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Start this statement with the following words:

This study discover the ---------- that can be beneficial for ……

And the last sentence of this statement could be such as:

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A Model Significance Statement:

This study discovers the possible synergistic effect of vitamin E, calcium, and vitamin D combination that can be beneficial for osteoporosis-induced ovariectomized rats. This study will help the researcher to uncover the critical area of postmenopausal bone loss that many researchers were not able to explore. Thus, a new theory on these micronutrients combination, and possibly other combinations, may be arrived at.

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The Effects of Adding Insoluble Non-Starch Polysaccharides and Exogenous Enzymes to a Commercial Broiler Diet on Growth Performance and Carcass Weight of Broiler Chickens

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Statement on Conflict of Interest: none declared

Abstract

It is possible that a commercial broiler ration that contains many fleas/insects and excess fine feed may have poor nutrient quality. This study aimed to investigate the effects of supplementing wheat pollard (WP), rice hull (RH), phytase, and cellulase in a commercial broiler diet on the growth performance, carcass weight, and gut characteristics of broiler chickens from 0-35days of age. A total of 175 one-day-old male broiler chicks (Lohmann) were fed using 7 dietary treatments (5replicates/treatment). The diets used were as follows:1.the commercial broiler diet, as a control diet (C), 2.C+WP (CWP)+phytase, 3.CWP+cellulase, 4.CWP+phytase+cellulase, 5.C+RH (CRH)+phytase, 6.CRH+cellulase, and7.CRH+phytase+cellulase. Wheat pollard/rice hull was added at 40 g/kg of diet. Phytase was added at 1250FTU/kg, and cellulase was added at 250unit/kg. The control diet contained a low level of Ca (0.5%) and total P (0.4%). Treatment diets did not affect growth performance of broiler chickens (P>0.05). Birds fed diets 5, 6, and 7 exhibited higher carcass weights (P<0.05), the lightest duodenum and jejunum weights (P<0.01), the lowest jejunum pHs (P<0.05), and the lowest caecal pHs (P<0.01), as well as higher caecal acetic and butyric acid concentrations (P<0.01). The duodenum digesta was the highest in the birds fed the control diet (P<0.05). In conclusion, adding 40 g/kg rice hull to the broiler diets containing low levels of Ca (0.5%) and total P (0.3-0.5%) enhanced carcass weight. The increase in carcass weight was due to the reduction in the gut weight, to which the addition of rice hull, as well as its physical structure, contributed more than the phytase supplementation. Cellulase supplementation did not enhance phytase efficacy.

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

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Introduction

In developing countries such as Indonesia, broiler farmers who live in small towns or villages often face the fact that broiler rations they purchased were “old”. The broiler ration in this condition usually contains many fleas/insects and its feed form is no longer intact[1]. Moreover, a broiler finisher ration in pellet form becomes a powder. Based on this, there is a suspicion that some kinds of rations have poor nutrient quality. The majority of feed ingredients used in poultry diets are derived from plant cereals such as soybean, corn, wheat, or barley[2]. 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 the nutrition obstacles for monogastric animals[3]. Some research has indicated that supplementation of broiler feeds with phytase can alleviate the effect of anti-nutritional factors and improve bird performance[4,5]. The use of exogenous phytase supplementation has been reported to improve the use of Phytate P,[6,7] which represents between 50% and80% of the total Phosphorus(P) content in cereals[8].

In addition to enzymes, the benefit of insoluble non-starch polysaccharides (iNSP) in broiler diets has also been reported in many studies. Many of the benefits have been associated with improving gut health[9-11], gizzard function[12], increasing starch digestibility[13], and growth performance in broilers[14,15] or in layers[16].

Lately, there is interest in combining phytase with cell wall-degrading enzymes in broiler diets based on the assumption that phytate hidden in the plant cell wall cannot be degraded by phytase. The use of phytase in combination with exogenous carbohydrase has been investigated in many

studies[17-19]. It was reported that the combination of phytase and carbohydrase was effective in improving broiler growth performance[17,18].

The inclusion of iNSP and/or the supplementation of enzymes to broiler diets are common in poultry research, but little data is available on the addition of iNSP and enzymes to the commercial broiler diet. In a previous study, Hartini and Massora[11]tried to add graded levels (20, 40, and 60 g/kg) of iNSP (wheat pollard and rice hull) to commercial broiler diets. They found that the addition of 40 g/kg of iNSP decreased the caecal microbial count in young broilers. Moreover, the addition of 40 g/kg of rice hull and 20 g/kg of wheat pollard reduced the total number of *Eschericia coli* and *Enterobacter* in the small intestine, respectively. As a source of iNSP, wheat pollard contains 33.6% DM of insoluble NSP and 1.7% soluble NSP[20]. Rice hulls are a byproduct of rice processing and contain mostly cellulose[21].

