## Evaluation Report for Article No. 81503-PJN-ANSI

Your revised research article has been received and evaluated. A large number of grammatical and sentence construction errors are observed in the article. Most of the sentences are haphazard. You please negotiate with American Journal Experts, or Bioscience Editing Solutions to provide you language editing services at competitive rates.

Non English authors may contact with American Journal Experts or Bioscience Editing Solutions to correct spelling, grammar, punctuation and word usage errors before submission or after acceptance of their manuscripts to eliminate (minimize) the chances of rejection due to poor English. Please re-submit your article after editing its language with editorial certificate for further processing.

Moreover, revise the Title of the article, Significance statement, and Results section according to the suggestions given in the text as side notes and re-submit your article after making proper modifications for further processing.

# The Effect ~~Impact~~ of Insoluble Non-Starch Polysaccharides and Exogenous Enzymes Addition to A Commercial Broiler Diet on Growth Performance and Carcass Weight~~Production Characteristics~~ of Broiler Chickens

S. Hartini and P. Purwaningsih

Faculty of Animal Husbandry, Papua University

Jl. Gunung Salju Amban, Manokwari 98314, Papua Barat-Indonesia

Corresponding E-mail: sri.hartini8877@gmail.com

**Comment [N1]:** Title of your research article is neither effective nor concise. It does not indicate accurately the purpose of the study. Use words that create a positive impression and stimulate reader interest.

Statement on Conflict of Interest: none declared

**Abstract**

A commercial broiler ration having a lot of fleas/insect and excess fine feed is possible to have poor nutrient quality. This study aimed to investigate the effects of wheat pollard (WP), rice hull (RH), phytase, and cellulase supplementation to the commercial broilers diet on growth performance, carcass weight, and gut characteristics of broiler chickens from 0-35 days of age. Total of 175 day-old male broiler chicks (Lohmann) were fed on 7 dietary treatments (5 replicates/treatment). The diets were: 1.the commercial broiler diet, as a control diet (C), 2.CWP+phytase, 3.CWP+cellulase, 4.CWP+phytase+cellulase, 5.CRH+phytase, 6.CRH+cellulase, 7.CRH+phytase+cellulase. Wheat pollard/rice hull was added at 40g/kg of diet. Phytase was given at 1250FTU/kg and cellulase at 250Unit/kg. The control diet by analyses 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, 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 broiler diets containing low levels of Ca (0.5%) and total P (0.3-0.5%) could enhance carcass weight. The mechanism was assumed through the reduction of the gut weight in which the addition of rice hull, as well as its physical structure, contributes more than the phytase supplementation. Cellulase supplementation did not enhance phytase efficacy.

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

Deleted: BWG, FI, and FCE

**Introduction**

In developing countries like Indonesia, broiler farmers who live in a small town or small village often face the fact that broiler rations they purchased were "old". The broiler ration in this condition usually contains a lot of fleas/insect and its feed form is no longer intact[1]. 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[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 nutrition obstacles for monogastric animals[3]. Some researches indicated that supplementation of broiler feeds with phytase can alleviate the effect of anti-nutritional factors and improve bird's 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% to 80% of the total Phosphorus (P) content in cereals[8].

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[9-11], gizzard function[12], increasing starch digestibility[13], and growth performance in broiler[14,15] or in layers[16].

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

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 commercial broiler diet. Hartini and Massora[11], in previous study, tried to add graded levels (20, 40, and 60g/kg) of iNSP (wheat pollard and rice hull) to the 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, as a source of iNSP, contains 33.6% DM of insoluble NSP and 1.7% soluble NSP[20]. Rice hulls are by product of rice processing which contain mostly cellulose[21].

Hence, the current study was designed to evaluate the effects of the utilization of exogenous enzymes (phytase and cellulase) to a commercial broiler diet added with iNSP (wheat pollard and rice hull) on growth performance, carcass weight, and gastrointestinal tract 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 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 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 starter and finisher diets diets at 40g/kg, respectively, the amount similar to the previous study by Hartini and Massora[11]. The 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 phosphorus (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 colorimetric 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 of experimental diets 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 coefficient variance (CV) of body weight (BW). ~~The result of the CV calculation of day old chick at the beginning of the experiment was 1.49.~~ The birds were fed a starter diet added with 40g/kg iNSP (wheat pollard or rice hull) and exogenous enzymes (phytase 1250FTU/kg and/or cellulase 250unit/kg of diets) to 21 d of age and then a finisher diet with the same additions from 22 d until the end of experiment at day 35. Both feed and water were offered *ad libitum* during the experiment. Pens were illuminated 24h per day.

