

Performances of KUB Chickens Fed Diets with Different Nutrient Densities and BS4 Enzyme Supplementation

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ABSTRACT

An experiment was conducted to study the effect of dietary enzyme supplementation (E) on the performance of KUB chickens fed different nutrient densities (ND). Diets with three densities: 70.7 g crude protein/Mcal or high (H), 66.1 g crude protein/Mcal or medium (M), and 59.3 g crude protein/Mcal or low (L), were formulated and supplemented with or without enzyme. Diets were given in four feeding programs, i.e., H-M-L, H-M-M, M-M-L, and M-L-L during the starter (1–28 d), grower (29–56 d), and finisher (57–84 d) periods, respectively. Each treatment was replicated five times. Bodyweight gain (BWG), feed intake, and FCR were measured each period. At the end of the trial, carcass yield and internal organs were measured. Results of the experiment (1–84 d period) showed that the feed intake was significantly affected by ND. Chickens fed the H-M-L diets have the highest feed intake, while the lowest was found in chickens fed M-M-L diets. A significant interaction was found in the FCR. The best FCR was found in chickens fed the H-M-M diets without enzymes, but the best FCR was found on the M-M-L diets with enzymes. Livability, carcass yield, abdominal fat, liver, proventriculus, and gizzard weights were not affected by the treatments. The jejunum sizes of chickens were significantly longer when fed the low-density diet than those fed the higher nutrient density diet. The ileum sizes of chickens were significantly shorter than chickens fed the diet without enzymes. The highest income over feed cost was achieved when chickens were fed the M-M-L diets supplemented with enzymes. It is concluded that the best performance of growing KUB chickens was obtained when fed M-M-L diets supplemented with BS4 enzymes (30 Units of saccharification/kg diet) and when fed H-M-M diets without enzyme supplementation.

Keywords: KUB chickens; growing performance; nutrient density; enzyme

INTRODUCTION

KUB chicken is a new breed chicken produced by a selection of local or kampung chickens. The chicken breed has been introduced to farmers in almost all provinces in Indonesia. Although the productive performances of the KUB chickens are not as superior as the improved breed chickens, the demand for this chicken is increasing due to the egg taste and the meat quality matches the preferential of the Indonesian majority. The chickens also have better productivity (eggs and meat) than the ordinary local chickens (Saragih *et al.*, 2019).

Farmers practiced various programs in feeding KUB chickens at present. Based on research, Hidayat *et al.* (2017) suggested the optimum ME: protein ratio in the diet for KUB chickens for the growing period (0–10 weeks) was 15.85, i.e., 2950 kcal ME/kg and 18.5% crude protein. Increasing or decreasing the ME: protein ratios resulted in poorer feed conversion ratios. Some studies have been conducted to determine the nutrient requirement of KUB chickens by mixing the commercial broiler diets with corn and rice bran (Mayora *et al.*, 2018; Sari *et al.*, 2017; Irawan *et al.*, 2018). These studies showed that the best performance of the KUB chickens was achieved by feeding a diet with 3089 kcal ME and 18.9% protein for the starter period (Mayora *et al.*, 2018), 12.8%–15.6% for the growing period (Sari *et al.*, 2017), and 8.4%–14.1% for the finishing period (Irawan *et al.*, 2018). On the other hand, the Indonesian Standard Bureau (BSN, 2013a; 2013b) has published two standards of feed for growing local chickens, i.e., starter diet for chickens age 0 to 4 weeks and grower diet for chickens age >4 to 20 weeks. Since KUB chickens grow faster than ordinary local chickens, the standard nutrient requirement recommended for local chickens may not be suitable for the KUB chickens. Therefore, it is necessary to identify the optimum nutrient requirement of the KUB chickens.

Based on the above information, there is a variation in the recommendation of the levels of nutrients requirement and feeding program for growing local chickens. The information on nutrients requirements is an essential key used in formulating diets to reach an optimum diet with a low cost. Formulating a high-density diet is usually considered a high-quality feed and produces

better performance when fed to chickens (Lamot *et al.*, 2019). Still, it will make the feed price expensive while formulating a diet with lower nutrients than the chickens required, which will eventually impair the performance of the chickens.

Enzyme supplementation in the feed may improve the quality of the low-density diet by increasing the availability of nutrients in the diet. A new enzyme named BS4 enzyme has been investigated in our laboratory. The enzyme consists of β -mannanase, cellulase, β -mannosidase, and α -galactosidase (Sinurat *et al.*, 2014). Supplementation of the enzyme in the diet is expected to increase nutrients digestibility of the feed. This condition implies that lower nutrients levels could be provided at a lower price to achieve similar performances. Nutrients requirement for local chickens is available, but there is no standard nutrient requirement for the selected local chickens (KUB). Therefore, this study is expected to find an appropriate nutrient density required by KUB chickens to support their growth performances during starter, grower, and finisher periods, especially if the enzyme is supplemented in the diet.

MATERIALS AND METHODS

All procedures in this experiment regarding the use of live animals were done according to the approval of The Animal Welfare Committee at the Indonesian Agency for Agricultural Research and Development: Balitbangtan/Balitnak/A/06/2020.

Animals and Management

A total of 440 day-old unsexed- KUB chicks were obtained from the hatchery of Balai Penelitian Ternak – Ciawi. The chicks were distributed randomly into 40 pens with 11 birds/pen. Each pen measuring 300 x 150 cm was covered with rice hull as deep litter and equipped with a light bulb as a heater during the starter period (1–28 d), a feeder, and a drinker. The chicks were reared and fed dietary treatments for starter (1 to 28 d), grower (29 to 56 d), and finisher (57 to 84 d) periods. The starter feed was in crumble form, while the grower and finisher feeds were made in pelleted form. The experimental chickens had free access to feed and drinking water at all times.

