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b) According to Shafqat and Saba[2], cuticular hydrocarbons can be used to identify termite species.

c) Variations in cuticular hydrocarbons may also assist for species recognition and foraging behaviour, investigated by Zeeshan and Pasha[3].

Indirect citation

a) Temperature affects cuticular hydrocarbons of termite[1]. Cuticular hydrocarbons can be used to identify termite species[2]. Variations in cuticular hydrocarbons may also assist for species recognition and foraging behavior[3].

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Impact of Insoluble Non-Starch Polysaccharides and Exogenous Enzymes Addition to An Old Commercial Broiler Diets on Performance, Carcass Weight, and Gastrointestinal Tract Characteristics of Broiler Chickens

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Statement on Conflict of Interest: none declare

Abstract

An old commercial broiler ration (ration which has fleas and excess fine feed) is possible to have poor nutrient quality. This study aimed to investigate the effects of wheat pollard, rice hull, phytase, and cellulase supplementation to the old commercial broilers diet on performance, carcass weight, and gut characteristics of broiler chickens from 0-35d of age. Total of 175 day-old male broiler chicks (Lohmann) were fed on 7 dietary treatments each contained 5 replicates of 5 chicks each. The diets were: 1) the old commercial broiler diet, as a control diet (C), 2) C + wheat pollard (CWP) + phytase, 3) CWP + cellulase, 4) CWP + phytase and cellulase, 5) C + rice hull (CRH) + phytase, 6) CRH + cellulase, 7) CRH + phytase and cellulase. Wheat pollard/rice hull was added at 40g/kg. Phytase was given at 1250FTU/kg and cellulase at 250Unit/kg. The control diet by analyses was found to contain a low level of Ca (0.5%) and total P (0.4%). Treatment diets did not affect broiler performance ($P>0.05$). Birds fed diets 5, 6, 7 showed the higher carcass weight ($P<0.05$), the lightest duodenum and jejunum weight ($P<0.01$), the lowest jejunum pH ($P<0.05$) and caecal pH ($P<0.01$), as well as the higher caecal acetic and butyric acid concentration ($P<0.01$). The duodenum digesta was the highest in the control diet ($P<0.05$). In conclusion, adding 40g/kg rice hull to the old commercial broiler diets containing low levels of Ca (0.5%) and total P (0.3-0.5%) could enhance carcass weight. Increasing carcass weight seems to be correlated with the reduction of microbial influence in the gut through increasing production of volatile fatty acids, in which the present of rice hull contribute more than the phytase supplementation. Cellulase supplementation did not enhance phytase efficacy.

Key words: phytase, cellulase, broiler chickens, performance, carcass weight

Introduction

In developing countries like Indonesia, broiler farmers who live in a small town or small village often face the fact that broiler rations purchased were "old". The so-called old broiler ration is when the broiler ration contains a lot of fleas and feed form is no longer intact. The broiler ration is categorized old when it contains a lot of fleas and its feed form is no longer intact. For example, pellet form in a broiler finisher ration has led to a powder. Based on this, there is a suspicion that the kind of rations has poor nutrient quality. The majority of feed ingredients used in poultry diets was derived from plant cereals such as; soybean, corn, wheat, or barley[1]. The anti-nutritional factors and indigestible components in plants become a factor that can affect the quality of a diet formulated. Phytate in plant material is one of nutrition obstacles for monogastric animals[2]. Some researches indicated that supplementation of broiler feeds with phytase can alleviate the effect of anti-nutritional factors and improve bird's performance[3,4] ~~(Shirley and Edwards, 2003; Selle *et al*., 2007)~~. The use of exogenous phytase supplementation has been reported to improve the use of Phytate P[5,6] ~~(Baidoo *et al*., 2003; Cowieson *et al*., 2006)~~ which represents between 50% to 80% of the total P content in cereals[7] ~~(Israel *et al*., 2006).~~

Besides enzymes, the benefit of insoluble non-starch polysaccharides (iNSP) used in broiler diets has also been reported in many studies. Many of the benefits has been associated with improving the gut health[8-10] ~~(Bao and Choct, 2010, Mateos *et al*., 2012, Hartini and Massora, 2014)~~, gizzard function[11] ~~(Svihus, 2011)~~, increasing starch digestibility[12] ~~(Hetland *et al*., 2004)~~, and growth performance in broiler[13,14] ~~(González -Alvarado *et al*., 2007, Jiménez-Moreno *et al*., 2016)~~ or in layers[15] ~~(Hartini *et al*., 2002)~~.

