p-ISSN 2615-787X e-ISSN 2615-790X
Accredited by Directorate General of Strengthening for Research and Development No: 30/E/KPT/2018

Tropical Animal Science Journal, September 2021, 44(3):316-326
DOI: https://doi.org/10.5398/tasj.2021.44.3.316
Available online at http://journal.ipb.ac.id/index.php/tasj

Performances of Crossbred Chickens Fed Fermented Papaya Leaf and Seed Powder at High Stocking Density

S. Sugiharto*, T. Yudiarti, E. Widiastuti, H. Wahyuni, T. Sartono, & A. R. Pratama
Department of Animal Science, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro
Semarang, Central Java Province 50275, Indonesia
*Corresponding author: sgh_undip@yahoo.co.id
(Received 04-11-2020; Revised 06-01-2021; Accepted 25-01-2021)

ABSTRACT

The study evaluated the influence of fermented papaya leaf and seed powder (FPLS) and/or multienzymes on the growth, physiology, antioxidant, and gut ecology of the Indonesian crossbred chicken (ICC) at high stocking density. Three hundred and seventy ICC were randomly allotted to LSD (chicks fed conventional feed at low stocking density), HSD (chicks fed conventional feed at high stocking density), HSD+mE (chicks fed conventional feed plus multienzyme at high stocking density), HSD+FPLS (chicks fed FPLS at high stocking density) and HSD+FPLS+mE (chicks fed FPLS plus multienzyme at high stocking density). Body weight and feed intake were determined weekly. Two ICC were taken from each pen (10 chicks per treatment group) at week 10 for sampling. The study was arranged based on a completely randomized design with five treatment groups, each consisted of five replicates. Except for performance, analysis of variance was conducted on two chicks from each replicate (10 chicks per treatment group). Chicks in LSD consumed more ($p<0.05$) feed and had a higher ($p<0.05$) feed conversion ratio. Platelet distribution width (PDW) was lower ($p<0.05$) in HSD, HSD+FPLS, and HSD+FPLS+mE chicks than in LSD chicks. Lymphocyte counts were lower ($p<0.05$) in HSD relative to HSD+mE chicks. Superoxide dismutase (SOD) was higher ($p<0.05$) in HSD and HSD+FPLS+mE chicks than in LSD, HSD+mE, and HSD+FPLS chicks. High-density lipoprotein was smaller ($p<0.05$) in HSD+mE chicks than in LSD, HSD, and HSD+FPLS chicks. Compared to the other treatment groups of chicks, HSD+FPLS chicks had a lower ($p<0.05$) albumin level. Creatinine level was lower ($p<0.05$) in HSD chicks than in the other group of chicks. *Enterobacteriaceae* **counts were lower ($p<0.05$) in HSD+FPLS cecal content of chicks than in LSD and HSD chicks. The redness values of breast meats were lower ($p<0.05$) in HSD+FPLS chicks than the chicks in HSD+mE and HSD+FPLS+mE dietary treatments. LSD chicks had higher ($p<0.05$) redness values of thigh meat than the other treatment groups of chicks. LSD chicks also had higher ($p<0.05$) yellowness values than HSD+mE and HSD+FPLS+mE chicks. In conclusion, high stocking density resulted in mild stress conditions, as was demonstrated by the increased SOD and decreased PDW and redness meat values. A combination of FPLS and multienzyme ameliorated the adverse influence of high stocking density in ICC.**

Keywords: Crossbred chicken; fermented papaya feed; multienzyme; stress-related high stocking density