Experimental Diets

The experiment was divided into three stages of growth, i.e., starter period, grower period, and finisher period. The detailed design of the treatment for each stage of growth is described in Table 1.

Starter period. Four experimental diets, i.e., factorial of 2 nutrient densities x 2 enzyme levels were tested for the starter period. Two different densities of diets, i.e., High (H) and Medium (M), were formulated. The nutrient content of the H density diet was formulated similar to the recommendation of the Indonesian Nasional Standard (SNI) for the pre-starter commercial layer chickens with the density of 70.7 g crude protein/Mcal

(BSN, 2016a). The M density was formulated similar to the SNI recommendation for grower commercial layer chickens with the density of 66.1 g crude protein/Mcal (BSN, 2016b). The ingredients composition and nutrient contents of the diets are shown in Table 2. Each diet was either supplemented with or without BS4 enzyme (30 Units of saccharification/kg diet). The dose has been reported to be effective in increasing nutrients digestibility of diets in laying hens (Sinurat *et al.*, 2014) and broilers (Sinurat *et al.*, 2015).

Each diet was fed to 10 pens of KUB chickens with 11 birds/pen from 1 d to 28 d old. The birds' performances, i.e., feed intake, body weight gain, and liveability, were measured.

Grower period. Three diets with different densities, i.e., High (H), Medium (M), and Low (L), were formulated. The nutrient content of the H and M densities was similar to those in the starter period. The low-density diet was formulated similar to the recommendation of the SNI for grower diets for commercial layer chickens with the density of 59.3 g crude protein/Mca (BSN, 2016c). Six dietary treatments, i.e., factorial of 3 densities (H-M, M-M, and M-L) x 2 enzyme levels (without and with enzyme supplementation) were tested. Birds fed with H-M were fed with the H density diet during the starter period, followed by M density during the grower period. Birds fed with M-M were fed with the M density diet during the starter and grower periods. Birds fed with M-L were fed with the M density diet during the starter period, followed by L density during the grower period. Each diet was either supplemented with or without BS4 enzyme, similar to the starter period.

The dietary treatments were fed to KUB chickens with 11 birds/pen from 29 d to 56 d old. The H-M treatment was given to 10 pens, while the M-M and M-L treatments were given to 5 pens, respectively. The performances of the birds, i.e., feed intake, body weight gain, and liveability, were measured.

Finisher period. During the finisher period, the factorial of 4 diet densities x 2 enzyme levels were tested. The nutrient densities consist of H-M-L, H-M-M, M-M-L, and M-L-L. The H-M-L was a diet with H and M densi-

Table 1. The arrangement of enzyme supplementation and nutrient density during the starter, grower, and finisher period

No	Enzyme supplemented	Starter	Grower	Finisher
1	Without	High	Medium	Low
2	Without	High	Medium	Medium
3	Without	Medium	Medium	Low
4	Without	Medium	Low	Low
5	With	High	Medium	Low
6	With	High	Medium	Medium
7	With	Medium	Medium	Low
8	With	Medium	Low	Low

Note: The high, medium, and low density diet was formulated with a density of 70.7 g crude protein/Mcal, 66.1 g crude protein/Mcal, and 59.3 g crude protein/Mcal, respectively.

Table 2. The composition and nutrient contents of the experimental diets

Feed ingredients	Nutrient density		
	Low	Medium	High
Maize, %	54.3	56.1	50.6
Wheat polard, %	15.00	10	10
Palm-kernel cake, %	8.50	7	5
Soybean meal, %	17.19	20.77	26.52
Vegetable oil, %	0.500	0.24	2.03
Meat and bone meal, %	1.00	4.35	4.3
Limestone, %	1.880	0.89	0.86
Lysine, %	0.020	0	0
Mono Calcium Phosphate, %	1.00	0	0
Methionine, %	0.100	0.11	0.19
Salt, %	0.200	0.2	0.2
Vitamin-mineral premixes, %	0.31	0.31	0.31
Total, %	100	100	100
Price, (IDR/kg)	5153	5407	5739
Nutrient composition*:			
Dry matter, %	87.9 (88.2)	87.6 (87.5)	88.9 (86.0)
Crude fibre, %	4.86 (4.56)	4.36 (4.36)	4.33 (3.55)
Crude protein (CP), %	16.0 (16.8)	18.5 (19.3)	20.5 (21.13)
Metabolizable energy, kcal/kg	2700	2800	2900
Nutrient density, g CP/Mcal ME	59.3	66.1	70.7
Lysine, %	0.739 (0.82)	0.900 (0.96)	1.000 (1.14)
Methionine, %	0.355 (0.24)	0.428 (0.24)	0.500 (0.25)
Methionine + Cystine, %	0.600 (0.40)	0.700 (0.42)	0.800 (0.43)
Threonine, %	0.583 (0.59)	0.674 (0.66)	0.750 (0.77)
Tryptophan, %	0.190	0.210	0.236
Calcium (Ca), %	1.10 (0.78)	0.90 (0.74)	0.90 (1.38)
Total Phosphorous (P), %	0.68 (0.85)	0.63 (0.88)	0.64 (0.89)
Available Phosphorous, %	0.40	0.35	0.35

Note: Numbers in brackets () are results of laboratory analyses; MCal= 1000 kcal. The high, medium, and low density diet was formulated with a density of 70.7 g CP/Mcal, 66.1 g CP/Mcal, and 59.3 g CP/Mcal, respectively.

ties given for the starter and grower period, followed by L density for the finisher period. The H-M-M was a diet with H and M densities given for the starter and grower period, followed by the M density diet for the finisher period. The M-M-L was a diet with M density given for the starter and grower period, followed by L density for the finisher period. Each diet was either supplemented with or without BS4 enzyme, similar to the starter period.