Hence, the current study was designed to evaluate the effects of the utilization of exogenous enzymes (phytase and cellulase) and the addition of iNSP (wheat pollard and rice hull) to a commercial broiler diet on growth performance, carcass weight, and gastrointestinal tract characteristics of broilers from 0 to 35 days of age. The hypothesis of this research was that the addition of exogenous enzymes and iNSP to commercial broiler diets could improve the nutritive values of the diets and consequently increase broiler performance. However, the degradation of nutrients in the commercial diets used was also considered as a factor that may influence the results of adding iNSP and enzymes.

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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 the Indonesian market and has characteristics as explained previously. The wheat pollard and rice hulls were used as sources of iNSP. Rice hulls were ground using a grinder fit with a 2 mm screen. The wheat pollard and rice hulls were added to the commercial starter and finisher diets at 40 g/kg, an amount similar to that in the study by Hartini and Massora[11].The commercial broiler diets, wheat pollard, and rice hulls were analyzed for dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF), gross energy (GE), calcium (Ca), and phosphorus (P). Dry matter, CP, EE, and CF were analyzed by the AOAC standard methods, GEwas analyzed by bomb calorimetry, Ca was analyzed by the AAS (Atomic Absorption Spectrophotometry) method, and P was analyzed by the colorimetric method. All of the chemical analyses were performed in the laboratory of the Nutrition and Food Study Center, Gadjah Mada University, Yogyakarta, Indonesia. The analyzed and calculated nutrient values of experimental diets are shown in Table 1.

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The phytase used was *E. coli* derived phytase (Quantum Blue, ABVista Feed Ingredients, Marlborough, UK).The standard recommended level of phytase was 100 g/ton to get the activity of 500FTU/kg. The cellulase used was a SQzyme CSP product at 20,000unit/g from Suntaq International Limited, Shenzhen, China.

Diets used in the experiment were:

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

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

3) CWP+250 unit/kg of cellulase,

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

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

6) CRH+250 unit/kg of cellulase, and

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

Bird management

One hundred 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 calculating the coefficient variance (CV) of body weight (BW). The birds were fed a starter diet with the addition of40 g/kg iNSP (wheat pollard or rice hull) and exogenous enzymes (1250 FTU/kg of phytase and/or 250 unit/kg of cellulase) until 21 days of age; then,they were fed a finisher diet with the same additions from 22 days until the end of experiment at day 35. Both feed and water were offered *ad libitum* during the experiment. Pens were illuminated 24 h per day.

Variablesmeasured

Variables measured in the experiment were body weight gain (BWG) (gram/bird/day), feed intake (FI) (g/b/d), feed conversion efficiency (FCE) (g/g), relative weight of digestive organs (gizzard, duodenum, jejunum, ileum, and caeca) expressed as g/100 g body weight (BW), relative length of digestive organs (duodenum, jejunum, and ileum) expressed as cm/100 g 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 days), twobirds from each of the five replicates per treatment were selected based on proximity to average bird weight per cage. The birds were fasted for approximately 8 hours before the slaughter. After determining the live weight, the birds were slaughtered by dissection at the jugular vein and used to measure the variables below.

Carcass

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

Caecal Volatile Fatty Acids (VFA)

The level of VFA in caecal digesta was determined by gas chromatography using the method described by Hartini[22]. Three grams of sample were diluted with 3 ml of 0.1 M H_2SO_4 and thoroughly mixed. The sample was then centrifuged (15,000 g, 15 min). 0.1 ml of a reference volatile fatty acid (caproic acid) was added to a 1 ml aliquot of supernatant. The volatile fatty acids were distilled using Thundberg tubes. The concentration of VFA was quantified using a GLC (Gas Liquid Chromatography).

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Gastrointestinal tract length, weight, digesta, and pH

The gastrointestinal tract (GIT) was removed and cut into the following 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. Next, the duodenum, jejunum, and ileum, gizzard and caeca were weighed prior to and after removal of contents. The pancreas was also removed and weighed. Digesta contents of the duodenum, jejunum, ileum, and caeca were measured, and then put in a 50 ml glass beaker. 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 homogenized, and a pH meter was inserted. The pH was read when the number was stable.