**Comment [N3]:** Don't mix results with procedures

***Variable measured***

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/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).

***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 samples were diluted with 3 ml of 0.1 M $H_2SO_4$ and

thoroughly mixed. The sample was centrifuged (15,000 g, 15 min). To an aliquot of 1 ml supernatant, 0.1 ml a reference volatile fatty acid (caproic acid) was added. The volatile fatty acids were distilled using Thundberg tubes. The concentration of VFA was quantified using a Hewlett Packard 427 GLC (Gas Liquid Chromatography).

***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[23]. After a significant F test, Duncan's multiple range test was used to inspect differences among group means. Statistical significance was accepted at $P<0.05$. Differences among means with $0.05< P <0.10$ were accepted as representing tendencies to differences.

## Results

### *Fiber sources and experimental diets*

Table 1 showed the nutrient values of 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 concentration of Ca and total P. The concentration of Ca and total P was not different among 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).~~

**Comment [N4]:** Results section does not show the important and key findings of the study. It includes material which does not belong to the results section such as interpretation and discussion; it focuses on the Tables representing the results, rather than the results themselves. No need to repeat the Table values in the text. Delete the data from the text that have already been presented in the table. An example is given at the end of this article which will signifiy how to present the key findings of the study with reference to the Table

### *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**

The reduction of nutrient quality in the control diets in this study has been predicted. However the fact that Ca and P levels which were drastically declined in the diet was far from the expectation. The growth of broilers needs the sufficient amount of Ca and P to be used for formation and maintenance of skeleton. Phytase supplementation significantly increased Ca digestibility regardless of Ca and P levels of the diets[24], so 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 demonstrated to improve BWG and feed conversion of young broiler chicks[14]. On the other hand, inclusion 4% oat hulls[25], or 10% cellulose[26] in the wheat-based diets was only found to increase FI but not 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 physico-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[27]. In addition, the supplementation of phytase or cellulase or combination of both seemed not working optimally. In contrast, a previous study by Meng *et al.*[28] demonstrated that multiple enzyme preparation statistically improved insoluble NSP digestibility. The effect of phytase was significant in increasing broiler weight gain when phytase added to a P-deficient diet[7,19]. Biehl and Baker[29] observed that supplementation of high dosage of microbial phytase increased FCE when diet deficient in the amino acid. Furthermore, Avilla *et al.*[18] 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).

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%[30]. 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 declined by around 44% each. For the finisher period, the Ca declined by around 44% and the total P around 17%. The exact cause of Ca and P reduction was unknown.

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 in 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]. Bedford *et al*.[32], in their review 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. Addition of phytase at either Ca level (0.18% Ca or 0.68% Ca) resulted in an increase

in Ca and P apparent absorption in the gastrointestinal tract of broiler chickens. Delezie *et al*.[31] 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 broiler growth 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 of young broilers chicks[34]. They suggested that addition of calcium caused a significant improvement 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 reduced 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[31]. 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[35,36].

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*.[37] reported that modification of the gut morphology related with increasing 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 microorganism in the caeca tend to migrate to the gut where nutrient absorption takes place, causing a thicker lining of the gut[41]. Supplementation of enzyme may reduce microbial influence on digestive tract of birds probably by reducing fermentative substrate[42,43], 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 influenced the increased of carcass weight. Previous study by Hartini and Massora[11] 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 iNSP *per se* had an influence by decreasing the microbial numbers and the action of enzyme was only addition into it.

Josefiak *et al*.[44] had 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 had higher 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 profile, 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 added with rice hull regardless of the enzyme showed 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.

