The effects of rice hull inclusion and enzyme supplementation on the growth performance, digestive traits, dry matter and phosphorus content of intestinal digesta and feces of broiler chickens

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ABSTRACT

The study was conducted to determine the effects of rice hull inclusion and enzyme supplementation on the growth performance, digestive traits, DM and phosphorus (P) content of intestinal digesta and feces of broiler chickens from 3 to 21 d of age. A total of 200 three-day-old male Lohmann chicks were allocated to 4 treatments (10 cages per treatment): corn-soybean-based diet (CON), 40 g/kg rice hull inclusion in the diet (RH), RH + phytase 1750 FTU/kg (RHP), or RHP + cellulase 500 unit/kg (RHPC). From 7 to 21 d of age, feeding the RH improved ADG, but enzyme addition reduced ADG, whereas phytase had a greater effect than phytase and cellulase ($P \leq 0.05$). Globally from 3 to 21 d of age, birds within the RH groups had better ADG and G:F than those within the CON group; the addition of phytase and cellulase improved G:F more than the individual phytase ($P \leq 0.05$). At 21 d of age, the feeding of RH tended to increase the weight of empty gizzards ($P = 0.057$), increased the jejunum content ($P \leq 0.05$) and the P-disappearance of digesta ($P \leq 0.05$), and reduced the P excretion ($P \leq 0.05$). Supplementation of phytase and cellulase increased the P-disappearance and reduced the P excretion more than the supplementation of phytase. This study demonstrated that the inclusion of 40 g/kg rice hulls can improve the growth performance of young broilers. Supplementation of phytase and cellulase had a better effect than phytase in increasing ADG, G:F, and P-disappearance in digesta and in reducing P excretion.

Keywords: Broiler chickens; Rice hulls; Phytase; Cellulase; Growth performance; Phosphorus

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1. Introduction

The use of a less expensive feed ingredient while still maintaining bird growth has been practiced over the past few years in poultry industries. Feed ingredients used to manufacture a diet could affect the development of the gastrointestinal tract (GIT) and the utilization of nutrients in broiler chickens, thereby affecting the production performance of birds (Yegani and Korver, 2008). Apart from what kind of feed ingredients are used in manufacturing broilers diets, it should be noted that the two main targets in poultry production are high growth rate and feed efficiency (Sugiharto, 2016). The ban of antibiotics as growth promoters in poultry diets has led to animal performance problems and a rise in the incidence of certain poultry diseases. The use of probiotics, prebiotics, and insoluble fiber in the diets has been explored as nutritional strategies to reduce the incidence of the problem. Previous research on insoluble fiber or insoluble nonstarch polysaccharides (iNSP) has demonstrated that iNSP had beneficial effects on nutrient utilization (Gonzaleź-Alvarado et al., 2007), starch digestibility (Hetland et al., 2003) of broiler chickens, and cannibalism in the laying hens (Hartini et al., 2002).

Phytic acids contained in major feed ingredients for poultry have the capacity to bind phosphorus (P) forming phytate-P, which reduces P utilization (Woyengo and Nyachoti, 2011). Approximately 50 to 80% of the total P content in cereals is in the form of phytate-P (Israel et al., 2006). Phytase supplementation has been reported to improve the use of phytate-P (Baidoo et al., 2003, Cowieson et al., 2006). The higher the dose of phytase used is, the more the P is released from phytate (Augspurger and Baker, 2004). Cowieson et al. (2006) found that high doses of phytase (>1,000 U/kg of diet) increased nutrient availability more than the use of lower (<1,000 U/kg) phytase activities. Recently, the combination of phytase and carbohydrase use is becoming of more growing interest than the use of

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phytase alone. It was assumed that phytase and carbohydrases can act synergistically in improving nutrient utilization (Woyengo and Nyachoti, 2011). This study was designed to determine the effect of rice hull inclusion and enzymes on the growth performance, digestive traits, dry matter and phosphorus content in feces and digesta of broiler chickens.

2. Materials and methods

2.1. Animal care

The study was carried out in compliance with Indonesia guidelines for animal handling and care (Keputusan Menteri Tenaga Kerja dan Transmigrasi RI, 2014).