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Lately, there is an interest in combining phytase with cell wall degrading enzymes in broiler diets, base on the assumption that some phytate hidden in the plant cell wall cannot be degraded by phytase. The use of phytase in a combination with exogenous carbohydrase has been investigated in many studies[16-18] ~~(Cowieson and Adola, 2005, Avila *et al*., 2012, Karimi *et al*., 2013)~~. It was reported that the combination of phytase and carbohydrase was highly effective in improving the broiler performance[17].

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The inclusion of iNSP and/or the supplementation of enzyme to broiler diets are common in poultry researches, but little data available on the addition of iNSP and enzymes to the old commercial broiler diet. Hartini and Massora[10] ~~(2014)~~, in previous study, tried to add graded levels (20, 40, and 60g/kg) of iNSP (wheat pollard and rice hull) to the old commercial broiler diets. They found that addition of 40g/kg iNSP decreased the caecal microbial numbers of young broilers. Moreover, the addition of 40g/kg rice hull and 20g/kg wheat pollard reduced the total number of *E.Coli* and *Enterobacter* in the small intestine, respectively. Wheat pollard and rice hulls were sources of iNSP. Wheat pollard contains 33.6% DM of insoluble NSP and 1.7% soluble NSP[19] ~~(Choct, 1997)~~. Rice hulls are by product of rice processing which contain mostly cellulose[20].

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Hence, the current study was designed to evaluate the effects of the utilization of exogenous enzymes (phytase and cellulase) to an old commercial broiler diet added with iNSP (wheat pollard and rice hull) on growth performance, carcass weight, and gastrointestinal tract ~~characteristics from 0 to 35 d of age~~ characteristics of broilers from 0 to 35 d of age. The hypothesis of this research was that the utilization of exogenous enzymes and iNSP to the old commercial broiler diets could improve the nutritive values of the diets and consequently increase

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broiler performance. However, the degradation of nutrients in the old commercial diets used was also considered as factors that may influence the activity of iNSP and enzymes additions.

Materials and Methods

Experimental diets and fiber sources

The commercial broiler diet used in this study was one of the commercial broiler diets circulated in Indonesia market and has characteristics as explained before. The wheat pollard and rice hulls were used as sources of iNSP. Rice hulls were ground using a grinder fitted with 2mm screen. The wheat pollard and rice hulls were added to the commercial broiler diets at 40g/kg, the amount similar to the previous study by Hartini and Massora[10] ~~(2014)~~. The old commercial broiler diets, wheat pollard, and rice hulls were analysed for dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF), gross energy (GE), calcium (Ca), and phosphor (P). Dry matter, CP, EE, CF were analysed by the AOAC standard methods, GE by bomb calorimetry, Ca by AAS (Atomic Absorption Spectrophotometry) method, and P by calorimetric method. All the chemical analyses were done in the laboratory of Nutrition and Food Study Center, Gadjah Mada University, Yogyakarta, Indonesia. The analysed and calculated nutrient values are shown in Table 1.

Inserted Table 1.

The phytase used was *E.Coli* derived phytase, Quantum Blue, ABVista Feed Ingredients, Marlborough, UK. The standard recommended level of phytase was 100g/tonne to get the activity

of 500FTU/kg. The cellulase used was SQzyme CSP product, 20.000unit/g, Suntaq International Limited, Shenzhen, China.

Diets used in the experiment were:

1) The old commercial broiler diets (corn-soybean based) as the control diet (C).

2) C + 40g/kg wheat pollard (CWP) + phytase 1250FTU/kg,

3) CWP + cellulase 250unit/kg,

4) CWP + enzyme complex (phytase 1250FTU/kg and cellulase 250unit/kg),

5) C + 40g/kg rice hull (CRH) + phytase 1250FTU/kg,

6) CRH + cellulase 250 unit/kg, and

7) CRH + enzyme complex (phytase 1250FTU/kg and cellulase 250unit/kg).

Birds management

One hundred and seventy five (175) one-day-old male broiler chicks (Lohmann) were randomly allocated to 35 floor pens with 5 birds per pen and 5 pens per treatment. The flock uniformity was determined by calculation on the CV (coefficient variance) of body weight (BW). The result of the CV calculation of doc at the beginning of the experiment was 1.49. Chick were given free access to both water and feed until 5 weeks of age. Pens were illuminated 24h per day.