Statistical analyses

The data obtained were analyzed statistically using a one-way Analysis of Variance[23]. After a significant F test, Duncan's multiple range test was used to inspect differences among group means. Statistical significance was accepted at $P<0.05$. In regards to differences among means, $0.05<P<0.10$ were accepted as representing tendencies and differences.

Results

Fiber sources and experimental diets

Table 1 showed the nutrient values of the fibers and the experimental diets used in this study. Rice hull contained crude fiber almost 7 times higher than wheat pollard. The addition of rice hull in the starter and finisher diet caused higher crude fiber content than the addition of wheat. The nutrient values of starter and finisher diets had low concentrations of Ca and total P. The concentrations of Ca and total P did not differ among diets.

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

The effects of the diets on performance, carcass weight, GIT pH, and GIT weight are summarized in Table 2. The 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 ($P<0.05$). The addition of the rice hulls with a single enzyme or a combination of enzymes resulted in 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

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wheat pollard with phytase or cellulase alone (Table 2). There was no mortality found during the experiment.

As shown in Table 2, CRH diets supplemented with phytase or with phytase and cellulase significantly lowered the jejunal pH ($P<0.05$) and caecal pH($P<0.01$) more than the other treatment groups. Among the CRH diets, supplementation of an enzyme combination (phytase and cellulase) had the lowest jejunal pH. The duodenal pH of birds fed CRH diets with the addition of an 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 weights of the gizzard, duodenum, jejunum, ileum, caeca and pancreas are expressed per 100 g of body weight (g/100g BW). Birds fed the control diet had significantly higher ($P<0.01$) duodenum and jejunum weights compared to those fed the other diets (Table 2). Supplementation of enzymes to either the CWP or CRH diets reduced the duodenal weight ($P<0.01$). Birds fed the CRH diets with the addition of phytase or with the addition of phytase and cellulase had the lightest duodenum and jejunum weights ($P<0.01$) (Table 2).

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Gastrointestinal tract length, digesta content, and caecal VFA

The effect of iNSP and enzyme additions on GIT length, digesta content, and caecal VFA are shown in Table 3.The length of the duodenum, jejunum, and ileum are expressed per 100 g body

weight (cm/100 g BW). Diets had no effect on the length of the 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 different (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 more than the other diets (P<0.01). Among the CRH diets, cellulase supplementation showed a higher concentration of butyric acids 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).

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Discussion

The reduction of nutrient quality in the control diets in this study has been predicted. But the low concentration of Ca and P in the diets was far from the expectation. The growth of broilers requires a sufficient amount of Ca and P for the formation and maintenance of their skeleton.

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Phytase supplementation significantly increased Ca digestibility regardless of the Ca and P levels of the diets[24], and significantly increased body weight. The inclusion of iNSP in broiler diets was also reported to improve growth performance of broiler chickens[14,15].

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. Insoluble non-starch polysaccharides inclusion (3% oat hulls or 3% soy hulls) in corn based-diets had been shown to improve BWG and feed conversion in young broiler chicks[14]. On the other hand, the inclusion of 4% oat hulls[25] or 10% cellulose[26] in the wheat-based diets was found to only increase the FI, but not the BWG and FCR, in broilers at 21d of age. The different results found indicated that the effect of iNSP source not only depended on its physicochemical 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[27]. Supplementation of phytase or cellulase or phytase and cellulase in this study did not show any significant effect. In contrast, a previous study by Meng *et al.*[28] demonstrated that multiple enzyme preparations statistically improved insoluble NSP digestibility. The effect of phytase was significant in increasing broiler weight gain when phytase was added to a P-deficient diet[7,19]. Biehl and Baker[29] observed that supplementation of a high dosage of microbial phytase increased FCE when a diet was deficient in the amino acid. Furthermore, Avilla *et al.*[18] found that the use of the multiple-enzyme complex on typical commercial sorghum-SBM broiler diets reformulated at −85 kcal/kg of AMEn, −1.5% CP, −1.5% amino acids, −0.153% of available P, and −0.12% of Ca reestablished broiler performance equivalent to that of the control diet.

The diets in the current study, by analysis, contained 0.5% Ca and 0.3%P in the starter period and 0.5%Ca and 0.5%P in the finisher period. In Indonesia, the standard nutrient requirements of meat type broiler chickens for the starter period (0-3wks of age) were 19-23%CP, 0.9-1%Ca, and

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0.6-1% total P, whereas for the finisher period (>3-6 wks of age) the requirements were 18-20%CP, 0.9-1.2%Ca, and 0.6-1% total P [30]. The concentration of Ca and P in the present study was below the standard requirements. In broiler starter diets, the concentration of Ca and P reduced by approximately 44%, respectively, whereas in broiler finisher diet the concentration of Ca and P reduced by approximately 44% and 17%, respectively. The exact cause of Ca and P reduction was unknown.