2.2. Experimental diets, animals, and bird management

The diets used in the experiment were as follows: 1) corn-soybean-based diet as a control diet (CON), 2) 40 g/kg rice hull inclusion in the diet (RH), 3) RH + phytase 1750 FTU/kg (RHP), and 4) RHP + cellulase 500 unit/kg (RHPC). Rice hulls were ground through a hammer mill (2 mm screen) before being included in the diet. The composition and calculation of nutrients for the experimental diets are shown in Table 1.

All diets were formulated isocaloric and isoprotein and met all nutrient recommendations of the NRC (1994) for broiler diets. All experimental diets were provided as mash feed and were fed to broiler chickens from 3 to 21 d of age. The phytase used was *Escherichia coli*-derived phytase, Quantum Blue, ABVista Feed Ingredients, Marlborough, UK. The standard recommended level of phytase was 100 g/tonne to achieve the activity of 500 FTU/kg. The cellulase used was SQzyme CSP product, 20.000 unit/g, Suntaq International Limited, Shenzhen, China.

A total of 200 male broiler chicks (strain Lohmann) at three days old (initial body weight of 54.6 ±

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2.3 g) were randomly placed in 40 cages with 5 birds per cage and 10 cages (50 birds) per treatment. Each cage was equipped with 1 waterer and 1 feeder. Feed and water were offered *ad libitum* throughout the experiment. Cages were illuminated 24 h per day.

2.3. Growth performance

Body weight (BW) and feed intake (FI) were weighed by cage at 3, 7, 14, and 21 d of age. Average daily gain (ADG) (g/b/d), average daily feed intake (ADFI) (g/b/d), and the gain-to-feed ratio (G:F) (g/g) were determined by treatment and globally. Mortality was recorded daily during the experiment. Birds that died were weighed, and their BW was included in the calculation of G:F. Feed intake was adjusted for mortality.

2.4. Sample collection and analyses

At 21 d of age and after a period of 8 hours of feed withdrawal, birds were weighed. One bird from each of ten replicates per treatment was selected based on proximity to average bird weight per cage and was slaughtered by dissecting the jugular vein and used to measure the variables below.

2.5. Digestive organs weight, length and digestive contents

After the birds were killed, the body cavity was immediately opened, and the GIT with content 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), and ileum (from Meckel's diverticulum to ileocecocolic junction). The pancreas was also removed. The length of the duodenum, jejunum, and ileum was measured to the nearest mm. After measuring the length, the content of duodenum, jejunum, and ileum was collected, weighed, and then mixed together to be analyzed for dry matter (DM) and phosphorus (P) content. The gizzard content was also collected. The weight of empty

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digestive organs (gizzard, duodenum, jejunum, and ileum) including pancreas was expressed relative to 100 g live BW (without digesta) (g/100 g BW), whereas the weight of digestive content and the length of duodenum, jejunum and ileum was expressed relative to 100 g live BW (g/100 g BW, cm/100 g BW, respectively).

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2.6. Dry matter and phosphorus content

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Dry matter digesta (mixed of duodenum, jejunum, and ileum content) and feces were analyzed using standard AOAC method (AOAC, 2000), whereas the P contained in the digesta and feces was analyzed using a colorimetric method. The analyses were performed in a laboratory at the Nutrition and Food Study Center, University of Gadjah Mada, Yogyakarta, Indonesia.

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2.7. Statistical analysis

The data obtained were analyzed statistically using one-way analysis of variance (SPSS, 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.

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3. Results

3.1. Growth performance

The results of growth performance are shown in Table 2. From 3 to 7 d of age, there was no significant difference (P > 0.05) found on ADG, ADFI and G:F, but from 7 to 21 d of age, broilers within the RH groups gained more weight than those within the CON group (30.65 vs 27.60 g/b/d from 7 to 14 d; 51.19 vs 43.32 g/b/d from 14 to 21 d, P ≤ 0.05, respectively) (Table 2). Furthermore, supplementation of phytase reduced (P ≤ 0.05) ADG more than phytase and cellulase but had no effect

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(P > 0.05) on ADFI or G:F. Globally, from 3 to 21 d of age, ADG and G:F within the RH groups were better than those within the CON group (P ≤ 0.05). Supplementation of enzymes reduced ADG (P ≤ 0.05) and had no effect on ADFI (P > 0.05) but improved G:F (P ≤ 0.05) with the phytase and cellulase supplementation more than phytase alone (Table 2). Mortality was 1.0% and was not related to any dietary treatment (data not shown).