Variable measured

Variables measured in the experiment were; body weight gain (BWG) (g/b/d), feed intake (FI) (g/b/d), feed conversion efficiency (FCE) (g/g), relative weight of digestive organs (gizzard,

duodenum, jejunum, ileum, and ceca) expressed as g/100g body weight (BW), relative length of digestive organs (duodenum, jejunum, and ileum) expressed as cm/100g BW, pH of digestive organs (duodenum, jejunum, ileum, caeca), carcass weight (% of live BW), and caecal volatile fatty acids (acetate, propionate, and butyrate). Body weight gain and feed intake were determined at the beginning and termination of the experiment. Body weight gain and FI were used to calculate feed conversion efficiency (FCE = BWG/FI) during the entire experimental period. Mortality was recorded daily.

Sample collection and analyses

At the end of the experiment (35 d), two birds from each of five replicates per treatment was selected based on proximity to average bird weight per cage. The birds were fasted approximately 8 hours before the slaughter. After weighing the live weight, the birds were slaughtered by dissecting at jugular's vein and used to measure the variables below.

Carcass

The broiler carcass was eviscerated, the neck with skin was cut off at the tip of the shoulders, and the shanks were removed at the hocks. Carcass weight was calculated as percentage of live body weight (g/100g BW).

Gastrointestinal tract length, weight, digesta and pH

The gastrointestinal tract (GIT) was removed and cut into segments: gizzard, duodenum (from gizzard to pancreo-biliary ducts), jejunum (measured from the end of duodenal loop to Meckel's diverticulum), ileum (from Meckel's diverticulum to ileocecocolic junction), and caeca. The length of the duodenum, jejunum, and ileum were measured to the nearest mm. After measuring the length, the duodenum, jejunum, and ileum, as well as the gizzard and caeca were weighed prior to and after removal of contents. The pancreas was also removed and weighed. Digesta contents of duodenum, jejunum, ileum, and caeca were measured, and then put in a 50 ml beaker glass. Distilled water was added in 1:1 ratio, except for caecal digesta in which the ratio between digesta and distilled water was 1:5. The mixture was stirred until homogen, and a pH meter was inserted. The pH was read when the number was stable.

Statistical analyses

The data obtained were analysed statistically using one-way Analysis of Variance[21] ~~(SPSS 16.0, 2007)~~. After a significant F test, Duncan's multiple range test was used to inspect differences among group means. Statistical significance was accepted at $P<0.05$. Differences among means with $0.05< P <0.10$ were accepted as representing tendencies to differences.

Results

Fiber sources and experimental diets

As shown on Table 1, the percentage of DM, CP, CF, Ca, P, and GE by analyses in the control starter diet was 90%, 20%, 3.5%, 0.5%, 0.3%, and 3845 kcal/kg, respectively. In the control

finisher diet, the percentage of DM, CP, CF, Ca, P, and GE was 90%, 20%, 4.8%, 0.5%, 0,5%, and 3898 kcal/kg, respectively (Table 1). The crude fiber (CF) of either wheat pollard (WP) or rice hulls (RH) by analyses were 7.4% and 51.6%. When iNSP added to the control diets, the calculated DM, CP, CF, Ca, P, and GE content became 93%, 21%, 3.8%, 0.5%, 0.3%, and 3984 kcal/kg, respectively (for WP addition) and 93%, 20.0%, 5.6%, 0.5%, 0.3%, and 3999 kcal/kg, respectively (for RH addition). For finisher diet, the calculated DM, CP, CF, Ca, P, and GE content became 93%, 21%, 5.1%, 0.5%, 0.5%, and 4037 kcal/kg (for WP addition), and 93%, 20%, 6.9%, 0.5%, 0,5%, and 4052 kcal/kg (for RH addition) (Table 1).

Growth performance, carcass weight, gastrointestinal tract (GIT) pH and weight

The effect of diets on performance, carcass weight, GIT pH, and GIT weight is summarized in Table 2. Diets did not have a significant effect on body weight gain, feed intake, and feed conversion efficiency ($P>0.05$), however, diets did affect the carcass weight ($P<0.05$). Addition of the rice hulls with a single or combination of enzymes showed significantly higher carcass weight than the other treatment groups. Supplementation of wheat pollard with phytase and cellulase showed a better carcass weight than the addition of wheat pollard with phytase or cellulase alone (Table 2). There was no mortality found during the experiment.