The dietary treatments were fed to KUB chickens from 57 d to 84 d old with 5 replicates per treatment. The birds' performances, i.e., feed intake, body weight gain, and liveability, were measured.

Variables and Data Analyses

The performances of the birds (feed intake, body weight gain, and liveability) were measured for starter (1-28 d), grower (29-56 d), finisher period (57-84 d), and the whole period (1-84 d of age). At the end of the trial, one male and one female chicken were taken from each pen and slaughtered to evaluate the effects of the treatments on the carcass yield, abdominal fat levels, and liver weight. Chickens were slaughtered by draining the blood from the jugular vein with a sharp knife. The

weights of the proventriculus and gizzard, the length of the duodenum, jejunum, and ileum, were also measured. Data of male and female chickens were pooled and not presented separately.

At the end of the feeding trial, one cockerel from each pen was removed to individual wire cages to measure the dry matter digestibility and metabolizable energy of the feed in a total collection method. Each day, the birds were fed 2 hours with 110 g/bird/d of the test diet for 3 days, followed by fasting for 24 h. The following day (4th day), the experimental birds were fed with 110 g/bird/d of test diet for 2 h, and a tray was placed underneath the cage. The feed intake was measured, and the excreta were collected and dried in an oven (70 °C) every day, and this procedure was repeated for 4 consecutive days. Dried excreta were pooled and weighed. The dry matter and gross energy were measured in a laboratory. The AME was calculated following the formula:

$$\text{AME (kcal/g)} = ((\text{Gef} \times X) - (\text{Gexc} - \text{Gend})) / X$$

where Gef is the gross energy of the feed (kcal/g); Gexc is the total gross energy voided in the excreta (kcal); X is the weight of the feed consumed (g). The dry matter digestibility (%) was calculated with a similar formula.

AME intake was calculated by multiplying the feed intake (1-84 d) with the AME of the feed and the AME efficiency ratio (AMEER) was calculated by dividing the total AME intake with the body weight gain during the trial.

The income over feed cost (IOFC) was calculated by subtracting the selling value of the chickens (the price of chicken/kg x weight of chicken) with the cost of feed consumed (the price of feed x total amount of feed consumed from 1 to 84 d). The price of feed without enzyme was IDR 5739/kg, IDR 5407/kg, and IDR 5153/kg, for the high-, medium- and low densities diets, respectively. Feed supplemented with enzyme was IDR 50/kg more expensive than feed without enzyme, and the selling price of the chicken was IDR 35000/kg.

All data were subject to analyses of variance (ANOVA) in a factorial design. Data on performance during the starter period (1- 28 d) were analyzed in 2 (nutrient density) x 2 (enzyme supplement) factorial design, and data on performance during grower (29-56 d) were analyzed in 3 (nutrient density) x 2 (enzyme supplement) factorial design. Data on performance during the finisher period (57-84 d), and performance for the whole period (1-84 d), dry matter digestibility, AME and AME Efficiency Ratio (AMEER), carcass yield, and gastrointestinal tract size was analyzed in 4 (nutrient density) x 2 (enzyme supplement) factorial design. Differences between treatments were calculated by Duncan's multiple range tests if the ANOVA was significant ($p < 0.05$).

RESULTS

Growth Performances of KUB Chickens during Starter Period

The performances of KUB chickens during the starter period (1-28 d) are presented in Table 3. Body weights of experimental chickens at one day old in the beginning of treatment were similar. Body weight gains of the experimental chickens during the starter period were similar. There were no significant effects of nutrient densities, enzyme supplementation, and interactions between nutrient densities (ND) and enzyme supplementation on body weight gain (BWG).

Feed intake was not significantly affected by the nutrient densities or enzyme supplementation. However, there was a significant ($p < 0.05$) interaction between nutrient densities and enzyme supplementation on feed intakes of experimental KUB chicks. Supplementation of the BS4 enzyme into the high-density diet did not affect the feed intake but significantly ($p < 0.05$) reduced the feed intake of chicks fed the medium-density diet.

The nutrient density of the diet did not significantly affect the FCR of the experimental chicks. However, enzyme supplementation in the diet during the starter period significantly ($p < 0.05$) affected the FCR of experimental chicks. In addition, there was an interaction effect of nutrient density and enzyme supplementation in the diet on FCR. Supplementation of enzymes into the high-density diet did not significantly affect the FCR, while supplementation into the medium density diet improved the FCR significantly ($p < 0.05$). The best FCR was achieved when the KUB chickens were fed medium nutrient density diet supplemented with BS4 enzyme. During the starter period, the livability of the KUB chickens was not significantly affected by the treatments with nutrient density and enzyme supplementation in the feed.

Growth Performance of KUB Chickens during Grower Period

The performances of the KUB chickens during the grower period (29 to 56 days old) are presented in Table 4. Nutrient density significantly affected ($p < 0.05$) bodyweight gain. Enzyme supplementation in the ration did not affect the body weight gain of experimental chickens during the grower period. However, the body weight gain was significantly ($p < 0.05$) affected by the interaction between nutrient density and enzyme supplementation. In the chickens fed ration without enzyme supplementation during the grower period, the heaviest body weight gain (452 g) was reached by chickens fed the H-M density diet. This BWG was not significantly different from chickens fed the M-M density diet (402 g) but significantly different ($p < 0.05$) from chickens fed the M-L diet (395 g). However, when the enzyme was supplemented in the diet, the heaviest BWG was

Table 3. Performances of KUB chickens during starter period (1-28 day) as were affected by nutrient densities and enzyme supplementation

Nutrient densities	Enzyme	Day old BW (g/bird)	BWG (g/bird)	Feed intake (g/bird)	FCR	Livability (%)
High	Without	31.7±1.7	255.4±19.3	617.4±81.4 ^a	2.41±0.20 ^a	99.1±2.9
Medium	Without	30.3±2.0	248.4±17.0	599.4±43.1 ^{ab}	2.42±0.23 ^a	96.4±8.8
High	With	29.9±2.3	255.6±11.0	592.0±27.2 ^{ab}	2.32±0.08 ^{ab}	99.1±2.9
Medium	With	31.1±2.3	256.4± 8.9	559.5± 8.6 ^b	2.19±0.10 ^b	100.0±0.0
<i>p-Value</i>						
Nutrient density (ND)		0.96	0.71	0.41	0.83	0.55
Enzyme (E)		0.56	0.3	0.12	0.03	0.08
ND x E		0.09	0.43	0.01	0.03	0.55

Note: Means in the same column with different superscripts differ significantly ($p < 0.05$). BW= body weight; BWG= body-weight gain; FCR= Feed-conversion ratio.