INTRODUCTION

The Indonesian crossbred chicken (ICC), which is a hybrid descent of male Indonesian native chicken and female commercial laying hen (a final laying stock of imported parent or grand-parent stock), has recently obtained wider attention from the Indonesian chicken-meat market. Compared to modern broiler chickens, the crossbred chicken has tastier and more delicacy meat (Chen *et al*., 2016). The recent increase in the domestic market hence encourages the farmers to intensify the rearing system of the ICC. In intensive broiler production, increasing the stocking density has commonly been practiced to decrease the production cost per house and to achieve higher profits (Jeong *et al*., 2020). Indeed, the rearing under high stocking density has also been applied for Thai crossbred chickens (Huo & Na-Lampang, 2016) as well as for indigenous chickens (Jobe *et al*., 2019). Apart from the resource efficiency concern, rearing the chicks under high stocking density may, however, lead to a stress condition causing oxidative stress, lowered final body weight, and health problems (Jobe *et al*., 2019; Jeong *et al*., 2020; Magnuson *et al*., 2020).

To deal with stress, poultry farmers have usually used synthetic antioxidants as dietary additives. However, Sugiharto *et al*. (2019a) pointed out that the long-term use of synthetic antioxidants for poultry may potentially risk human health due to its carcinogenic and mutagenic effects on humans. Furthermore, the enzyme is the other dietary additive that has been reported to alleviate the negative effect of stress in poultry. A former study by Madrid *et al*. (2010) showed that

a multi-enzyme complex of protease and carbohydrase was capable of ameliorating the negative effects of stress on the growth rate and nutrient utilization of broilers under commercial farm conditions. Dietary supplementation of xylanase was also beneficial in ameliorating the destructive impact of high temperature-related stress on the productive outcome and physiological conditions of broilers (Hosseini & Afshar, 2017). In the previous study, we incorporated the fermented papaya leaf and seed powder (FPLS; by using the fungus *Chrysonilia crassa* as the fermentation starter) into the rations of the ICC. We found that such dietary treatment improved the growth, immune competencies, physiological condition, and intestinal functions of the ICC (Sugiharto *et al*., 2020a). In such cases, the antioxidant capacities of papaya leaf (Asghar *et al*., 2016), seed (Sugiharto, 2020), and the fungus *C. crassa* (Sugiharto *et al*., 2017) seemed to partly contribute to the improvement in poultry performance and health. The enzymes contained in papaya leaf (Welde & Worku, 2018) and seed (Sugiharto, 2020) were also responsible for the improved performance, digestibility, and antioxidative status of the animal (Oloruntola *et al*., 2018). To the best of our understanding, no other work has investigated the use of FPLS and its combination with enzymes to mitigate the detrimental effects of high stocking density on the ICC. The goal of this current work was to investigate the influence of FPLS and/or multienzymes on the growth, physiology, antioxidant status, and intestinal ecology of the ICC reared under high stocking density.

MATERIALS AND METHODS

Preparation of Starter for Fermentation

The fermentation starter was prepared according to Sugiharto *et al*. (2020a). The pure isolates of *C. crassa* were initially rejuvenated from the culture stock (nurtured on potato dextrose agar [PDA; Merck KGaA, Darmstadt, Germany] and kept at 4°C) and then re-cultured on PDA under the aerobic condition for 48 hours at 38°C. The spores were harvested using 10 mL of sterilized water. To make the starter for fermentation, 100 g of rice was steamed for about 1 hour and spread on a tray. The steamed rice was then cultivated with 10 mL of spore suspension as previously prepared. The cultivated rice was aerobically incubated for 48 hours at room temperature. Then, it was dried under the sun, ground, and sifted. The sample was taken for counting of the fungal colony, and the remainder was used as a starter of fermentation. Plate count procedure (using PDA; aerobically incubated for 48 hours at 38°C) showed that the starter of fermentation contained >1 × 10^8 cfu/g *C. crassa*.