Jamroz *et al*.[48], moreover, 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 only 4%. So it is possibly that not only the level of rice hull in the diet but also the physical structure of rice hull which might affect the reduction of the intestinal wall, and consequently reducing the gut weight. Montagne *et al*.[49] in their review reported that the effect of dietary fiber on epithelial morphology and cell turnover is variable, and depends on the physico-chemical characteristics of the fiber, their level of incorporation in the diet, and the duration of ingestion.

Hetland and Svihus[25] 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[13]. 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 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. Increase in carcass weight was due to the reduction in the gut weight, in 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 a commercial broiler diet to increase carcass weight. However, there is a possibility that the physical structure of the rice hull influenced (reduced) gut weight and eventually increased carcass weight, which warrants further study.

~~Supplementation of phytase 1250FTU/kg in the broiler diets having a very low concentration of P was not able to enhance performance.~~

- ~~The level as well as the physical structure of the rice hulls could influence the gut weight of broiler chickens, which indirectly affected the carcass weight.~~
- ~~Each insoluble polysaccharide has a different physiological function depends on its physico-chemical property and its interaction with other cell wall components in the diets.~~

**Comment [N5]:** Significance Statement does not show the novelty aspect and significance of this research work with respect to the existing literature and more generally to the society. It should be a short summary which describe what this paper adds to and what was already known.

**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:**

This study will help the researcher to uncover the critical areas of ---------- that many researchers were not able to explore. Thus a new theory on ---------- may be arrived at.

A model significance statement is given below:

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.

**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.

**References**


1. Retnani, Y., E. D. Putra, and L. Herawati, 2011. The effect of different water spraying level and storage period on endurance of pellet broiler finisher. Agripet., 11 (1): 10-14.
2. NRC (NATIONAL RESEARCH COUNCIL),1994. Nutrient requirements of poultry. 9th revised Edn., Washington D.C.: National Academic Press.
3. Cabahug, S., V. Ravindran, W.L. Bryden, and P.H. Selle, 1999. Response of broilers to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus levels. I. Effects on broiler performance and toe ash content. Br. Poult. Sci., 40: 660-666.
4. Shirley, R.B., and H.M. Edwards Jr., 2003. Graded levels of phytase past industry standards improves broiler performance. Poult. Sci., 82: 671-680.
5. Selle, P.H., V. Ravindran, G. Ravindran, and W.L. Bryden, 2007. Effect of dietary lysine and microbial phytase on growth performance and nutrient utilization of broiler chickens. Asian-Aust. J. Anim. Sci., 20 (7): 1100-1107.
6. Baidoo, S.K., Q.M. Yang, and R.D. Walker, 2003. Effects of phytase on apparent nutrient digestibility of organic phosphorus and nutrients in maize-soya bean meal-based diets for sows. Anim. Feed Sci. Technol., 104:133–141.
7. Cowieson, A.J., T. Acamovic, and M.R. Bedford, 2006. Supplementation of corn–soy-based diets with an *Eschericia coli*-derived phytase: effects on broiler chick performance and the digestibility of amino acids and metabolizability of minerals and energy. Poult. Sci., 85:1389–1397.

8. Israel, D.W., P. Kwanyuen, and J.W. Burton, 2006. Genetic variability for phytic acid phosphorus and inorganic phosphorus in seeds of soybeans in maturity groups V, VI and VII. Crop Sci., 46: 67–71.
9. Bao, Y.M., and M. Choct, 2010. Dietary NSP nutrition and intestinal immune system for broiler chickens. World's Poult. Sci.J., 66: 511-517.
10. Mateos, G.G., E. Jiménez-Moreno, M.P. Serrano, and R. Lázaro. 2012. Poultry response to high levels of dietary fiber sources varying in physical and chemical characteristics. J. Appl. Poult. Res., 21: 156-174.
11. Hartini, S., and M. Massora, 2014. Supplemental insoluble non-starch polysaccharides affect performance and intestinal microflora of broilers. Proceeding of the XIVth European Poultry Conference, June 24th-27th 2014, Stavanger, Norway.
12. Svihus, B, 2011. The gizzard: function, influence of diet structure and effects on nutrient availability. World's Poult. Sci. J., 67: 207-224.
13. Hetland, H., M. Choct, and B. Svihus, 2004. Role of insoluble non-starch polysaccharides in poultry nutrition. World's Poult. Sci. J., 60: 415-422.
14. González-Alvarado, J.M., E. Jimenez-Moreno, and G.G. Mateos, 2007. Effect of type of cereal, heat processing of cereal, and inclusion of fiber in the diet on productive performance and digestive traits of broilers. Poult. Sci., 86: 1705-1715.
15. Jiménez-Moreno, E., A. de Coca-Sinova, J.M. González-Alvarado, and G.G. Mateos. 2016. Inclusion of insoluble fiber sources in mash or pellet diets for young broilers. 1. Effects on growth performance and water intake. Poult. Sci., 95: 41-52.
16. Hartini, S., M. Choct, G. Hinch, A. Kocher, and J.V. Nolan, 2002. Effects of light intensity during rearing, beak trimming and dietary fibre sources on mortality, egg