Table 4. Performances of KUB chickens during grower period (age 29-56 days) as were affected by nutrient densities and enzyme supplementation

Nutrient densities	Enzyme	BWG (g/bird)	Feed intake (g/bird)	FCR	Livability (%)
High-Medium	Without	452 ±27 ^a	1780 ± 80 ^{ab}	3.93±0.29 ^b	100.0± 0.0
Medium-Medium	Without	402 ±46 ^a	1657 ±154 ^c	4.20±0.81 ^{ab}	96.4±13.8
Medium-Low	Without	395 ±38 ^b	1622 ± 77 ^{cd}	4.13±0.35 ^b	98.2± 4.1
High-Medium	With	441±35 ^a	1704 ±136 ^{bc}	3.87±0.29 ^b	99.1± 2.9
Medium-Medium	With	456 ±24 ^a	1594 ± 94 ^d	3.50±0.20 ^c	100.0± 0.0
Medium-Low	With	406±34 ^b	1857 ± 83 ^a	4.47±0.33 ^a	98.2± 4.1
<i>p-Value</i>					
Nutrient density (ND)		0.01	0.03	0.02	0.72
Enzyme (E)		0.31	0.39	0.30	0.61
ND x E		0.05	0.002	0.02	0.00

Note: Means in the same column with different superscripts differ significantly ($p < 0.05$). BWG= body-weight gain; FCR= Feed-conversion ratio.

achieved by chickens fed with the M-M density diet (456 g). In the chickens fed diet supplemented with BS4 enzyme, chickens fed with M-M density diet had similar BWG with chickens fed the H-M density diet (441 g) but significantly higher ($p < 0.05$) than chickens fed the M-L density diet (406 g). The highest BWG was found in chickens fed ration with M-M nutrient densities supplemented with enzyme (456 g/bird), followed by the chickens fed ration with H-M nutrient densities without enzyme supplementation (452g/bird) and chickens fed ration with H-M nutrient densities with enzyme supplementation (441 g/bird).

Feed intake of birds during the grower period was significantly ($p < 0.01$) affected by the nutrient density. There was no significant effect of enzyme supplementation in the diet on the feed intakes of experimental birds. However, there was a significant interaction effect between nutrient density and enzyme supplementation on feed intake. When feed was not supplemented with enzyme, the lowest feed intake was found in chickens fed the M-L density diet (1622 g/bird). Feed intake was significantly ($p < 0.05$) higher in birds fed the H-M density diet (1780 g/bird) than those fed M-M and M-L densities diets. However, when the feed was supplemented with enzyme, the lowest feed intake was found in chickens fed the M-M density diet (1594 g/bird). The highest feed intake was found in chickens fed M-L densities diets (1857 g/bird) that was significantly ($p < 0.05$) higher compared to chickens fed H-M (1704 g/bird). In general, the highest feed intake was found in chickens fed diet with M-L nutrient densities supplemented with enzyme (1857 g/bird). The second highest feed intake was found in chickens fed the diet with H-M nutrient densities without enzyme supplementation (1780 g/bird), and the third was found in chickens fed the diet with H-M nutrient densities with enzyme supplementation (1704 g/bird).

During the grower period, the FCR of experimental chickens was significantly affected by the nutrient density. However, the enzyme supplementation did not affect the FCR. There was a significant interaction effect of nutrient density and enzyme supplementation in the diet on FCR during the grower period ($p < 0.05$). The best FCR (3.50) was found in the chickens fed the M-M

nutrient density diet with enzyme supplementation. In general, the livability of the chickens during the grower period was good and was not significantly affected by treatments.

Growth Performance of KUB Chickens during Finisher Period

The growth performances of the KUB chickens during the finisher period are presented in Table 5. Nutrient density and enzyme supplementation in the diet did not significantly affect the body weight gain of experimental chickens during the finisher period. In addition, there was no interaction effect between nutrient density and enzyme supplementation on the bodyweight of experimental chickens.

During the finisher period, nutrient density significantly affected experimental chickens' feed intake ($p < 0.01$). However, enzyme supplementation did not affect the feed intake of the experimental chickens. In addition, there was no interaction effect between nutrient density and enzyme supplementation. The highest feed intake (2556 g/bird) was found in chickens fed H-M-L nutrient density diet without enzyme supplementation, followed by chickens fed M-L-L nutrient density diet with enzyme supplementation (2527 g/bird), chickens fed M-L-L nutrient density without enzyme supplementation (2507 g/bird) that were significantly higher ($p < 0.05$) than those chickens fed H-M-M nutrient density diet supplemented with enzyme (2346 g/bird), and followed by chickens fed M-M-L nutrient density diet without enzyme supplementation (2324 g/bird), followed by chickens fed M-M-L nutrient density diet with enzyme supplementation (2313 g/bird), and chickens fed H-M-M nutrient density diet without enzyme supplementation (2278 g/bird).