Preparation of FPLS

The papaya leaves were picked from the local campus gardens. The harvested leaves were dried at room temperature, grounded, and stored until being used. The seeds were obtained from the fruit vendors around the campus. The seeds were separated from the papaya flesh, and the collected seeds were then sun-dried, ground, and stored prior to being used. According to Sugiharto *et al*. (2020a), the manufacture of FPLS was carried out with few modifications. In short, 645 g of papaya-leaf powder, 345 g of papaya-seed powder, and 10 g of starter were thoroughly blended. The autoclaved water was added to the blend (1:1) to operate the solid-state fermentation. For four days, the culture was aerobically incubated at room temperature and then sun-dried afterward. The FPLS production was carried out in multiple batches using identical protocols for each batch. Samples from each sample were collected and then pooled for proximate examination (AOAC, 1995) and counting of colonies of fungi. The rest of FPLS was used for *in vivo* trials. Table 1 presents the proximate components of the leaf, seed, and FPLS. The number of fungi in FPLS was around 1 × 10^6 cfu/g.

In Vivo Experiment

The *in vivo* experiment was approved by the Animal Ethics Committee of the Faculty of Animal and Agricultural Sciences, Universitas Diponegoro (No. 57-01/A3/KEP/FPP) and carried out in accordance with the basic animal husbandry and health protocols referred to Legislation of the Republic of Indonesia No. 18, 2009.

Four hundred day-old ICCs, purchased from the local hatchery, were raised under the standard intensive-rearing system for the ICC from arrival until week 4. The chicks were solely weighed at the end of week 4, and the *in vivo* study used 370 chicks with slightly similar body weight (236.4±4.33 g; mean ± standard deviation). They were shared to one of five groups (each consisted of five replicates or pens), i.e., LSD (chicks provided with conventional feed ingredients and raised at low stocking density [10 chicks in 1×1 m^2 pen]), HSD (chicks provided with conventional feed ingredients and raised at high stocking density [16 chicks in 1×1 m^2 pen]), HSD+mE (chicks provided with conventional feed ingredients supplemented with commercial multi-enzyme [0.5 g/kg diet] and raised at high stocking density), HSD+FPLS (chicks provided with feed containing 7.5% of FPLS and raised at high stocking density), and HSD+FPLS+mE (chicks provided with feed containing 7.5% of FPLS and supplemented with commercial multienzyme (0.5 g/kg diet) and maintained at high stocking density). In each pen, half of the chicks were male, and the other half were female. The treatments were applied from weeks 5 to 10. To date, there is no published

Table 1. Chemical ingredients of leaf and seed powders of papaya and FPLS

Nutrients (%, DM basis)	Leaf powder	Seed powder	FPLS
Dry matter	87.6	89.9	87.9
Crude protein	27.7	24.6	28.6
Crude fat	6.30	15.2	9.20
Crude fiber	31.7	35.6	31.4
Crude ash	13.9	9.00	12.2

Note: DM= dry matter; FPLS= fermented papaya leaf and seed powders.

study reporting the standard/ideal stocking density for ICC. The only study was reported by Patria *et al*. (2016), of which high stocking density of Kampong-broiler crossbred chickens was defined as 12 chicks per m^2, while low stocking density was 8 chicks per m^2 (reared until 12 weeks of age). Given that ICC was raised only until 10 weeks of age, in this study, low stocking density was defined as the rearing of 10 ICC per m^2, while the high density was 16 ICC per m^2. Treatment diets were produced in mash form (Table 2) and were provided *ad libitum*. The commercial multienzyme (Natuzyme, Bioproton Europe Oy, Kaarina, Finland) consisted of cellulose (6,000,000 u/kg), xylanase (10,000,000 u/kg), *β*-glucanase (700,000 u/kg), protease (3,000,000 u/kg), *α-amylase (*700,000 u/kg), pectinase (70,000 u/kg), phytase (1,300,000 u/kg), and lipase (5,000 u/kg). The multienzyme was added 'on top' at the end of the feed mixing process. The drinking water was also provided *ad libitum* throughout the experiment.