3.2. Weight and length of digestive organs and weight of digestive content

Diets did not affect (P > 0.05) the weight of pancreas, length and relative weight of duodenum, jejunum, and ileum, except for the gizzard (Table 3). At 21 d of age, broilers fed the RH treatment tended to have heavier gizzards without content (P = 0.057) than those fed the CON treatment (Table 3). Supplementation of phytase, but not supplementation of phytase and cellulase, caused a reduction in the weight of empty gizzard.

The inclusion of rice hulls did not affect (P > 0.05) the contents of gizzard, duodenum, and ileum but increased (P ≤ 0.05) the contents of the jejunum (Table 3). The addition of enzymes with the RH reduced the contents of jejunum the same as those on the CON treatment (P ≤ 0.05).

3.3. Dry matter and P content of digesta and feces

The inclusion of rice hulls did not affect (P > 0.05) digesta DM or fecal DM, but supplementation of enzymes on RH affected (P ≤ 0.05) fecal DM (Table 3). Phytase and cellulase supplementation caused higher (P ≤ 0.05) fecal DM than other treatments, which were not significantly different. Broilers fed the RH treatment had higher (P ≤ 0.05) P-disappearance in digesta than those fed the CON treatment. Supplementation of enzymes increased the P-disappearance in the digesta, an effect that was more pronounced for phytase and cellulase mix than an individual phytase. The results on P-excretion

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showed that broilers fed the RH treatment had the same P-excretion as those fed the CON treatment (Table 3). Supplementation of phytase increased the P-excretion, whereas supplementation of phytase and cellulase decreased the P-excretion ($P \leq 0.05$).

4. Discussion

Growth performance was affected by rice hull inclusion in the diet. From 3 to 21 d of age, the improvement observed was approximately 12% for ADG and approximately 8% for G:F. Similar findings were observed in previous studies with sunflower and rice hulls (Jimenez-Moreno et al., 2016) or oat hulls and soy hulls (Gonzaleź-Alvarado et al., 2007). During this period, the ADFI of the birds was not affected by the diets, indicating that young broilers respond positively to the inclusion of 40 g/kg rice hulls in the diet by improving feed utilization. Jimenez-Moreno et al. (2016) found that the inclusion of 25 g/kg rice hulls improved the feed to gain ratio when diet was offered in mash form, but no difference was detected when the level was increased to 50 g/kg in the same form. There was a decrease in G:F when diet was offered in pelleted form. Hartini and Purwaningsih (2017) found that addition of 40 g/kg rice hulls in a commercial starter diet did not improve ADG and ADFI but observed an increase in carcass weight. However, Sadeghi et al. (2015) supplemented 30 g/kg rice hulls in the basal diet and found no effects on BW and carcass weight compared to the control diet. The information provided and the results found in the present study suggest that the effects of rice hulls inclusion on growth performance depends on the type of diet offered, the composition of the basal diets used and the manner in which the rice hulls were incorporated into the diet.

Supplementation of either phytase and cellulase or phytase on the RH improved G:F but reduced ADG. The improvement on G:F was greater for the phytase-cellulase mix than the individual phytase,

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and it has been suggested that this is due to synergistic action between phytase and carbohydrase in improving nutrient utilization (Woyengo and Nyachoti, 2011).

There was a reduction in the ADG by increasing G:F with phytase; phytase had a greater effect than combined phytase and cellulase supplementation. The reason for this finding is unknown but might be related to the release of P due to phytase supplementation. Waldroup et al. (2000) found that at higher levels of P at which the dietary level was sufficient, the addition of phytase had no significant effect on body weight. In the current study, the nutrient level was formulated to meet the broilers requirement. The P-phytate degraded by phytase might increase the absorption of P, which in addition, can interfere with homeostasis of the other nutrients and result in reduced growth rate (Shafey et al., 1990). Our data support the above suggestion; the P-disappearance in digesta of broilers fed diets supplemented with enzymes was higher compared to others.

Increases in gizzard size due to insoluble fiber have been reported due to the stimulative effect of the increased grinding activity of the gizzard for the need of reduced particle size (Svihus, 2011). In the present study, the inclusion of 40 g/kg rice hulls (2 mm in size) resulted in a 7% increase in empty gizzard weight. In a previous study, the inclusion of 30 g/kg ground oat hulls (2 mm in size) resulted in a 35% increase in gizzard weight (Gonzaleź-Alvarado et al., 2007). Sadeghi et al. (2015) reported that addition of 30 g/kg rice hulls (2 mm in size) did not affect gizzard weight. This finding indicates that the effect of rice hulls on gizzard activity was less pronounced than that of oat hulls at the same particle size.