During the finisher period, the FCR of experimental chickens was similar and was not affected by nutrient density and enzyme supplementation. In addition, there were no significant interaction effects of nutrient density and enzyme supplementation on the FCR of experimental chickens during the finisher period. During the finisher period, the FCR in the experimental KUB chickens ranged from the lowest level (4.07) in

M-M-L with enzyme supplementation to the highest level (4.92) in H-M-L nutrient density without enzyme supplementation.

The livabilities of experimental KUB chickens during the finisher period were good and were not significantly affected by the treatments of nutrient density and enzyme supplementation in the diet.

Growth Performance of KUB Chickens from 1-84 Days

The growth performances of the KUB chickens from 1 to 84 days of age are presented in Table 6. The body weight gain and the livability of the KUB chickens from 1 to 84 d old were not significantly affected by the nutrient density, enzyme supplementation. There was no interaction effect between nutrient density and enzyme supplementation on the growth performances of KUB chickens from 1 to 84 d old.

The feed intake was significantly ($p<0.05$) affected by the nutrient density of the ration. However, the feed intake was not significantly affected by the enzyme supplementation. In addition, there was no interaction effect of nutrient density and enzyme supplementation on the feed intake during 84 days of raising KUB chickens. The highest feed intake (4956 g/bird) was found in

birds fed the high-density starter diet followed by medium-density grower diet and the low-density finisher diet (H-M-L) and was significantly different from those fed medium-density starter diet followed by medium-density grower diet and the low-density finisher diet (M-M-L).

The feed conversion ratio of experimental KUB chickens was not affected by the nutrient density and enzyme supplementation. However, there were significant interaction effects of nutrient density and enzyme supplementation in the diet on the FCR of experimental KUB chickens during 84 days of rearing ($p<0.05$). When the feed was not supplemented with the enzyme, the best FCR (3.77) was found by feeding chickens with the high-density starter diet followed by the medium-density grower and medium-density finisher diets (H-M-M). However, when feed was supplemented with the enzyme, the best FCR (3.48) was found by feeding the medium-density starter diet followed by the medium-density grower diet and the low-density finisher diet (M-M-L). The livability of all experimental KUB chickens during 84 days of rearing was good and was not affected by the nutrient density and enzyme supplementation.

Table 5. Performances of KUB chickens during grower period (age 57-84 days) as were affected by nutrient densities and enzyme supplementation

Nutrient densities	Enzyme	BWG (g/bird)	Feed intake (g/bird)	FCR	Livability (%)
High-Medium-Low	Without	526± 60	2556±132 ^a	4.92±0.69	96.4±5.0
High-Medium-Medium	Without	508± 78	2278± 43 ^b	4.56±0.73	96.4±4.5
Medium-Medium-Low	Without	516± 72	2324±297 ^b	4.53±0.40	96.4±5.0
Medium-Low-Low	Without	575±104	2507± 80 ^a	4.49±0.90	96.4±8.2
High-Medium-Low	With	558± 47	2413±132 ^a	4.35±0.39	98.2±4.1
High-Medium-Medium	With	536± 68	2346± 84 ^b	4.44±0.60	98.2±4.1
Medium-Medium-Low	With	571± 41	2313±152 ^b	4.07±0.48	94.5±5.0
Medium-Low-Low	With	563± 26	2527± 57 ^a	4.58±0.19	100.0±0.0
<i>p-Value</i>					
Nutrient density (ND)		0.46	0.003	0.64	0.67
Enzyme (E)		0.22	0.72	0.16	0.39
ND x E		0.71	0.41	0.57	0.67

Note: Means in the same column with different superscripts differ significantly ($p<0.05$). BWG= body-weight gain; FCR= Feed-conversion ratio.

Table 6. Performances of KUB chickens from 1-84 day as were affected by nutrient densities and enzyme supplementation

Nutrient densities	Enzyme	BWG (g/bird)	Feed intake (g/bird)	FCR	Livability (%)
High-Medium-Low	Without	1248±116	4956±229 ^a	4.07±0.40 ^a	96± 5
High-Medium-Medium	Without	1225± 78	4603± 54 ^{bc}	3.77±0.28 ^{ab}	96± 5
Medium-Medium-Low	Without	1159± 77	4612±437 ^c	3.99±0.35 ^a	93±12
Medium-Low-Low	Without	1225± 90	4696±172 ^{ab}	3.85±0.29 ^{ab}	95± 5
High-Medium-Low	With	1253± 54	4730±300 ^a	3.78±0.19 ^{ab}	98± 4
High-Medium-Medium	With	1234± 68	4619±101 ^{bc}	3.75±0.27 ^{ab}	98± 4
Medium-Medium-Low	With	1285± 31	4462±243 ^c	3.48±0.24 ^b	95± 5
Medium-Low-Low	With	1246± 63	4947± 88 ^{ab}	4.05±0.15 ^a	98± 4
<i>p-Value</i>					
Nutrient density (ND)		0.86	0.01	0.23	0.49
Enzyme (E)		0.10	0.72	0.09	0.24
ND x E		0.25	0.13	0.04	0.98

Note: Means in the same column with different superscripts differ significantly ($p<0.05$). BWG= body-weight gain; FCR= Feed-conversion ratio.

Dry Matter Digestibility, AME and AME Efficiency Ratio (AMEER)

Dry matter (DM) digestibility and metabolizable energy (AME) of the diets are presented in Table 7. The DM digestibility and the AME were not significantly affected by the interaction between ND and E. However, the DM digestibility and the AME of the diet were significantly ($p < 0.01$) affected by nutrient density but not by the enzyme supplementation. High nutrient density has the lowest DM digestibility, and significantly ($p < 0.05$) lower than the DM digestibility of the low- and medium-density diets. The determined AME values of the diets have a similar trend but were higher than the calculated values used in the diet formulation. The determined AME values of low-, medium-, and high-density diets were 2883, 2925, and 3009 Kcal/kg, respectively.