As shown on Table 2, CRH diets supplemented with phytase or phytase and cellulase significantly lower the jejunal pH ($P <0.05$) and caecal pH ($P <0.01$) more than the other treatment groups. Among CRH diets, supplementation of enzyme combination (phytase and cellulase) had the lowest jejunal pH. The duodenal pH of birds fed CRH diets added with enzyme

combination (phytase and cellulase) also tended to be lower than those fed the other diets (P=0.095). The ileal pH was not affected by diets (P> 0.05) (Table 2).

The weight of gizzard, duodenum, jejunum, ileum, caeca and pancreas are expressed per 100 g body weight (g/100g BW). The diets significantly affected the relative weight of duodenum and jejunum (P<0.01), but not for the other digestive organs including pancreas (Table 2). Birds fed the control diet had the heavier duodenum and jejunum weight compared to those fed the other diets. Supplementation of enzymes to either the CWP or CRH diets reduced the duodenal weight (P<0.01). Birds fed the CRH diets added with phytase or phytase and cellulase had the lightest duodenum and jejunum weight (P<0.01) (Table 2).

Inserted Table 2.

Gastrointestinal tract length, digesta content, and caecal VFA

The effect of iNSP and enzymes additions on GIT length, digesta content, and caecal VFA is shown in Table 3. The length of duodenum, jejunum, and ileum are expressed per 100g body weight **(**cm/100g BW). Diets had no effect on the length of gastrointestinal tract measured (P>0.05).

Diets affected the duodenal content (P<0.05), but the effect on jejunal and caecal content only tended to be difference (P=0.08 and P=0.07, respectively) (Table 3). Birds fed the control diets had the higher duodenal content than those fed the other diets (P<0.05). While the jejunal content tended to be higher in birds fed the control diet (P=0.08), the caecal content tended to be higher in

those fed the CRH diet supplemented with enzyme complex (phytase and cellulase) (P=0.07) (Table 3).

The caecal VFA, except propionate, were highly affected by the diets (P<0.01) (Table 3). Addition of rice hull and enzymes increased the concentration of caecal acetic and butyric acids higher than the other diets (P<0.01). Among the CRH diets, cellulase supplementation showed the higher concentration of butyric acids rather than phytase or a combination of phytase and cellulase supplementation. Birds fed the control diets had the lowest concentration of caecal acetic and butyric acids (Table 3).

Inserted Table 3.

Discussion

Insoluble non-starch polysaccharides inclusion (3% oat hulls or 3% soy hulls) in corn based-diets had been demonstrated to improve BWG and feed conversion of young broiler chicks[13] ~~(González -Alvarado *et al*., 2007)~~. On the other hand, inclusion 4% oat hulls[22] ~~(Hetland and Svihus, 2001)~~, or 10% cellulose[23] ~~(Svihus and Hetland, 2001)~~ in the wheat-based diets was only found to increase FI but not BWG and FCR in broilers at 21d of age. In the current study, the BWG, FI, and FCE were not affected by the addition of 4% wheat pollard or 4% rice hulls and exogenous enzymes. The different results found indicated that the effect of iNSP source not only depended on its physyco-chemical properties of the iNSP source *per se*, but also depended on the interaction between the individual iNSP sources with other cell wall components in the diet[24] ~~(Hartini *et al*., 2003)~~. In addition, the supplementation of phytase or cellulase or combination of

both seemed not working optimally. ~~In the opposite with the previous study by Meng et al (2005) who found that multiple-enzyme preparation statistically improved insoluble NSP digestibility.~~ In contrast, a previous study by Meng *et al*.[25] demonstrated that multiple-enzyme preparation statistically improved insoluble NSP digestibility. The effect of phytase was significant when phytase added to a P-deficient diet[6,18] ~~(Cowieson *et al*., 2006; Karimi *et al*., 2013)~~. Biehl and Baker[26] ~~(1997)~~ observed that supplementation of high dosage of microbial phytase increased FCE when diet deficient in the amino acid. Furthermore, Avilla *et al*.[17] ~~(2012)~~ found that addition of the multiple-enzyme complex containing carbohydrases and phytase could reestablish the low performance of broiler chickens equal to the control diets when supplemented in a negative commercial sorghum-SBM broiler diets (-85kcal/kg of AMEn, -1.5% CP, -1.5% amino acids, -0.153% of available P, and -0.12% of Ca).