production and performance of ISA brown laying hens. J. Appl. Poult. Res., 11: 104-110.

17. Cowieson, A. J., and O. Adeola. 2005. Carbohydrases, protease, and phytase have an additive beneficial effect in nutritionally marginal diets for broiler chicks. Poult.Sci., 84:1860–1867.
18. Avila, E., J. Arce, C. Soto, F. Rosas, M. Ceccantini, and D.R. McIntyre, 2012. Evaluation of an enzyme complex containing nonstarch polysaccharide enzymes and phytase on the performance of broilers fed a sorghum and soybean meal diet. J. Appl. Poult. Res., 21 :279–286
19. Karimi, A., C. Coto, F. Mussini, S. Goodgame, c. Lu, J. Yuan, M.R. Bedford, and .W. Waldroup, 2013. Interaction between phytase and xylanase enzymes in male broiler chicks fed phosphorus-deficient diets from 1 to 18 days of age. Poult. Sci., 92: 1818-1823.
20. Choct, M., 1997. Feed non-starch polysaccharides: Chemical structures and nutritional significance. Feed Milling Int. (June): 13-26.
21. Valchev, I., V. Lasheva, Tz. Tzolov, and N. Josifov, 2009. Silica products from rice hulls. J. U. Chem. Technol. Metallurgy, 44 (3): 257-261.
22. Hartini, S. 2003. Dietary amelioration of cannibalism in laying hens. PhD thesis, University of New England, Australia.
23. SPSS 16.0, 2007. Command Syntax Reference. Chicago Ill: SPSS Inc.
24. Paiva, D., C. Walk., and A. McElroy, 2004. Dietary calcium, phosphorus, and phytase effects on bird performance, intestinal morphology, mineral digestibility, and bone ash during a natural necrotic enteritis episode. Poult. Sci., 93: 2752-2762.

25. Hetland, H., and B. Svihus, 2001. Effect of oat hulls on performance, gut capacity and feed passage time in broiler chickens. Br. Poult. Sci., 42: 354-361.
26. Svihus, B., and H. Hetland, 2001. Ileal starch digestibility in growing broiler chickens fed a wheat-based diet is improved by mash feeding, dilution with cellulose or whole wheat inclusion. Br. Poult. Sci., 42: 633-637.
27. Hartini, S., M. Choct, G. Hinch, and J.V. Nolan, 2003. The relationship between physico-chemical properties of fibre and their effects on the gut weight of chickens. Proc. Aust. Poult. Sci. Sym., 15: 135-138.
28. Meng, X., B.A. Slomiski, C.M. Nyachoti, L.D. Campbell, and W. Guenter, 2005. Degradation of cell wall polysaccharides by combinations of carbohydrases enzymes and their effect on nutrient utilization and broiler chicken performance. Poult. Sci., 83: 37-47.
29. Biehl, R.R., and D.H. Baker, 1997. Microbial phytase improves amino acid utilization in young chicksfed diets based on soyabean meal but not diets based on peanut meal. Poult. Sci., 76: 355-360.
30. SNI (STANDAR NASIONAL INDONESIA), 2008. Kumpulan SNI bidang pakan. Jakarta, Direktorat Budidaya Ternak Non Ruminansia, Direktorat Jenderal Peternakan, Departemen Pertanian.
31. Delezie, E., K. Bierman, L. Nollet, and I. Maertens, 2015. Impacts of calcium and phosphorus concentration, their ratio, and phytase supplementation level on growth performance, foot pad lesions, and hock burn of broiler chickens. J. Appl. Poult. Res., 24: 115-126.
32. Bedford, M.R., C.L. Walk, and H.V. Masey O'Neill, 2016. Assessing measurements in feed enzyme research: Phytase evaluations in broilers. J. Appl. Poult. Res., 25: 305-314.