For the entire study period, the ICC was raised on the litter of rice husk in an open-sided chicken shelter. Each pen has one manual feeder and one drinker. The light was scheduled for 24 hours per day. The temperature and humidity of the chicken house were partially controlled by the plastic curtains, light bulbs, and blower fans. The chicks were inoculated with the Newcastle disease vaccine (NDV) on day 4 by eye drops and on days 18 and 30 by drinking water. From week 5 to 10, live weight, the amount of feed intake, as well as feed conversion ratio (FCR) were reported on a weekly basis. At the ultimate of week 10, two ICC were taken from each pen, and blood was then withdrawn from the veins of their wings. The blood was preserved in containers containing ethylenediaminetetraacetic acid (EDTA) for the assessment of whole blood numbers, and the remainder of the blood was placed in vacutainers without EDTA. The remaining blood was allowed to clot and then centrifuged at 5,000 rpm for 10 minutes to create the serum. The serum was frozen before the biochemical analysis was carried out. The same ICC used for blood sampled was killed, the feathers were removed, and the internal organs were eviscerated. Digesta was taken from the ileal and cecal segments of the ICC for the enumeration of selected bacteria and stored in sterile sample bottles. Subsequently, the empty weight of the internal organs was measured. The small intestinal segments (each roughly 2 cm) were collected and placed into 10% neutral formalin buffer solution (Leica Biosystems Richmond, Inc., Richmond, USA) to assess small intestinal morphology. The ICC's eviscerated carcass and commercial proportions were also calculated. The skinless muscles of the breast and thigh were obtained to determine the meat color.

Laboratory Analysis

By using Prima Fully-auto Hematology Analyzer (PT. Prima Alkesindo Nusantara, Jakarta, Indonesia), the complete blood profiles of the ICC were calculated according to the electrical impedance method based on the manufacturer instructions. Based on the hemagglutination inhibition (HI) method (Villegas, 1987), the calculation of antibody titers toward NDV was performed. Briefly, NDV antigen was mixed with two-fold serial dilutions of the test samples. Following the addition of chicken red blood cells, the dilutions were tested for

Table 2. Chemical compositions of experimental feeds

Items (%, unless otherwise noted)	Starter (week 1-4)	Finisher (week 5-10)	
		Conventional feed	Feed with FPLS
Maize	55.9	62.5	58.8
Soybean meal (SBM)	37.1	29.5	25.7
Fermented papaya leaf and seed powder (FPLS)	-	-	7.50
Palm oil	2.22	3.22	3.22
DL-methionine	0.19	0.19	0.19
Bentonite	1.00	1.00	1.00
Limestone	1.34	1.34	1.34
Monocalcium phosphate (MCP)	1.51	1.51	1.51
Premix[1]	0.27	0.27	0.27
Chlorine chlorite	0.07	0.07	0.07
NaCl	0.40	0.40	0.40
Nutrient content:			
ME, (kcal/kg)[2]	2,900	3,042	3,041
Crude protein	21.0	18.0	18.0
Crude fiber	5.50	5.50	6.50
Ca	1.30	1.29	1.26
P	0.60	0.62	0.59

Note: [1]Premix composed (per kg of feed) of vitamin A (7,750 IU), vitamin E (1.88 mg), vitamin D3 (1,550 IU), vitamin B1 (1.25 mg), vitamin B2 (3.13 mg), vitamin B6 (1.88 mg), vitamin B12 (0.01 mg), vitamin C (25 mg), folic acid (1.50 mg), Ca-d-pantothenate (7.5 mg), niacin (1.88 mg), biotin (0.13 mg), Co (0.20 mg), Cu (4.35 mg), Fe (54 mg), I (0.45 mg), Mn (130 mg), Zn (86.5 mg), Se (0.25 mg), L-lysine (80 mg), Choline chloride (500 mg), methionine (900 mg), CaCO3 (641.5 mg), DCP (1500 mg).
[2]ME (metabolizable energy) was counted with respect to formula (Bolton, 1967)= 40.81 {0.87 [crude protein + 2.25 crude fat + nitrogen-free extract] + 2.5}

full hemagglutination inhibition. The HI titers were recorded as geometric mean titers (log_2).