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In contrast, a previous study by Meng et al (2005) demonstrated that multiple-enzyme preparation statistically improved insoluble NSP digestibility.

The diets in the current study by analysis contained Ca 0.5% and P 0.3% in the starter period and Ca 0.5% and P 0.5% in the finisher period. In Indonesia the standard nutrient requirements of meat type broiler chickens for the starter period (0-3wks of age) were CP 19-23%, Ca 0.9-1%, total P 0.6-1%, whereas for the finisher period (>3-6 wks of age) the requirements were CP 18-20%, Ca 0.9-1.2%, total P 0.6-1%[27] ~~(SNI, 2008)~~. Compared with this standard nutrient requirement, the nutritive values in the present study was at very deficient concentration of Ca and P. For the starter period the Ca and P concentration of the diets diminished by around 44% each. For the finisher period, the Ca diminished by around 44% and the total P around 17%.

Calcium and phosphor, particularly the ratio between Ca:P, were factors that influenced phytase responses[28] ~~(Delezie *et al*., 2015)~~. Bedford *et al*.[29] ~~(2016)~~, in their review included Ca and P as major determinants of phytase response. Delezie *et al*.[28] ~~(2015)~~ concluded that Ca and P concentration can be reduced by 20% if done in a balanced way, and supplementation of phytase in this condition will improve performance even more. Adding graded level of Ca and phytase in

a diet deficient in Ca but adequate in total P was found to improve weight gain and BW of young broilers chicks[30] ~~(Schöner *et al*., 1994 in Schöner and Hoppe, 2002)~~. They suggested that calcium addition caused a significant improve in weight gain, and addition of phytase caused more calcium to be available, so significantly increased body weight. In the current study, although the high dosage of phytase (1250FTU/kg) was used, the diminished concentration of both Ca and P by around 44% from normal requirements in the starter period was more likely to be a constraint for phytase to response optimally. Even at normal concentration of Ca, the liberated phytate-P by phytase may not sufficient to meet the broiler requirements of P when diet had low P concentration[28] ~~(Delezie *et al*., 2015)~~. Moreover, the growth rate of broiler is relatively high during the starter period (1-21d), indicating that the demand of Ca and P in this period is also higher than the finisher period. Reduction of Ca and P concentration during the starter period was assumed to greatly affect the growth performance. Addition of cellulase in the current study did not give any different results on BWG indicated that the cellulase supplementation was failed to increase phytase efficacy. The GE content in the present study was in the range of 3845-3999kcal/kg for starter and 3898-4052 kcal/kg for finisher. The fact that the FI was found similar amongst diets implied that the contribution of iNSP and feed enzymes had not yet affected energy availability. Birds would compensate by increasing FI when fed diets low in energy[31,32] ~~(Savory, 1984, Newcombe and Summers, 1985)~~.

The carcass weight was found higher in broiler chickens fed the CRH diets added with phytase or phytase and cellulase than those fed the other diets. The broiler carcass weight is part of a bird's body without blood, feathers, heads, legs, and visceral organs. The lighter weight of duodenum and jejunum, also duodenum content of birds fed the CRH diets plus phytase, cellulase, or pytase and cellulase might responsible for increasing carcass weight. Rolls *et al*.[33]