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Addition of iNSP (wheat pollard or rice hull) and exogenous enzymes (phytase and/or cellulase) did not enhance growth performance. In broiler diets, calcium and phosphorus are essential minerals for growth. A deficiency or excess of one may interfere with the proper utilization of the other. Therefore, in the feed formulation, Ca and P should be included in a definite relationship for bone formation in the bird. The SNI[30]recommended a calcium-phosphorus ratio of approximately 1.5:1 for broilers, based on total phosphorus. Calcium and phosphorus, particularly the ratio between Ca:P, were factors that influenced phytase responses[31]. In their review, Bedford *et al*.[32], included Ca and P as major determinants of phytase response. Tamim *et al*.[33] demonstrated that dietary calcium can precipitate with phytate P, forming insoluble-phytate complexes. The addition of phytase at either Ca level (0.18% Ca or 0.68% Ca), resulted in an increase in the apparent absorption of Ca and P in the gastrointestinal tract of broiler chickens. Delezie *et al*.[31] concluded that Ca and P concentrations can be reduced by 20% if done in a balanced way, and supplementation of phytase in this condition will further improve broiler growth. Adding graded levels of Ca and phytase in a diet deficient in Ca but adequate in total P was found to improve weight gain in young broiler chicks[34]. They suggested that the addition of calcium caused a significant improvement in weight gain and addition of phytase caused more calcium to be available, which significantly increased body weight. In the current

study, although a high dosage of phytase (1250FTU/kg) was used, the reduced concentrations of both Ca and P by approximately 44% from the normal requirements in the starter period was more likely to be a constraint for phytase to respond optimally. Even at normal concentrations of Ca, the liberated phytate-P by phytase may not be sufficient to meet the broiler requirements of P if the diet had a low P concentration[31]. Moreover, the growth rate of broilers are relatively high during the starter period (1-21d), indicating that the demand of Ca and P in this period is also higher than in the finisher period. The reduction of Ca and P concentrations during the starter period was assumed to greatly affect the growth performance. The addition of cellulase in the current study did not give any different results in BWG, which indicated that the cellulase supplementation failed to increase phytase efficacy. The GE content in the present study was in the range of 3845-3999kcal/kg for the starter diet and 3898-4052 kcal/kg for the finisher diet. The fact that the FI was found to be similar among the diets implied that the contributions of iNSP and feed enzymes had not yet affected energy availability. Birds would compensate by increasing FI when fed diets low in energy[35,36].

The carcass weight was found to be higher in broiler chickens fed the CRH diets with the addition of phytase or phytase and cellulase than those fed the other diets. The broiler carcass weight is a bird's body without blood, feathers, head, legs, and visceral organs. The lighter weight of the duodenum and jejunum and the duodenum content of birds fed the CRH diets plus phytase, cellulase, or pytase and cellulase might be responsible for increasing the carcass weight. Rolls *et al*.[37] reported that modification of the gut morphology was associated with an increase of gut microflora. Cereal by-products contain little water soluble NSP but substantial concentrations of insoluble NSP[38]. The insoluble β-glucans pass into the large intestine, where they are fermented by bacteria[39]. 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[40]. Large numbers of microorganisms in the caeca tend to migrate to the gut where nutrient absorption occurs, causing a thicker lining of the gut[41]. Supplementation of enzyme may reduce the microbial influence on the digestive tract of birds possibly by reducing the fermentative substrate[42,43] and consequently reducing the gut weight. We only agreed in part with this idea. The differing results on the effect of the addition of wheat pollard and enzyme on carcass weight implied that there was another factor besides enzymes that influenced the increase in carcass weight. A previous study by Hartini and Massora[11] found that the addition of 40 g/kg iNSP (rice hull or wheat pollard) in commercial broiler diets without enzyme supplementation significantly reduced the total number of microbes in the small intestine. Therefore, it seemed that iNSP had an influence by decreasing the microbial count, and the action of enzyme was the only addition to it.