The total serum triglyceride was calculated using glycerol-3-phosphate oxidase (GPO) based on the enzymatic colorimetric procedure. Total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) cholesterol were assessed using the cholesterol oxidase/p-amino phenazone (CHOD-PAP) enzymatic colorimetric tool. The triglycerides were assessed following enzymatic separation with lipoprotein lipase, whereas total cholesterol was assessed following enzymatic hydrolysis and oxidation. Using hydrogen peroxide and peroxidase as a catalyst, both triglyceride and cholesterol used a quinoneimine indicator synthesized from 4-aminoantipyrine and 4-chlorophenol (DiaSys Diagnostic System GmbH, Holzheim, Germany). Heparin was used to precipitate the LDL. After centrifugation, the HDL remained in the supernatant and was enzymatically processed using CHOD-PAP technique. The difference between total cholesterol and cholesterol in the supernatant was used to determine the LDL concentration. Using the Reflotron system (Roche Diagnostics Corporation, Indianapolis, IN, USA), the alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes were spectrophotometrically determined. The serum and the ALT or AST reagents were mixed at room temperature. The absorbance was then measured at 340 nm after 60 seconds of incubation at 37°C. Total serum protein was measured by a photometric examination based on the biuret kit process (total serum protein kit, DiaSys Diagnostic System GmbH, Holzheim, Germany) according to the instructions from the producer. In principle, proteins in alkaline solution created a violet blue color complex with copper ions. The absorbance of the color (measured at 545 nm) was directly proportionate to the protein concentration. The serum albumin was assessed with bromocresol green (DiaSys Diagnostic Device GmbH, Holzheim, Germany) using a photometric examination. The color of serum albumin changed from yellow-green to green-blue in the presence of bromocresol green at a slightly acid pH. The globulin concentration was determined from the overall protein subtracted by albumin. The uric acid level was determined according to the enzymatic colorimetric method by mixing 4-aminoantipyrin with 2-hydroxy-2,4,6-tribromobenzoic acid (TBHBA; DiaSys Diagnostic Device GmbH, Holzheim, Germany) and hydrogen peroxide in the presence of peroxide to produce a chromogen measured at 520 nm. The serum creatinine was determined according to the enzymatic colorimetric test (DiaSys Diagnostic Device GmbH, Holzheim, Germany), and the absorption of the red dye produced at 545 nm was proportional to the serum level of creatinine.

The concentration of malondialdehyde (MDA) in the serum was determined based on the reaction of MDA with thiobarbituric acid (TBA) (Sigma-Aldrich, St. Louis, USA). The concentration of MDA was quantified spectrophotometrically at 532 nm. The serum superoxide dismutase (SOD) concentration was measured using SOD kits (Sigma-Aldrich, St. Louis, USA) according to an indirect assay procedure based on xanthine oxidase. A spectrophotometer (absorbance at 550 nm) was used to measure the concentration of SOD in the serum.

On 5 μm duodenal, jejunal, or ileal slices stained with hematoxylin and eosin, histological analyses were performed on the small intestinal segments. An optical microscope with a digital camera (Leica Microsystems GmbH, Wetzlar, Germany) was used to calculate the height of the villus and the depth of the crypt in each segment. The mean value of villus height and crypt depth of each sample was derived from the five measurements. As defined by Sugiharto *et al*. (2019b), the populations of bacteria in the ileal and cecal digesta of the ICC were enumerated based on the total viable count procedure. The numbers of coliform and lactose-negative enterobacteria were counted as red and colorless colonies on MacConkey agar (Merck KGaA, Darmstadt, Germany), respectively, following aerobic incubation at 38°C for 24 hours. *Enterobacteriaceae* is known to be the cumulative amount of coliform and lactose-negative enterobacteria. The counts of lactic acid bacteria (LAB) were determined on de Man, Rogosa, and Sharpe agar (MRS; Merck KGaA) after anaerobic incubation for 48 hours at 38°C.