33. Tamim, N.M., R. Angel., and M. Christman. 2004. Influence of dietary calcium and phytase on phytate phosphorus hydrolysis in broiler chickens. Poult. Sci., 83: 1358-1367.
34. Schöner, F.J., and P.P. Hoppe, 2002. The Effect of Phytase in Poultry Nutrition. In: Poultry Feedstuffs Supply, Composition and Nutritive Value, McNab, J.M. and K.N. Boorman (Eds). Poult. Sci. Sym. Series; vol. 26, pp: 363-373.
35. Savory, C.J, 1984. Regulation of food intake by Brown Leghorn cockerels in response to dietary dilution with kaolin. Br. Poult. Sci., 25: 253-258.
36. Newcombe, M., and J.D. Summers, 1985. Effect of increasing cellulose in diets fed as crumbles or mash on the food intake and weight gain of broiler and leghorn chicks. Br. Poult. Sci., 26: 35-42.
37. Rolls, B.A., A. Turvey, and M.E. Coates, 1978. The influence of the gut microflora and of dietary fiber on epithelial cell migration in the chick intestine. Br. J. Nut., 39: 91-98.
38. Bao, Y.M., L.F. Romero, and A.J. Cowieson, 2013. Functional patterns of exogenous enzymes in different feed ingredients. World's Poult. Sci. J., 69: 759-774.
39. Lynch, M.B., T. Sweeney, J.J. Callan, and J.V. O'Doherty, 2007. Effects of increasing the intake of dietary β-glucans by exchanging wheat for barley on nutrient digestibility, nitrogen excretion, intestinal microflora, volatile fatty acid concentration and manure ammonia emissions in finishing pigs. Animal, 1: 812–819.
40. Choct, M. and A. Kocher, 2000. Xylanases of different origins: "Effect on nutritive value of wheat in poultry. A Poultry Research Report. Armidale, NSW, Australia; University of New England
41. Campbell, G.L., and M.R. Bedford, 1992. Enzyme applications for monogastric feeds: A review. Can. J. Anim. Sci., 72: 449-466.

42. Choct, M., R.J. Hughes, J. Wang, M.R. Bedford, A.J. Morgan, and G. Annison, 1996. Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. Br. Poult. Sci., 37: 609-621.
43. Brenes, A., M. Smith, W. Guenter, and R.R. Marquardt, 1993. Use of enzymes in wheat- and barley-based broiler diets. Poult. Sci., 72: 1731-1739.
44. Jozefiak, D., A. Rutkowski, and S. A. Martin, 2004. Carbohydrate fermentation in the avian ceca: a review. Anim. Feed Sci. Technol., 113:1-15.
45. Raninen, K., J. Lappi, H. Mykkanen, and K. Poutanen, 2011. Dietary fiber type reflects physiological functionality: Comparison of grain fiber, inulin, and polydextrose. Nutr. Rev., 69: 9-21.
46. He, L.W., Q.X. Meng, D.Y. Li, Y.W. Zhang, and L.P. Ren. 2015. Influence of feeding alternative fiber sources on the gastrointestinal fermentation, digestive enzyme activities and mucosa morphology of growing Greylag geese. Poult. Sci., 94: 2464-2471.
47. Meynell, G.G., 1963. Antibacterial mechanisms of the mouse gut II. The role of pH and volatile fatty acids in the normal gut. Br. J. Exp. Pathol., 44: 209-219.
48. Jamroz, D., A. Wiliczkiewicz, and J. Skorupińska, 1992. The effect of diets containing different levels of structural substances on morphological changes in the intestinal walls and the digestibility of the crude fibre fractions in geese (Part III). J. Anim. Feed. Sci., 1: 37-50.

