentation metabolites in the ceca.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2018.12.001.

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