The ICC meat color was measured in Mac OS X (set to CIE Lab) by means of the digital color meter as defined by Sugiharto *et al*. (2019b). The color was given as L* values (lightness), a* values (redness), and b* values (yellowness). The color determination was conducted in triplicate.

Statistical Analysis

The study was arranged based on a completely randomized design with five treatment groups, each consisted of five replicates. Except for performance, statistical analysis was conducted on two chicks from each replicate (10 chicks per treatment group). Data were examined according to the analysis of variance (SPSS 16.0 version). As means and standard error of the means (SEM), findings are presented. When notable variations ($p<0.05$) occurred within the group of treatments, posthoc analysis using Duncan's multiple-range test was performed.

RESULTS

Performances of the ICC

Details on the growth and economical traits of the ICC are provided in Table 3. The chicks in LSD group ingested more ($p<0.05$) feed than those of the other chicks. The LSD chicks had higher ($p<0.05$) FCR and feed cost per weight gain than those of the other chicks. Income over feed cost was greater ($p<0.05$) in HSD+FPLS+mE than that in LSD and HSD, but did not vary from HSD+mE and HSD+FPLS chicks.

Complete Blood Counts of the ICC

Results regarding the whole blood numbers of the ICC are listed in Table 4. The levels of PDW were lower ($p<0.05$) in HSD HSD+FPLS, and HSD+FPLS+mE than

Table 3. Growth and economic performances of Indonesian crossbred chickens given experimental diet up to 10 weeks of age

Variables	Treatments					SEM	p value
	LSD	HSD	HSD+mE	HSD+FPLS	HSD+FPLS+mE		
Final BW, g	863	857	868	863	889	5.45	0.43
Weight gain, g	621	622	633	628	653	5.51	0.37
Cumulative FI, g	2,503[a]	2,160[b]	2,103[b]	2,175[b]	2,131[b]	48.8	0.04
FCR	4.03a	3.47[b]	3.33[b]	3.47[b]	3.26[b]	0.08	0.01
Feed cost per weight gain (IDR)[1]	25,081[a]	21,558[b]	20,732[b]	20,033[b]	18,850[b]	565	<0.01
Income over feed cost (IDR)[2]	2,559[c]	4,552[b]	5,138[ab]	5,553[ab]	6,351[a]	343	<0.01

Note: Means in the same row with different superscripts differ significantly (p<0.05).
[1]= Calculations were conducted at the period of investigation as the cost of feed ingested to reach a kilogram of live body-weight gain; [2]= Calculations were conducted at the period of investigation as the entire income minus entire feed cost; LSD= chicks fed conventional feed at low stocking density; HSD= chicks fed conventional feed at high stocking density; HSD+mE= chicks fed conventional feed plus multienzyme at high stocking density; HSD+FPLS= chicks fed FPLS at high stocking density; HSD+FPLS+mE= chicks fed FPLS plus multienzyme at high stocking density; BW= body weight; FCR= feed conversion ratio; FI= feed intake, IDR= Indonesian Rupiah (Indonesian currency); SEM= standard error of means.

Table 4. Complete blood profile of Indonesian crossbred chickens given experimental diet up to 10 weeks of age