The increase in gizzard size, however, was not followed by an increase in gizzard content. These results underline that not all insoluble fibers which increase gizzard size would have an increase in gizzard content as reported by Svihus (2011). Based on our data, it seems that inclusion of rice hulls

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caused a faster gizzard emptying but a lower digesta passage rate in the jejunum, as shown by an increase in the jejunum content, but further studies are needed to prove this. Savory (1985) showed that the gizzard of quails fed on a diluted (40% cellulose) diet emptied faster compared to those fed undiluted food. These results accentuate the statement that different physio-chemical properties of fiber will produce different effects on digestive organs (Montagne et al., 2003).

Supplementation of phytase, but not supplementation of phytase and cellulase, in the RH treatment reduced the weight of empty gizzard. The reason for this finding is unknown but might be that the addition of phytase counteracted the effect of rice hulls. Hetland and Svihus (2001) also found a reduction in weight of digestive tract when oat-based diets were supplemented with enzymes.

The fecal DM in broilers fed the RH did not differ from those fed the CON diet. If insoluble fibers results in more rapid passage (Cao et al., 1998), the RH treatment should have a higher fecal DM than the CON treatment. The high fecal DM was observed only when phytase and cellulase were added with the RH, implying that the addition of enzyme counteracted the effect of rice hulls, which tended to slow the rate of digesta passage.

The reduction of P excretion found in the present study is in agreement with the results reported by Powell et al. (2008) and Lie et al. (2016) that microbial phytase addition could increase P-disappearance in the gut and reduce P excretion in the environment.

5. Conclusion(s)

The inclusion of 40 g/kg rice hulls can improve growth performance of young broilers. Supplementation of phytase and cellulase mix increases the P-disappearance in the digesta and reduces the P excreta better than the supplementation of phytase alone. An increase in the jejunum content

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might be related to a faster gastric emptying and a lower digesta passage rate, but more studies are needed to prove this.

Conflict of interest

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript. We confirm that the manuscript has been read and approved by all named authors. The manuscript has not been published previously.

Acknowledgments

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PBPM	14.31	15.56	15.56	15.56
Palm olein	0.62	6.38	6.38	6.38
Rice hulls	-	4.00	4.00	4.00
NaCl	0.33	0.33	0.33	0.33
Lysin	0.03	0.01	0.01	0.01
DL-methionin	0.11	0.12	0.12	0.12
Mineral mix	0.50	0.50	0.50	0.50
Ca_2PO_4	1.27	1.14	1.14	1,14
$CaCO_3$	0.56	0.50	0.50	0,50
Phytase 1750 FTU/kg	-	-	+	+
Cellulase 500 unit/kg	-	-	-	+
Calculated Analyses[1)]				
ME, kcal/kg	3,000	3,000	3,000	3,000
Protein, %	23	23	23	23
Fat, %	4.74	10.31	10.31	10.31
Crude Fiber, %	3.09	5.53	5.53	5.53
Ca, %	1.00	0.94	0.94	0.94
Total P, %	0.90	0.98	0.98	0.98
Na, %	0.20	0.20	0.20	0.20
Lysin, %	1.10	1.10	1.10	1.10
Methionin, %	0.50	0.50	0.50	0.50

[1)]Calculated to meet the nutrient requirement of broiler diets (NRC, 1994)

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Table 2. Growth performance response of diets

	3 to 7 d of age	7 to 14 d of age	14 to 21 d of age	3 to 21 d of age
ADG, g/b/d				
CON	14.87	27.60^{a}	43.32^{a}	28.60^{a}
RH	15.45	30.65^{b}	51.19^{c}	32.43^{c}
RHP	14.43	28.56^{ab}	45.62^{ab}	29.26^{ab}
RHPC	14.88	30.19^{b}	49.37^{bc}	31.48^{bc}
SEM[1]	0.180	0.424	0.899	0.438
P-value	0.27	0.03	0.00	0.00
ADFI, g/b/d				
CON	21.84	49.46	46.72	46.51
RH	23.09	52.07	45.55	48.19
RHP	21.18	47.44	42.34	43.55
RHPC	22.10	49.55	44.46	45.91
SEM[1]	0.286	0.744	2.625	0.717
P-value	0.12	0.18	0.48	0.14
G:F, g/g				
CON	0.682	0.560	0.939	0.617^{a}
RH	0.670	0.590	1.126	0.674^{ab}
RHP	0.681	0.603	1.091	0.685^{b}
RHPC	0.674	0.632	1.046	0.697^{b}
SEM[1]	0.008	0.015	0.041	0.011
P-value	0.94	0.42	0.42	0.05