The total AME intake and AME efficiency ratio (AMEER) during the experimental period (1-84 d) are presented in Table 8. The AME intake was not significantly affected by the interaction between ND and

E. However, it was significantly ($p < 0.05$) affected by nutrient density but not by enzyme supplementation. Chickens fed the H-M-L diets consumed the highest dietary AME (14,199 Mcal/bird), and chickens fed the M-M-L diets consumed the lowest dietary AME (13,237 Mcal/bird). The AMEER was not significantly affected by the interaction between ND and E. However, the AMEER was significantly ($p < 0.05$) affected by enzyme supplementation but not by nutrient density. Chickens fed with a diet supplemented with enzyme (10.58 Mcal/g BWG) were more efficient in converting dietary energy to body weight than those fed diets without enzyme supplementation (11.26 Mcal/g BWG).

Carcass Yield and Gastrointestinal Tract Size

Data on the carcass yield, abdominal fat weight, and liver weight of KUB chickens are presented in Table 9. The results showed that the percentage of carcass yield, abdominal fat level, and relative liver weight were

Table 7. Dry matter digestibility and apparent metabolizable energy of feed with different nutrient densities and enzyme supplementation

	Dry matter digestibility (%)	AME (kcal/kg)
Nutrient density:		
Low	70.38±1.43 ^a	2883±59 ^b
Medium	70.23±1.84 ^a	2925±73 ^b
High	67.14±1.67 ^b	3009±63 ^a
Enzyme supplementation:		
With	70.0±2.54	2937±89
Without	69.8±1.88	2942±79
p-values		
Enzyme supplementation (E)	0.73	0.83
Nutrient density (D)	0.00	0.00
E x D	0.79	0.79

Note: Means in the same column and factor with different superscripts differ significantly ($p < 0.05$). AME= apparent metabolizable energy.

Table 8. Total AME intake (Mcal/bird) and AME efficiency ratio of KUB chickens fed with different nutrient densities and enzyme supplementation

	AME intake (Mcal/bird)	AMEER (Mcal/g BWG)
Nutrient density:		
High-Medium-Low	14199±810 ^a	11.22±0.93
High-Medium-Medium	13654±300 ^{ab}	10.87±0.77
Medium-Medium-Low	13237±928 ^b	10.36±1.61
Medium-Low-Low	14055±401 ^a	11.22±0.62
Enzyme supplementation:		
With	13899±781	11.26±0.90 ^a
Without	13673±703	10.58±1.15 ^b
p-values		
Enzyme supplementation (E)	0.28	0.029
Nutrient density (D)	0.01	0.148
E x D	0.243	0.053

Note: Means in the same column and factor with different superscripts differ significantly ($p < 0.05$). AME= apparent metabolizable energy; AMEER= AME efficiency ratio.

Table 9. Carcass yield, abdominal fat, and liver weight of KUB chickens fed diets with different nutrient densities and enzyme supplementation

	Carcass (%BW)	Abdominal fat (%BW)	Liver (%BW)
Nutrient density (ND)			
High-Medium-Low	68.50±2.29	1.13±0.78	2.13±0.41
High-Medium-Medium	68.52±2.57	1.00±0.57	2.10±0.29
Medium-Medium-Low	68.84±4.70	1.24±1.14	2.11±0.25
Medium-Low-Low	68.70±2.22	0.98±0.65	2.17±0.57
Enzyme supplementation (E)			
With	68.39±3.81	1.12±0.92	2.13±0.49
Without	68.89±2.13	1.06±0.69	2.12±0.26
p-Value			
Nutrient density (ND)	0.99	0.85	0.46
Enzyme (E)	0.38	0.78	0.68
ND x E	0.86	0.40	0.79

Note: BW= body weight.

not significantly affected by the interaction between ND and E, nutrient density, or enzyme supplementation.

The size of some gastrointestinal tracts of KUB chickens measured at the end of the experiment is shown in Table 10. The interaction between ND and E, nutrient density, or enzyme supplementation was not significantly affected by the proventriculus weight, gizzard weight, and duodenum length. However, the jejunum size was significantly ($p < 0.05$) affected by the nutrient density. The jejunum of chickens fed medium-low-low (M-L-L) density diets (55.0 cm) and M-M-L density (54.9 cm) was significantly ($p < 0.05$) longer than those fed H-M-M density diets (49.1 cm). Enzyme supplementation in the diet did not significantly affect the jejunum size. The ileum size was significantly ($p < 0.05$) affected by the enzyme supplementation in the diet. Chickens-fed diets supplemented with enzymes have a significant ($p < 0.5$) shorter ileum (50.7 cm) as compared with those fed diets without enzymes supplementation

(54.1 cm). Nutrient density did not significantly affect the ileum size.

Income Over Feed Cost

The effects of the treatments on the income over feed cost (IOFC) are shown in Figure 1. The interactions between the ND and E significantly ($p < 0.05$) affect the IOFC. The highest IOFC was obtained when the KUB chickens were fed the M-M-L density diets and supplemented with the enzyme (IDR 22311/bird). The lowest IOFC was obtained when the chickens were fed similar (M-M-L) density diets without enzyme supplementation.