Variables	Treatments					SEM	p value
	LSD	HSD	HSD+mE	HSD+FPLS	HSD+FPLS+mE		
Hemoglobin (g/dL)	10.3	10.5	12.7	10.3	10.9	0.38	0.24
Erythrocytes (10^6/μL)	2.89	2.99	3.54	2.93	3.06	0.11	0.28
Hematocrit (%)	34.9	37.0	42.7	35.0	37.1	1.27	0.29
MCV (fl)	121	125	122	122	122	0.51	0.20
MCH (pg)	35.6	34.5	35.8	35.4	35.5	0.24	0.47
MCHC (g/dL)	29.5	28.4	29.6	29.5	29.3	0.22	0.36
RDW-SD (fl)	54.3	54.5	49.8	53.0	53.5	0.72	0.25
RDW-CV (%)	11.8	11.6	10.8	11.6	11.6	0.15	0.24
MPV (fl)	9.23	9.07	9.12	9.22	9.14	0.11	0.99
PDW (%)	4.42[a]	1.78[b]	2.08[ab]	0.73[b]	0.92[b]	0.41	0.03
Leukocytes (10^3/μL)	87.6	101	114	92.6	95.2	4.43	0.41
Heterophils (10^3/μL)	6.00	6.95	6.55	7.05	7.35	0.43	0.89
Lymphocytes (10^3/μL)	81.4	79.7	107	85.6	87.9	3.51	0.09
Thrombocytes (10^3/μL)	10.1	9.30	8.10	8.50	8.40	0.52	0.76

Note: Means in the same row with different superscripts differ significantly (p<0.05).
LSD= chicks fed conventional feed at low stocking density; HSD= chicks fed conventional feed at high stocking density; HSD+mE= chicks fed conventional feed plus multienzyme at high stocking density; HSD+FPLS= chicks fed FPLS at high stocking density; HSD+FPLS+mE= chicks fed FPLS plus multienzyme at high stocking density; MCH= mean corpuscular hemoglobin; MCV= mean corpuscular volume; MCHC= mean corpuscular hemoglobin concentration; RDW-SD= red blood cell distribution width-standard deviation; RDW-CV= red blood cell distribution width-variation coefficient; MPV= mean platelet volume, PDW= platelet distribution width; SEM= standard error of means.

that in LSD, but did not vary from HSD+mE chicks. The number of lymphocytes appeared (p=0.09) to be lower in HSD relative to HSD+mE chicks in particular. The treatment had no influence (p>0.05) on the other blood indices.

Serum SOD, MDA, Biochemical Indices, and Antibody Titers against NDV of the ICC

The concentrations of SOD were greater (p<0.05) in HSD and HSD+FPLS+mE c than that in the other chicken groups, and HSD+mE had the lowest serum SOD concentration. There was no major impact of treatments on MDA concentrations of chicks (Table 5). The HDL values were lower (p<0.05) in HSD+mE in comparison to LSD, HSD, and HSD+FPLS, but not different from HSD+FPLS+mE chicks. In comparison to the other chicks, HSD+FPLS had a lower (p<0.05) concentration of albumin. The level of creatinine was slightly lower (p≥0.05) in HSD than in the other group of treatments. Other biochemical indices were not impacted (p>0.05) by the applied treatments. Antibody titers against NDV were not significantly different across the treatment groups (Table 5).

Selected Bacterial Counts and Intestinal Morphology of ICC

The number of *Enterobacteriaceae* was shown to be reduced (p<0.05) in HSD+FPLS cecal content than in LSD and HSD, but did not vary significantly from HSD+mE and HSD+FPLS+mE chicks. There was no impact (p>0.05) of treatments on the bacterial populations in the ICC ileal digesta (Table 6).

Table 5. Serum superoxide dismutase, malondialdehyde, biochemical variables, and antibody titers toward Newcastle Disease vaccine of Indonesian crossbred chickens given dietary experimental up to 10 weeks of age