**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 |

**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 (%)** | $74.43 \pm 0.65^{ab}$ | $73.53 \pm 1.18^{a}$ | $75.02 \pm 1.15^{ab}$ | $76.49 \pm 0.40^{b}$ | $76.63 \pm 1.11^{b}$ | $76.52 \pm 0.43^{b}$ | $77.22 \pm 0.32^{b}$ | * |
| **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** | $1.12 \pm 0.07^{c}$ | $0.81 \pm 0.04^{a}$ | $1.01 \pm 0.06^{bc}$ | $0.88 \pm 0.09^{ab}$ | $0.77 \pm 0.03^{a}$ | $0.88 \pm 0.03^{ab}$ | $0.87 \pm 0.05^{ab}$ | ** |
| **Jejunum wt** | $2.80 \pm 0.19^{c}$ | $2.14 \pm 0.18^{b}$ | $2.49 \pm 0.20^{bc}$ | $2.19 \pm 0.13^{b}$ | $2.06 \pm 0.12^{ab}$ | $2.19 \pm 0.13^{b}$ | $1.61 \pm 0.17^{a}$ | ** |
| **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** | $0.506 \pm 0.10^{b}$ | $0.216 \pm 0.03^{a}$ | $0.308 \pm 0.06^{a}$ | $0.248 \pm 0.05^{a}$ | $0.214 \pm 0.04^{a}$ | $0.333 \pm 0.05^{ab}$ | $0.308 \pm 0.07^{a}$ | * |
| **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** | $53.1 \pm 16.55^{a}$ | $48.7 \pm 22.55^{a}$ | $81.6 \pm 32.95^{ab}$ | $103.9 \pm 34.31^{ab}$ | $151.3 \pm 9.92^{b}$ | $149.77 \pm 20.8^{b}$ | $146.56 \pm 52.8^{b}$ | ** |
| **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** | $7.8 \pm 2.49^{a}$ | $14.4 \pm 2.13^{ab}$ | $14.7 \pm 5.72^{ab}$ | $21.3 \pm 2.06^{bc}$ | $37.3 \pm 0,81^{d}$ | $29.0 \pm 2.53^{cd}$ | $31.1 \pm 3.16^{cd}$ | ** |

*(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.

Example: How to present the key findings of the study with reference to the Table

Table 1. Combined main effects of light sources and light-intensity on blood physiological selected variables of broilers grown to heavy weights[1]