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Josefiak *et al*.[44]demonstrated that supplementation of oligosaccharides increased fermentation in the caeca, consequently increasing the production of volatile fatty acids (acetate, propionate, and butyrate). In the present study, the addition of rice hull, regardless of the enzymes, resulted in a greater production of acetic and butyric acids than the addition of wheat pollard. Different fiber sources affected VFA production in different segments of the gastrointestinal tract[45]. He *et al*.[46] also demonstrated that different fiber sources resulted in different VFA profiles, especially in the gizzard and caeca of growing Greylag geese. The butyric acid was reported to reduce the small intestinal pH and had anti-bacterial properties[47]. Indeed, in the current study, birds fed the control diets with the addition of rice hull, regardless of the enzyme, showed a low pH in the jejunum and caeca. This may also explain the lower duodenal and jejunal digesta in birds fed the control diets with the addition of rice hull and enzymes.

Moreover, Jamroz *et al*.[48] reported that feeding geese a large proportion (50%) of rye resulted in an increase in the thickness of the intestinal wall, while feeding rapeseed meal (20%) decreased the thickness of the intestinal wall. The addition of rice hull in this present study was only 4%. Therefore, it is possible that the level of rice hull in the diet and the physical structure of rice hull might affect the reduction of the intestinal wall, consequently reducing the gut weight. In their review, Montagne *et al*.[49]reported that the effect of dietary fiber on epithelial morphology and cell turnover is variable and depends on the physicochemical characteristics of the fiber, the level of incorporation into the diet, and the duration of ingestion.

Hetland and Svihus[25] suggested that a high fiber diet tended to stay in the gizzard longer. The result on gizzard weight in this current study was not significant. The addition of 4% iNSP may not be sufficient to stimulate the gizzard function. The activity of the gizzard depends on its content[13]. However, in birds fed the commercial broiler diets with the addition of rice hull and enzymes, the lower duodenal and jejunal digesta indicated that the presence of rice hull actually caused a slower passage rate of digesta than in the other diets.

Based on the results, it can be concluded that adding 40 g/kg iNSP and enzymes (phytase or both phytase and cellulase) to commercial broiler diets containing low concentrations of Ca (0.5%) and P (0.3-0.5%) did not affect the growth performance of broiler chickens, but did affect carcass weight. The increase in carcass weight was due to the reduction in the gut weight, to which the addition of rice hull, as well as its physical structure, contributed more than the phytase supplementation. Cellulase supplementation did not enhance phytase efficacy.

Significance Statements

This study demonstrates the possible effect of rice hull addition in decreasing the thickness of the intestinal wall that can be beneficial for increasing the carcass weight of broiler chickens. This study will help the researcher to uncover the importance of rice hull in influencing the epithelial morphology and cell turnover that many researchers were not aware of. Thus, a new finding on the important use of rice hull in broiler diets may be arrived at.

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Acknowledgments

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

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

Table 2. Responses of performance, carcass weight (%) and gastrointestinal tract (GIT) weight (g/100 g BW) to diet type

Variable Measured \ Diet	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 ± 0,14^{b}	6,02 ± 0,11^{b}	6,04 ± 0,05^{b}	6,20 ± 0,09^{b}	5,90 ± 0,23^{b}	5,68 ± 0,26^{ab}	5,25 ± 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 ± 0,23^{bc}	7,54 ± 0,14^{c}	7,18 ± 0,07^{bc}	7,28 ± 0,17^{bc}	6,68 ± 0,29^{ab}	6,23 ± 0,37^{a}	6,63 ± 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 40 g/kg of WP (CWP) and 1250 FTU/kg of phytase, 3) CWP added with 250 unit/kg of cellulase, 4) CWP added with 1250 FTU/kg of phytase and 250 unit/kg of cellulase, 5) C added with 40 g/kg of RH (CRH) and 1250 FTU/kg of phytase, 6) CRH added with 250 unit/kg of cellulase, 7) CRH added with 1250 FTU/kg of phytase and 250 unit/kg of cellulase.

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

Table 3. Response of diets on GIT length (duodenum, jejunum, ileum) (cm/100 g BW) and GIT digesta (gizzard, duodenum, jejunum, ileum, caeca) (g/100 g 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 with40 g/kg of WP (CWP) and 1250 FTU/kg of phytase, 3) CWP added with 250 unit/kg of cellulase, 4) CWP added with1250 FTU/kg of phytase and 250 unit/kg of cellulase, 5) C added with 40 g/kg of RH (CRH) and 1250 FTU/kg of phytase, 6) CRH added with 250 unit/kg of cellulase, 7) CRH added with 1250 FTU/kg of phytase and 250 unit/kg of cellulase.

[abc]mean values within a row bearing different superscripts differ significantly.