[1]SEM = standard error of the mean. CON = control diet, RH = 4% rice hull inclusion, RHP = RH + phytase 1750 FTU/kg, RHPC = RHP + cellulase 500 unit/kg

[a,b,c]mean values within a column with different superscripts differ significantly ($P \leq 0.05$)

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Table 3. Diet response on all digestive traits measured at 21 d of age

	Treatment				SEM[1]	P-value
	CON	RH	RHP	RHPC		
Empty gizzard, g/100 g BW	1.97^{ab}	2.10^{b}	1.67^{a}	2.14^{b}	0.068	0.057
Empty duodenum, g/100 g BW	0.79	0.88	0.83	0.83	0.026	0.68
Empty jejunum, g/100 g BW	1.50	1.66	1.54	1.70	0.049	0.45
Empty ileum, g/100 g BW	1.05	1.18	1.06	1.16	0.036	0.47
Pancreas weight, g/100 g BW	0.05	0.05	0.05	0.06	0.002	0.36
Duodenum length, cm/100 g BW	3.18	3.49	3.38	3.50	0.064	0,26
Jejunum length, cm/100 g BW	8.20	8.54	8.06	8.51	0.172	0,72
Ileum length, cm/100 g BW	8.60	8.78	8.52	8.68	0.181	0,97
Gizzard content, g/100 g BW	1.16	1.24	1.18	1.30	0.063	0.87
Duodenum content, g/100 g BW	0.31	0.38	0.34	0.35	0.020	0.68
Jejunum content, g/100 g BW	0.57^{a}	0.89^{b}	0.46^{a}	0.55^{a}	0.056	0.03
Ileum content, g/100 g BW	0.46	0.90	0.63	0.71	0.085	0.33
DM digesta, %	13.9	17.9	19.2	16.0	0.949	0.28
DM feces, %	24.6^{a}	23.9^{a}	24.1^{a}	27.3^{b}	0.432	0.01
P digesta, %	0.64^{c}	0.58^{b}	0.53^{ab}	0.50^{a}	0.012	0.00
P feses, %	0.66^{ab}	066^{ab}	0.71^{b}	0.62^{a}	0.011	0.03

[1]SEM: standard error of the mean. CON = control diet, RH = 4% rice hull inclusion, RHP = RH + phytase 1750 FTU/kg, RHPC = RHP + cellulase 500 unit/kg.

[a,b,c]mean values within a row with different superscripts differ significantly (P ≤ 0.05).

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was approximately 12% for ADG and approximately 8% for G:F. Similar findings were observed in previous studies with sunflower and rice hulls[14] or oat hulls and soy hulls[3]. During this period, the ADFI of the birds was not affected by the diets, indicating that young broilers respond positively to the inclusion of 40 g kg^{-1} rice hulls in the diet by improving feed utilization. Jimenez-Moreno *et al.*[14] found that the inclusion of 25 g kg^{-1} rice hulls improved the feed to gain ratio when diet was offered in mash form but no difference was detected when the level was increased to 50 g kg^{-1} in the same form. In addition, there was a decrease in G:F when diet was offered in pelleted form. Hartini and Purwaningsih[15] found that addition of 40 g kg^{-1} rice hulls in a commercial starter diet did not improve ADG and ADFI but observed an increase in carcass weight. However, Sadeghi *et al.*[16] supplemented 30 g kg^{-1} rice hulls in the basal diet and found no effects on BW and carcass weight compared to the control diet. The information provided and the results found in the present study suggest that the effects of rice hulls inclusion on growth performance depends on the type of diet offered, the composition of the basal diets used and the manner in which the rice hulls were incorporated into the diet. Supplementation of either phytase and cellulase or phytase on the RH improved G:F. The improvement on G:F was greater for the phytase-cellulase than the individual phytase and it has been suggested that this is due to synergistic action between phytase and carbohydrase in improving nutrient utilization[6].