~~(1978)~~ reported that modification of the gut morphology related with increasing gut microflora. Cereal by-products contain little water soluble NSP but substantial concentrations of insoluble NSP[34] ~~(Bao, *et al*., 2013)~~. The insoluble β-glucans pass into the large intestine, where they are fermented by bacteria[35] ~~(Lynch *et al*., 2007)~~. Significant disaccharides, low-molecular weight polysaccharides and oligosaccharides derived from either water-soluble or insoluble NSP due to the use of exogenous enzymes may be fermented in caeca[36] ~~(Choct and Kocher, 2000)~~. Large numbers of microorganism in the caeca tend to migrate to the gut where nutrient absorption takes place, causing a thicker lining of the gut[37] ~~(Campbell and Bedford, 1992)~~. Supplementation of enzyme may reduce microbial influence on digestive tract of birds probably by reducing fermentative substrate[38,39] ~~(Choct *et al*., 1996, Brenes *et al*., 1993)~~, and consequently reducing the gut weight. We only partly agreed with this idea. The different results found on the addition of wheat pollard and enzyme on carcass weight implied that there was another factor beside enzymes that influencing. Previous study by Hartini and Massora[10] ~~(2014)~~ found that the addition of 40g/kg iNSP (rice hull or wheat pollard) in the commercial broiler diets without enzymes supplementation significantly reduced the total number of microbia in the small intestine. So, it seemed that the iNSP *per se* had an influence on reducing the gut weight by decreasing the microbial numbers and the action of enzyme was only addition into it. Josefiak *et al*.[40] ~~(2004)~~ had demonstrated that supplementation of oligosaccharides increased fermentation in the caeca, consequently increasing the production of volatile fatty acids (acetate, propionate, and butyrate). It was likely that in the present study ~~the present of rice hull~~ the addition of rice hull increased the fermentation in the caeca. The butyric acid was reported to reduce the small intestinal pH and had anti-bacterial properties[41] ~~(Meynell, 1963)~~. Indeed, birds fed the control diets added with rice hull regardless of the enzyme in the current study showed a high concentration of acetic and butyric

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acids as well as a low pH in the jejunum and caeca. This may also explained the lower duodenal and jejunal digesta in birds fed the control diets added with the rice hull and enzymes.

Hetland and Svihus[22] ~~(2001)~~ suggested that high fiber diet tended to stay longer in the gizzard. The result on gizzard weight in this current study was not significant. The addition of 4% iNSP may not sufficient to stimulate the gizzard function. The activity of the gizzard depends on its content[12] ~~(Hetland *et al*., 2004)~~. However, the lower duodenal and jejunal digesta found in birds fed the commercial broiler diets added with the rice hull and enzymes indicated that the present of rice hull actually caused a slower passage rate of digesta than the other diets.

Based on the results, it can be concluded that adding 40g/kg iNSP and enzymes (phytase or phytase and cellulase) to the old commercial broiler diets containing low concentration of Ca (0.5%) and P (0.3-0.5%) did not affect growth performance of broiler chickens, but affecting carcass weight. Increasing carcass weight seems to be correlated with reduction of microbial influence in the gut through increasing production of volatile fatty acids, in which the present of rice hull contribute more than the phytase supplementation. Cellulase supplementation did not enhance phytase efficacy.

Significance Statements

- An old commercial broiler ration (ration which has fleas and excess fine feed) by analyses consisted of low concentration of Ca and P. The diminish concentration of Ca and P was more pronounced in the old commercial broiler starter ration.
- Supplementation of phytase 1250FTU/kg in the broiler diets having a very low concentration of P was not able to enhance performance.

- Addition a minimum level of rice hulls in the old commercial broiler rations affected the gut weight by reducing the microbial population and consequently increased carcass weight.
- Each insoluble polysaccharides has a different physiological function depends on its physicochemical properties and its interaction with other cell wall components in the diets.

Acknowledgments

The experimental work presented was made possible through the financial assistance provided by The Directorate General of Higher Education through DIPA DP2M No. 023.04.1.673453/2015, November 14, 2014 and contract no 150/SP2H/PL/ DIT.LITABMAS/11/2015, April 19, 2015, under the BOPTN Research Funding, Hibah Bersaing research.

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Table 1. Chemical analyses of fiber sources and commercial diets and calculation analyses of basic experimental diets before added with exogenous enzymes

Chemical analyses	Wheat pollard	Rice hull	Commercial diet (as a control diet)		Commercial Starter diet		Commercial Finisher diet	
	(WP)	(RH)	Starter	Finisher	WP	RH	WP	RH
Dry matter (%)	91.0	92.0	89.7	89.7	93.3	93.4	93.3	93.4
Crude protein (%)	16.6	3.1	19.9	19.8	20.6	20.0	20.5	19.9
Ether extract (%)	4.9	1.4	4.6	3.5	4.8	4.7	3.7	3.6
Crude fiber (%)	7.4	51.6	3.5	4.8	3.8	5.6	5.1	6.9
Ash (%)	4.7	15.3	6.1	6.8	6.3	6.7	7.0	7.4
Gross energy (kcal/kg)	3467	3852	3845	3898	3984	3999	4037	4052
Ca (%)	0.5	0.7	0.5	0.5	0.5	0.5	0.5	0.5
Total P (%)	0.3	0.4	0.3	0.5	0.3	0.3	0.5	0.5