DISCUSSION

Enzymes are known as biological products with a specific biochemical reaction such as breaking down a

Table 10. Gastrointestinal size of KUB chickens fed diets with different nutrient densities and enzyme supplementation

	Proventriculus (g)	Gizzard (g)	Duodenum (cm)	Jejunum (cm)	Ileum (cm)
Nutrient density					
High-Medium-Low	7.31±1.84	2.79±0.49	24.47±3.20	54.88±5.80 ^a	52.94±6.61
High-Medium-Medium	6.92±1.66	2.84±0.40	24.27±2.37	49.06±5.91 ^b	50.53±7.88
Medium-Medium-Low	6.52±1.73	2.75±0.54	25.53±3.66	51.16±6.73 ^{ab}	52.16±7.63
Medium-Low-Low	7.06±1.87	2.75±0.49	25.88±3.60	55.00±6.09 ^a	53.56±5.88
Enzyme supplementation (E)					
With	6.99±1.72	2.82±0.50	24.57±3.26	52.97±7.15	54.09±7.31 ^a
Without	6.91±1.84	2.75±0.46	25.58±3.21	51.84±5.78	50.70±6.43 ^b
<i>p-Value</i>					
Nutrient density (ND)	0.56	0.88	0.42	0.02	0.56
Enzyme (E)	0.86	0.98	0.17	0.69	0.04
ND x E	0.33	0.53	0.49	0.61	0.50

Note: Means in the same column and factor with different superscripts differ significantly ($p < 0.05$).

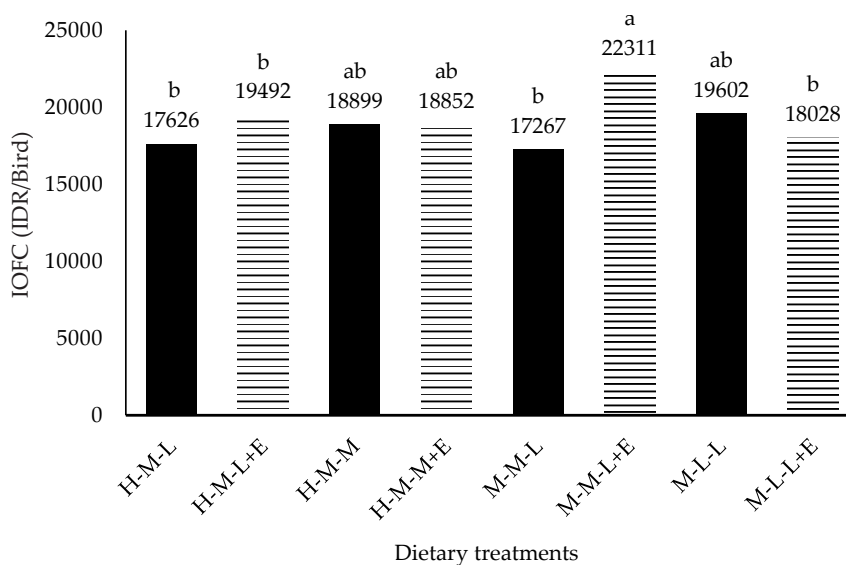


Figure 1. Income over feed cost gained after feeding KUB chickens fed diet with different densities and enzyme supplement from 1-84 days, without enzyme (■), with enzyme (≡). H-M-L= High-medium-low density diet; H-M-M= High-medium-low density diet; M-M-L= Medium-medium-low density diet; M-L-L= Medium-low-low density diet; Different characters above the values show significantly difference ($p < 0.05$).

complex molecule structure become simple molecules structure. Although chickens naturally produce digestive enzymes endogenously, supplementation of exogenous enzymes in the commercial poultry feed is commonly practiced. The beneficial effect of dietary enzyme supplementation is normally pronounced in better efficiency of feed utilization as an effect of reduction in feed intake (Hajati, 2010), higher body weight gain (El-Kelawy *et al.*, 2017), or the combination between feed intake reduction and body weight gain improvement (Amerah *et al.*, 2017). The BS4 enzyme used in this experiment has been reported to increase dry matter, protein-digestibility, and the ME of palm kernel meal (Sinurat *et al.*, 2013; 2015), ileal protein, and amino acids digestibility of fermented palm kernel cake (Sinurat *et al.*, 2014). This is in line with results that showed that feeding chickens with lower nutrient levels achieved similar performances as those fed with nutrient density diets (Abudabos, 2012; Jabbar *et al.*, 2021).

The bodyweight of the KUB chickens achieved in this experiment was higher than those reported by Puteri *et al.* (2020) at the same age. The effect of enzymes on the FCR in this trial, is consistently and significantly affected by the interaction between the enzyme supplementation and nutrient density during starter, grower, and finisher periods. This means that the enzyme supplementation has different effects on the FCR when the nutrient densities are different. Enzyme supplementation in the starter diet only effectively improves the FCR of medium-density diet significantly (10.5% improvement), while supplementation of the enzyme in the high-density diet only slightly but not significantly improved the FCR (3.9% improvement). This improvement resulted from the reduction in feed intake without altering the body weight gain (Table 3). Enzymes are well known to enhance the digestibility of low-quality feedstuffs; hence more nutrients available could be absorbed in the gastrointestinal tracts. Since the medium-density diet contains a higher level of low-quality feedstuff such as palm-kernel meal than the high-density diet (Table 2), the effect of adding enzymes in the medium-density diets was more pronounced than in the high-density diet. A similar finding was also reported in broiler chickens (Abudabos, 2012).

The classical theory stated that nutrient (especially energy) density is negatively correlated with the feed intake in poultry. However, breeding selection has evolved the physiological mechanism of feed intake control (Classen, 2017). The results of this trial did not show a clear effect of nutrient density on the feed intake, as shown by the significant interaction between enzyme supplement and nutrient density. During the starter period, feed intake was slightly lower in birds fed the medium-density diet than those fed the high-density diet. Kim *et al.* (2016) showed that the feed intake of broilers fed a high-density diet was lower than those fed a low-density diet. The feed intake responses of slow-growing chickens to different density diets may differ from the fast-growing chickens. Sun *et al.* (2017) and Wang *et al.* (2013) showed that the feed intake of slow-growing broilers was not different when fed a medium- or a high-density diet.