There was a reduction in the ADG by increasing G:F with phytase. The reason for this finding is unknown but might be related to the release of P due to phytase supplementation. Waldroup *et al.*[17] found that at higher levels of P at which the dietary level was sufficient, the addition of phytase had no significant effect on body weight. In the current study, the nutrient level was formulated to meet the broilers requirement. The P-phytate degraded by phytase might increase the absorption of P, which in addition, can interfere with homeostasis of the other nutrients and result in reduced growth rate[18]. Our data support the suggestion; the P-disappearance in digesta of broilers fed diets supplemented with enzymes was higher compared to others.

Increases in gizzard size due to insoluble fiber have been reported due to the muscular activity of the gizzard during its physiological function to reduce particle size[19]. The result in our study supports the suggestion. There was a 7% increase in empty gizzard weight by the inclusion of 40 g kg^{-1} rice hulls (2 mm in size). The increase, however, was 28% lower than the results when 30 g kg^{-1} ground oat hulls (2 mm in size) was included in the diet[3]. Sadeghi *et al.*[16], on the other hand, found that addition of 30 g kg^{-1} rice hulls (2 mm in size) did not affect gizzard weight. This finding indicates that the effect of insoluble fiber on gizzard activity was more depended on chemical characteristics of the fiber[20] and diet composition than the particle size and that the stimulatory effect of rice hull on the gizzard was less than that of oat hulls at the same particle size.

All insoluble fibers which increase the gizzard size would substantially increase the gizzard content[19]. The gizzard content in the present study was not different among diets. This is understandable since the gizzard's weight increase in this study was only 7%, therefore the increase in its content was also barely noticed, in contrast to Gonzalez-Alvarado *et al.*[3] where the increase of the gizzard weight was 35%. However, the improvement of ADG on birds given the RH treatment indicated that the presence of insoluble fiber in the gizzard is important to enhance the gizzard physiological activity in improving nutrient utilization. Supplementation of phytase but not supplementation of phytase and cellulase, in the RH treatment reduced the weight of empty gizzard. Hetland and Svihus[21] also found a reduction in weight of digestive tract when oat-based diets were supplemented with enzymes. The reduction of empty gizzard weight might be related to the improvement of enzyme production and nutrient digestibility due to the inclusion moderate amount of fiber[22]. The supplementation of enzymes would further improve the nutrient digestibility and thereby reduced the muscular activity of the gizzard.

The inclusion of rice hulls increased the contents of the jejunum. Although, it was generally known that insoluble fibers results in more rapid passage, however inclusion of insoluble fibers in moderate amounts could slow the feed passage at least in the front part of the GIT[19]. The slow of digesta passage rate might result in reducing the fecal DM of broilers fed the RH. Supplementation of phytase and cellulase increased the rate of digesta passage and as a consequence increased the DM of the feces.

The reduction of P excretion found in the present study is in agreement with the results reported by Powell *et al.*[23] and Li *et al.*[24] that microbial phytase addition could increase P-disappearance in the gut and reduce P excretion in the environment.

CONCLUSION

The inclusion of 40 g kg^{-1} rice hulls can improve growth performance of young broilers. Supplementation of phytase and cellulase increases the P-disappearance in the digesta and reduces the P-excreta better than the supplementation of phytase alone.

nutrient recommendations of the NRC[11] for broiler starter diets. All experimental diets were provided as mash feed and were fed to broiler chickens from 3-21 days of age. The phytase used was *Escherichia coli*-derived phytase, Quantum Blue, ABVista Feed Ingredients, (Marlborough, UK). The standard recommended level of phytase was 100 g tonne^{-1} to achieve the activity of 500 FTU kg^{-1}. The cellulase used was SQzyme CSP product, 20.000 unit g^{-1}, (Suntaq International Limited, Shenzhen, China).

A total of 200 male broiler chicks (strain Lohmann) three days old (initial body weight of 54.6±2.3 g) were randomly placed in 40 cages with 5 birds per cage and 10 cages (50 birds) per treatment. Each cage was equipped with 1 drinker and 1 feeder. Feed and water were offered *ad libitum* throughout the experiment. Cages were illuminated 24 h per day.

Growth performance: Body weight (BW) and feed intake (FI) were weighed by cage at 3, 7, 14 and 21 d of age. Average daily gain (ADG) (g b^{-1} day^{-1}), average daily feed intake (ADFI) (g b^{-1} day^{-1}) and the gain-to-feed ratio (G:F) (g g^{-1}) were determined periodically and cumulatively. Mortality was recorded daily during the experiment. Birds that died were weighed and their BW was included in the calculation of G:F. Feed intake was adjusted for mortality.