Diet 1= Commercial broiler diets (starter or finisher) (C)

Diet 2= C-wheat pollard (CWP) + phytase 1250FTU/kg

Diet 3= CWP + cellulase 250 unit/kg

Diet 4= CWP + enzyme complex (phytase 1250 FTU/kg and cellulase 250 unit/kg)

Diet 5= C –rice hulls (CRH) and phytase 1250FTU/kg

Diet 6= CRH + cellulase 250 unit/kg

Diet 7= CRH + enzyme complex (phytase 1250 FTU/kg and cellulase 250 unit/kg)

Table 2. Response of diets on performance, carcass weight (%) and gastrointestinal tract (GIT) weight (g/100g BW)

Variable / Diet / Measured	1	2	3	4	5	6	7	P-value
BWG (g/b/d)	60.17 ± 1.08	57.54 ± 2.02	59.09 ± 1.32	59.01 ± 1.96	60.04 ± 1.53	60.44 ± 1.87	60.36 ± 2.68	NS
FI (g/b/d)	102.51 ± 2.44	103.89 ± 2.66	103.41 ± 2.65	104.05 ± 3.32	104.01 ± 4.39	107.35 ± 3.47	102.97 ± 8.77	NS
FCE (g/g)	0.587 ± 0.01	0.554 ± 0.01	0.572 ± 0.01	0.567 ± 0.01	0.579 ± 0.01	0.563 ± 0.01	0.593 ± 0.03	NS
Carcass weight (%)	74.43 ± 0.65^{ab}	73.53 ± 1.18^{a}	75.02 ± 1.15^{ab}	76.49 ± 0.40^{b}	76.63 ± 1.11^{b}	76.52 ± 0.43^{b}	77.22 ± 0.32^{b}	*
pH duodenum	5,76 ± 0,09	6,06 ± 0,16	5,82 ± 0,17	5,96 ± 0,22	6,22 ± 0,18	5,65 ± 0,106	5,50 ± 0,20	0.09
pH jejunum	$6,04 \pm 0,14^{b}$	$6,02 \pm 0,11^{b}$	$6,04 \pm 0,05^{b}$	$6,20 \pm 0,09^{b}$	$5,90 \pm 0,23^{b}$	$5,68 \pm 0,26^{ab}$	$5,25 \pm 0,22^{a}$	*
pH ileum	6,56 ± 0,32	7,10 ± 0,27	7,00 ± 0,27	7,28 ± 0,08	6,80 ± 0,44	6,18 ± 0,49	6,43 ± 0,26	NS
pH caeca	$7,26 \pm 0,23^{bc}$	$7,54 \pm 0,14^{c}$	$7,18 \pm 0,07^{bc}$	$7,28 \pm 0,17^{bc}$	$6,68 \pm 0,29^{ab}$	$6,23 \pm 0,37^{a}$	$6,63 \pm 0,21^{ab}$	**
Gizzard wt	1.45 ± 0.16	1.57 ± 0.14	1.65 ± 0.33	1.69 ± 0.20	1.87 ± 0.17	1.79 ± 0.33	1.62 ± 0.17	NS
Duodenum wt	1.12 ± 0.07^{c}	0.81 ± 0.04^{a}	1.01 ± 0.06^{bc}	0.88 ± 0.09^{ab}	0.77 ± 0.03^{a}	0.88 ± 0.03^{ab}	0.87 ± 0.05^{ab}	**
Jejunum wt	2.80 ± 0.19^{c}	2.14 ± 0.18^{b}	2.49 ± 0.20^{bc}	2.19 ± 0.13^{b}	2.06 ± 0.12^{ab}	2.19 ± 0.13^{b}	1.61 ± 0.17^{a}	**
Ileum wt	1.76 ± 0.12	1.77 ± 0.13	1.97 ± 0.16	1.84 ± 0.15	1.85 ± 0.08	1.72 ± 0.06	1.82 ± 0.21	NS
Caeca wt	0.48 ± 0.04	0.46 ± 0.03	0.35 ± 0.03	0.47 ± 0.05	0.48 ± 0.05	0.47 ± 0.01	0.48 ± 0.04	NS
Pancreas wt	0.222 ± 0.012	0.204 ± 0.012	0.224 ± 0.013	0.196 ± 0.005	0.202 ± 0.013	0.210 ± 0.020	0.213 ± 0.011	NS