| Variables[2] | Light Sources[3] | | | | P-value | Light Intensity[4] (lx) | | P-value | Pooled SEM |
|---|---|---|---|---|---|---|---|---|---|
| | ICD | CFL | Neutral-LED | PSF-LED | | 5 | 20 | | |
| BW (kg) | 4.080$^{b}$ | 4.119$^{ab}$ | 4.114$^{ab}$ | 4.226$^{a}$ | 0.011 | 4.162 | 4.123 | 0.213 | 0.014 |
| pH | 7.39$^{a}$ | 7.38$^{ab}$ | 7.37$^{b}$ | 7.39$^{a}$ | 0.023 | 7.38 | 7.37 | 0.065 | 0.003 |
| $pCO_2$ (mmHg) | 49.37$^{b}$ | 49.51$^{b}$ | 50.79$^{a}$ | 50.24$^{b}$ | 0.026 | 49.42 | 50.01 | 0.494 | 0.698 |
| $pO_2$ (mmHg) | 41.85$^{a}$ | 42.03$^{a}$ | 40.10$^{b}$ | 40.38$^{ab}$ | 0.035 | 41.19 | 41.19 | 0.792 | 0.520 |
| $HCO_3^-$ (mmHg) | 28.10 | 27.96 | 27.53 | 27.83 | 0.059 | 27.88 | 27.83 | 0.722 | 0.194 |
| $SaO_2$ % | 73.15$^{a}$ | 72.98$^{ab}$ | 70.24$^{b}$ | 70.94$^{ab}$ | 0.041 | 72.25 | 71.9 | 0.601 | 0.473 |
| Hct (%) | 24.70 | 24.67 | 24.92 | 24.72 | 0.728 | 24.89 | 24.62 | 0.113 | 0.170 |
| Hb (g/dL) | 7.93 | 7.92 | 8.00 | 7.94 | 0.738 | 7.99 | 7.91 | 0.120 | 0.120 |
| McHc (g/dL) | 32.06$^{b}$ | 32.07$^{b}$ | 32.11$^{ab}$ | 32.13$^{a}$ | 0.036 | 32.11 | 32.10 | 0.361 | 0.010 |
| $Ca^{2+}$ (mEq/L) | 3.46 | 3.48 | 3.45 | 3.45 | 0.896 | 3.46 | 3.46 | 0.986 | 0.094 |
| $Na^+$ (mEq/L) | 148.36$^{b}$ | 149.15$^{a}$ | 148.49$^{b}$ | 148.28$^{b}$ | 0.001 | 148.65 | 148.49 | 0.274 | 0.144 |
| $K^+$ (mEq/L) | 4.66$^{b}$ | 4.67$^{b}$ | 4.91$^{a}$ | 4.82$^{ab}$ | 0.035 | 4.89 | 4.84 | 0.386 | 0.054 |
| $Cl^-$ (mEq/L) | 104.92$^{ab}$ | 105.51$^{a}$ | 105.13$^{ab}$ | 104.81$^{b}$ | 0.024 | 105.2 | 105.0 | 0.444 | 0.172 |
| Angap (mmol/L) | 20.81 | 20.68 | 20.82 | 20.47 | 0.529 | 20.56 | 20.57 | 0.877 | 0.275 |
| GLU (mg/dL) | 233.99 | 233.97 | 231.49 | 230.30 | 0.064 | 232.93 | 232.0 | 0.384 | 0.796 |
| Osmo (mmol/kg) | 309.71$^{b}$ | 311.29$^{a}$ | 309.84$^{b}$ | 309.37$^{b}$ | 0.001 | 310.23 | 309.88 | 0.204 | 0.222 |
| CORT (pg/mL) | 1,719.25 | 1,849.18 | 2,026.93 | 1,938.4 | 0.182 | 1,926.0 | 1,839.9 | 0.403 | 72.650 |
| $T_3$ (ng/mL) | 3.04 | 3.08 | 3.00 | 3.00 | 0.633 | 3.01 | 3.06 | 0.338 | 0.053 |
| $T_4$ (µg/dl) | 2.07 | 2.08 | 2.02 | 2.07 | 0.811 | 2.08 | 2.04 | 0.369 | 0.048 |

[ab]Means within a row and treatment that lack common superscripts differ significantly ($P \leq 0.05$)

[1]Values are least squares of 8 replicate rooms with 60 birds per room.

[2]BW= Body Weight; $pCO_2$ = partial pressure of $CO_2$; $pO_2$ = partial pressure of $O_2$; $HCO_3^-$= Bicarbonate; $SaO_2$ = saturated $O_2$; Hct = haematocrit; Hb = hemoglobin; McHc = mean corpuscular hemoglobin concentration; GLU = glucose; Osmo = osmolality; angap = anion gap; CORT = Corticosterone; $T_3$ = triiodothyronine; $T_4$ = thyroxine.

[3]Light Sources: ICD = Incandescent light (Standard), CFL = Compact Fluorescent light, Neutral-LED = Light Emitting Diode, Cool-PSF-LED = Poultry Specific Filtered LED.

[4]Light intensity: 1 = 5 lx, 2 = 20 lx

Table 1 shows the combined main effects of light sources and light intensity on major selected blood physiological variables. In comparison with ICD light, birds in the CFL group had higher $Na^+$ ($P < 0.001$) and Osmo ($P < 0.001$); birds reared under Neutral-LED light sources had lower pH ($P < 0.023$), $pO_2$ ($P < 0.035$), $SaO_2$ ($P < 0.041$), and higher $pCO_2$ ($P < 0.026$), $K^+$ ($P < 0.035$), while birds reared under Cool-PSF-LED light sources had higher McHc ($P < 0.036$) and BW ($P < 0.011$). There was no effect of light intensity and no difference between 5 and 20 lx on all examined variables. In addition, no main effects of light sources, light intensity, or their interaction on $HCO_3^-$, Hct, Hb, $Ca^{2+}$, angap, GLU, CORT, $T_3$ and $T_4$ were observed.