Sample collection and analyses: At the end of experiment (21 days of age) and after a period of 8 h of feed withdrawal, birds were weighed. One bird from each of ten replicates per treatment was selected based on proximity to average bird weight per cage and was slaughtered by dissecting the jugular vein, ~~and variables measured were~~ Digestive organs weight, length and digestive contents. After the birds were killed, the body cavity was immediately opened and the GIT with content was removed and the following segments: gizzard, duodenum (from gizzard to pancreo-biliary ducts), jejunum (measured from the end of duodenal loop to Meckel's diverticulum) and ileum (from Meckel's diverticulum to ileocecocolic junction) was cut. The pancreas was also removed. The length of the duodenum, jejunum and ileum was measured to the nearest mm. After measuring the length, the content of duodenum, jejunum and ileum was collected, weighed and then mixed together to be analyzed for dry matter (DM) and phosphorus (P) content. The gizzard content was also collected. The weight of empty digestive organs (gizzard, duodenum, jejunum and ileum) including pancreas was expressed relative to 100 g live BW (without digesta) (g/100 g BW), whereas the weight of digestive content and the length of duodenum, jejunum and ileum was expressed relative to 100 g live BW (g/100 g BW, cm/100 g BW, respectively).

Dry matter and phosphorus content: Dry matter digesta (mixed of duodenum, jejunum and ileum content) and feces were analyzed using standard AOAC method[12], whereas the P-contained in the digesta and feces was analyzed using a colorimetric method. The analyses were performed in a laboratory at the Nutrition and Food Study Center, University of Gadjah Mada, Yogyakarta, Indonesia.

Statistical analysis: All treatment data obtained were analyzed statistically using one-way analysis of variance[13]. When F tests were significant, Duncan's multiple range test was applied to examine differences among group means. Statistical significance was accepted at $p \leq 0.05$.

RESULTS

Growth performance: The results of growth performance are shown in Table 2. From 3-7 days of age, there was no significant difference ($p>0.05$) found in ADG, ADFI and G:F but from 7-14 and 14-21 days of age, broilers within the RH groups gained more weight than those within the CON group (30.65 vs 27.60 g b^{-1} day^{-1} from 7-14 days; 51.19 vs 43.32 g b^{-1} day^{-1} from 14-21 days, $p \leq 0.05$, respectively). Furthermore, supplementation of phytase reduced ($p \leq 0.05$) ADG more than phytase and cellulase but had no effect ($p>0.05$) on ADFI or G:F. Globally, from 3-21 days of age, ADG and G:F within the RH groups were better than those within the CON group ($p \leq 0.05$). Mortality was 1.0% and was not caused by any dietary treatment (data not shown).

Weight and length of digestive organs and weight of digestive content: Diets did not affect ($p>0.05$) the weight of pancreas, length and relative weight of duodenum, jejunum and ileum, except for the gizzard (Table 3). At 21 days of age, broilers fed the RH treatment tended to have heavier gizzards without content ($p = 0.057$) than those fed the CON treatment (Table 3). Supplementation of phytase but not supplementation of phytase and cellulase, caused a reduction in the weight of empty gizzard. The inclusion of rice hulls did not affect ($p>0.05$) the contents of gizzard, duodenum and ileum but increased ($p \leq 0.05$) the contents of the jejunum (Table 3). The addition of enzymes on the RH reduced the contents of jejunum the same as those in the CON treatment ($p \leq 0.05$).