*(P<0.05), ** (P<0.01), NS: not significant, Diets: 1) Commercial diet as a control diet (C), 2) C added with WP 40g/kg (CWP) and phytase1250 FTU/kg, 3) CWP added with cellulase 250unit/kg, 4) CWP added with phytase1250 FTU/kg and cellulase 250unit/kg, 5) C added with RH 40g/kg (CRH) and phytase1250 FTU/kg, 6) CRH added with cellulase 250unit/kg, 7) CRH added with phytase1250 FTU/kg and cellulase 250unit/kg.

^{abc}mean values within a row bearing different superscripts differ significantly

Table 3. Response of diets on GIT length (duodenum, jejunum, ileum) (cm/100g BW) and GIT digesta (gizzard, duodenum, jejunum, ileum, caeca) (g/100g BW), and caecal volatile fatty acids (acetic, propionic, butyric) (µM/ml)

Variable measured \ Diet	1	2	3	4	5	6	7	P-value
Duodenum length	1.486 ± 0.04	1.352 ± 0.06	1.406 ± 0.05	1.308 ± 0.05	1.298 ± 0.05	1.330 ± 0.06	1.440 ± 0.05	NS
Jejunum length	3.544 ± 0.13	3.206 ± 0.09	3.204 ± 0.12	3.162 ± 0.15	3.154 ± 0.08	3.143 ± 0.13	3.208 ± 0.07	NS
Ileum length	3.576 ± 0.16	3.222 ± 0.07	3.810 ± 0.27	3.564 ± 0.22	3.500 ± 0.05	3.685 ± 0.25	3.620 ± 0.28	NS
Gizzard digesta	0.414 ± 0.14	0.454 ± 0.09	0.660 ± 0.21	0.704 ± 0.19	0.752 ± 0.11	0.708 ± 0.36	0.668 ± 0.15	NS
Duodenum digesta	0.506 ± 0.10^{b}	0.216 ± 0.03^{a}	0.308 ± 0.06^{a}	0.248 ± 0.05^{a}	0.214 ± 0.04^{a}	0.333 ± 0.05^{ab}	0.308 ± 0.07^{a}	*
Jejunum digesta	1.602 ± 0.15	1.108 ± 0.18	1.308 ± 0.21	1.144 ± 0.15	1.114 ± 0.09	1.355 ± 0.16	0.845 ± 0.14	0.08
Ileum digesta	0.830 ± 0.12	0.970 ± 0.12	1.066 ± 0.16	0.976 ± 0.11	1.072 ± 0.07	1.025 ± 0.16	1.228 ± 0.23	NS
Caeca digesta	0.216 ± 0.04	0.210 ± 0.04	0.094 ± 0.02	0.220 ± 0.05	0.228 ± 0.04	0.230 ± 0.01	0.258 ± 0.03	0.07
Acetic acid	53.1 ± 16.55^{a}	48.7 ± 22.55^{a}	81.6 ± 32.95^{ab}	103.9 ± 34.31^{ab}	151.3 ± 9.92^{b}	149.77 ± 20.8^{b}	146.56 ± 52.8^{b}	**
Propionic acid	29.9 ± 3.50	29.0 ± 7.29	42.8 ± 3.47	33.9 ± 2.27	31.2 ± 3.75	41.5 ± 9.77	31.7 ± 0.33	NS
Butyric acid	7.8 ± 2.49^{a}	14.4 ± 2.13^{ab}	14.7 ± 5.72^{ab}	21.3 ± 2.06^{bc}	$37.3 \pm 0,81^{d}$	29.0 ± 2.53^{cd}	31.1 ± 3.16^{cd}	**

*(P<0.05), ** (P<0.01), NS: not significant, Diets: 1) Commercial diet as a control diet (C), 2) C added with WP 40g/kg (CWP) and phytase1250 FTU/kg, 3) CWP added with cellulase 250unit/kg, 4) CWP added with phytase1250 FTU/kg and cellulase 250unit/kg, 5) C added with RH 40g/kg (CRH) and phytase1250 FTU/kg, 6) CRH added with cellulase 250unit/kg, 7) CRH added with phytase1250 FTU/kg and cellulase 250unit/kg.

^{abc}mean values within a row bearing different superscripts differ significantly.