The significant interactions between nutrient density and enzyme supplementation in the diet on the performance, especially the FCR of the KUB chickens, suggest that different feeding methods should be applied when the enzyme is supplemented. Based on the performance from 1–84 d, the KUB chickens should be fed with a high-density starter diet followed by a medium density diet for the grower and finisher period (H-M-M) when the enzyme was not supplemented. However, a medium-density diet during starter and grower periods followed by a low-density finisher diet (M-M-L) should be fed when the enzyme was supplemented.

Carcass percentage, relative abdominal fat, and liver weight were not affected by treatments. Results on broilers (Zhai *et al.*, 2013; Kim *et al.*, 2016) or slow-growing chickens (Sun *et al.*, 2017) also showed that carcass yield and abdominal fat level were not affected by the nutrient density of the feed. Hajati (2010) reported that dietary enzyme enhanced the carcass percentages but not the abdominal fat levels and liver weights of broilers. On the other hand, Hussein *et al.* (2020) showed no effect of enzyme supplementation on carcass and fat weight. Still, the liver weight was increased when the enzyme was supplemented into a low energy diet but not into a normal or higher energy diet.

Among the gastrointestinal tracts, the length of the jejunum was significantly affected by nutrient density, and the length of the ileum was significantly affected by enzyme supplementation. The results showed that chickens fed a lower density diet (M-L-L) have a longer jejunum size than those fed with higher density diets (H-M-M). This finding is in agreement with Hussein *et al.* (2020). The results also found that enzyme supplementation produced shorter ileum. This is in agreement with Kalmendal & Tauson (2012) but contradicting to the finding of Hussein *et al.* (2020). Increasing the size of intestinal organs normally indicate their higher activities. A shorter jejunum size of chickens fed a high-density diet may indicate that the digestion and nutrient absorption is easier than the low-density diet in the jejunum. A shorter ileum size may indicate that the absorption of nutrients was easier in the ileum due to enzyme supplementation. This is supported by findings that a lower viscosity of ileal digesta in chickens fed the dietary enzyme (Balasubramanian *et al.*, 2018). The results also showed that birds fed with M-M-L supplemented with enzyme have shorter ileum and best FCR among the treatments.

The determined AME values of the diets were higher than the calculated values used in the diet formulation. The determined AME values of low-, medium-, and high-density diets were 2883, 2925, and 3009 Kcal/kg, respectively, while the calculated AME values were 2700, 2800, and 2900 kcal/kg (Table 2), respectively. The AME values used in the diet formulation were based on values usually obtained from hybrid or fast growth chickens which may differ from the local or slow-growth chickens. Zaefarian *et al.* (2015) showed that bird genetics might be another factor causing the variability in nutrient digestibility.

The AMEER is an indicator of the conversion of dietary energy to bodyweight gain. This study showed

that nutrient density affected the AME intake but not the AMEER. This finding was contradicted with finding on broilers (Shu-Biao *et al.*, 2019). Information on AMEER on slow growth chickens and fast growth chickens is scant. Therefore, no explanation could be given for this discrepancy. The KUB chickens less efficient in converting energy to body weight gain with the AMEER varied from 10.22-11.22 Mcal/g BWG as compared to the broiler chickens with the AMEER varied from 4.41-4.56 Mcal/g BW (Shu-Biao *et al.*, 2019).

Although the AME of the diet and the AME intake was not significantly affected by enzyme supplementation, the AMEER was improved 6.4% by enzyme supplementation. This indicates that the dietary enzyme may not only increase nutrient digestibility of feed as commonly declared but may also increase the energy anabolism or conversion of metabolized energy into bodyweight or body cells. However, further study needs to prove this hypothesis. Abdallah *et al.* (2020) also reported that enzyme supplementation improved the efficiencies of ME use for body energy or energy retention in broilers.

Income over feed cost (IOFC) is a simple indicator of the profitability of a poultry farm. Increasing the nutrient density and supplementation of the enzyme into a diet increased the price of feed. However, the IOFC is not affected by the feed price only but also the FCR. In this trial, the highest IOFC (IDR 22,311/bird) was obtained when the birds were fed diet H-M-L and supplemented with the enzyme. This treatment also produced the lowest FCR. El-Kelawy *et al.* (2017) also showed an increase in the net revenue of broilers fed with an enzyme supplemented diet.

The Indonesian Nasional Standard (SNI) recommends the nutrient density for local chickens age 0 to 4 weeks was 65.5 g CP/Mcal ME (BSN, 2013a). However, based on the performance achieved in this experiment, the best nutrient density for KUB chickens age 0 to 4 weeks was 70.7 g CP/Mcal ME if enzymes were not supplemented. The recommendation is still valid if the enzymes are supplemented in the diet. The nutrient density for the grower diet (age >4 to 20 weeks) recommended by the SNI was 56g CP/Mcal ME (BSN, 2013b). This experiment showed the best nutrient density for grower and finisher diet was 66.1 g CP/Mcal ME if enzymes were not supplemented. However, the best nutrient density for the grower (age 5 to 8 weeks) and finisher (age 9 to 12 weeks) period was 66.1 and 59.3 g CP/Mcal ME, respectively, when enzymes were supplemented in the diet. Different growth rates due to the breeding selection may be the reason for the difference in nutrient density requirement of KUB chickens with the SNI recommendations. Therefore, the SNI recommendation needs to be adjusted if applied to formulate feed for KUB chickens.

CONCLUSION

Feeding KUB chickens with different nutrient densities and enzyme supplementations influenced feed efficiency utilization. Different nutrient densities should be applied when enzymes are supplemented in the diet.

The best performance of KUB chickens was achieved when fed with a high-density diet for the starter period, followed by a medium-density diet for the grower and finisher period without enzyme supplementation. However, the best performance of KUB chickens was achieved when fed with a medium density diet for starter and grower and followed by a low (L) density diet for the finisher period with enzyme supplementation.

CONFLICT OF INTEREST

The authors declared no conflict of interest with financial support and materials used in conducting the experiment and writing this paper.

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