Table 2: Growth performance response of diets

	3-7 days of age	7-14 days of age	14-21 days of age	3-21 days of age
ADG (g b^{-1} day^{-1})				
CON	14.870	27.600^{a}	43.320^{a}	28.600^{a}
RH	15.450	30.650^{b}	51.190^{c}	32.430^{c}
RHP	14.430	28.560^{ab}	45.62^{ab}	29.26^{ab}
RHPC	14.880	30.190^{b}	49.37^{bc}	31.480^{bc}
SEM[1]	0.180	0.424	0.899	0.438
p-value	0.270	0.030	0.000	0.000
ADFI (g b^{-1} day^{-1})				
CON	21.840	49.460	46.720	~~46.510~~ 39.570
RH	23.090	52.070	45.550	~~48.190~~ 40.240
RHP	21.180	47.440	42.340	~~43.550~~ 37.560
RHPC	22.100	49.550	44.460	~~45.910~~ 38.700
SEM[1]	0.286	0.744	2.625	~~0.717~~ 0.503
p-value	0.120	0.180	0.480	~~0.140~~ 0.300
G:F (g g^{-1})				
CON	0.682	0.560	0.939	~~0.617^{a}~~ 0.728^{a}
RH	0.670	0.590	1.126	~~0.674^{ab}~~ 0.807^{b}
RHP	0.681	0.603	1.091	~~0.685^{b}~~ 0.781^{ab}
RHPC	0.674	0.632	1.046	~~0.697^{b}~~ 0.819^{b}
SEM[1]	0.008	0.015	0.041	0.011
p-value	0.940	0.420	0.420	0.050

[1]SEM: Standard error of the mean, CON: Control diet, RH: 4% rice hull inclusion, RHP: RH+phytase 1750 FTU kg^{-1}, RHPC: RHP+cellulase 500 unit kg^{-1}, ^{a,b,c}mean values within a column with different superscripts differ significantly (p≤0.05)

Table 3: Diet response on all digestive traits measured at 21 days of age

Treatments	SEM[1]	P-value	CON	RH	RHP	RHPC
GIT weight (g/100 g BW)						
Empty gizzard	1.97^{ab}	2.10^{b}	1.67^{a}	2.14^{b}	0.068	0.057
Empty duodenum	0.79	0.88	0.83	0.83	0.026	0.680
Empty jejunum	1.50	1.66	1.54	1.70	0.049	0.450
Empty ileum	1.05	1.18	1.06	1.16	0.036	0.470
Pancreas	0.05	0.05	0.05	0.06	0.002	0.360
GIT length (cm/100 g BW)						
Duodenum length	3.18	3.49	3.38	3.50	0.064	0.260
Jejunum length	8.20	8.54	8.06	8.51	0.172	0.720
Ileum length	8.60	8.78	8.52	8.68	0.181	0.970
GIT content (g/100 g BW)00						
Gizzard content	1.16	1.24	1.18	1.30	0.063	0.870
Duodenum content	0.31	0.38	0.34	0.35	0.020	0.680
Jejunum content	0.57^{a}	0.89^{b}	0.46^{a}	0.55^{a}	0.056	0.030
Ileum content	0.46	0.90	0.63	0.71	0.085	0.330
DM digesta (%)	13.90	17.90	19.20	16.00	0.949	0.280
DM feces (%)	24.60^{a}	23.90^{a}	24.10^{a}	27.30^{b}	0.432	0.010
P-digesta (%)	0.64^{c}	0.58^{b}	0.53^{ab}	0.50^{a}	0.012	0.000
P-feces (%)	0.66^{ab}	066.00^{ab}	0.71^{b}	0.62^{a}	0.011	0.030

[1]SEM: Standard error of the mean, CON: Control diet, RH: 4% rice hull inclusion, RHP: RH+phytase 1750 FTU kg^{-1}, RHPC: RHP+cellulase 500 unit kg^{-1}, ^{a,b,c}mean values within a row with different superscripts differ significantly (p≤0.05)

SEM values move to CON

P-values move to RH

CON values move to RHP

RH values move to RHPC

RHP values move to SEM

RHPC values move to p-value

0.66^{ab}

Dry matter and P content of digesta and feces: The inclusion of rice hulls did not affect (p>0.05) digesta DM or fecal DM but supplementation of enzymes on RH affected (p≤0.05) fecal DM (Table 3). Phytase and cellulase supplementation caused higher (p≤0.05) fecal DM than other treatments, which were not significantly different. Broilers fed the RH treatment had higher (p≤0.05) P-disappearance in digesta than those fed the CON treatment. Supplementation of enzymes increased the P-disappearance in the digesta, an effect that was more pronounced for phytase and cellulase than an individual phytase. The results on P-excretion showed that broilers fed the RH treatment had the same P-excretion as those fed the CON treatment (Table 3). Supplementation of phytase increased the P-excretion, whereas supplementation of phytase and cellulase decreased the P-excretion (p≤0.05).

DISCUSSION

Growth performance was affected by rice hull inclusion in the diet. From 3-21 days of age, the improvement observed