Variables	Treatments					SEM	p value
	LSD	HSD	HSD+mE	HSD+FPLS	HSD+FPLS+mE		
SOD (U/mL)	24.0^{c}	26.8^{a}	22.6^{d}	25.5^{b}	27.0^{a}	0.29	<0.01
MDA (nmol/mL)	7.71	6.99	7.01	7.97	7.79	0.49	0.95
Total triglyceride (mg/dL)	85.9	72.7	93.9	76.2	88.6	3.07	0.15
Total cholesterol (mg/dL)	125	112	124	115	129	2.79	0.25
LDL (mg/dL)	12.9	10.2	24.2	10.7	23.4	2.35	0.14
HDL (mg/dL)	99.8^{a}	95.4^{a}	80.9^{b}	92.8^{a}	89.0^{ab}	1.82	<0.01
Total protein (g/dL)	4.04	3.84	4.03	3.67	3.98	0.06	0.17
Albumin (g/dL)	1.55^{a}	1.59^{a}	1.61^{a}	1.41^{b}	1.57^{a}	0.02	0.04
Globulin (g/dL)	2.50	2.25	2.42	2.25	2.41	0.05	0.45
A/G ratio	0.62	0.71	0.69	0.63	0.65	0.01	0.22
AST (U/L)	255	263	229	232	247	5.71	0.27
ALT (U/L)	1.39	1.44	1.03	0.77	1.46	0.17	0.64
Uric acid (mg/dL)	6.12	6.60	5.72	5.50	7.00	0.26	0.35
Creatinine (mg/dL)	0.03	0.02	0.03	0.03	0.03	<0.01	0.05
Antibody titers against NDV (Log_2 GMT)	3.90	2.90	3.70	3.20	3.00	0.32	0.83

Note: Means in the same row with different superscripts differ significantly (p<0.05).
LSD= chicks fed conventional feed at low stocking density; HSD= chicks fed conventional feed at high stocking density; HSD+mE= chicks fed conventional feed plus multienzyme at high stocking density; HSD+FPLS= chicks fed FPLS at high stocking density; HSD+FPLS+mE= chicks fed FPLS plus multienzyme at high stocking density; SOD= superoxide dismutase; MDA; malondialdehyde; LDL= low-density lipoprotein; HDL= high-density lipoprotein; A/G= albumin to globulin ratio; AST= aspartate transaminase; ALT= alanine transaminase; GMT= geometric mean titer; NDV= Newcastle disease vaccine; SEM= standard error of means.

Table 6. Bacterial counts in intestinal segments of Indonesian crossbred chickens given dietary experimental up to 10 weeks of age

Variables (Log cfu/g)	Treatments					SEM	p value
	LSD	HSD	HSD+mE	HSD+FPLS	HSD+FPLS+mE		
Ileum							
Coliform	7.13	6.80	7.25	6.79	6.48	0.25	0.89
Enterobacteriaceae	6.71	6.84	7.28	7.00	6.90	0.25	0.97
LAB	10.8	11.1	11.2	11.2	11.3	0.10	0.60
Caecum							
Coliform	8.80	7.82	8.13	7.63	7.70	0.19	0.28
Enterobacteriaceae	9.10^{a}	9.06^{a}	8.49^{ab}	8.10^{b}	8.23^{ab}	0.14	0.04
LAB	11.7	11.7	11.6	11.6	11.7	0.02	0.74

Note: Means in the same row with different superscripts differ significantly (p<0.05).
LSD= chicks fed conventional feed at low stocking density; HSD= chicks fed conventional feed at high stocking density; HSD+mE= chicks fed conventional feed plus multienzyme at high stocking density; HSD+FPLS= chicks fed FPLS at high stocking density; HSD+FPLS+mE= chicks fed FPLS plus multienzyme at high stocking density; LAB= lactic acid bacteria; cfu= colony forming unit; SEM= standard error of means.

The data on intestinal morphology of the ICC are provided in Table 7. In general, the treatments possessed no noticeable impact on the height of the villus, depth of crypt, and villus height to crypt depth ratio of the ICC.

Internal Organ Weights and Carcass Traits of the ICC

Findings on the weights of internal organs of ICC are provided in Table 8. In general, the weights of the internal organs of the ICC were not impacted (p>0.05) by the treatments. The carcass traits and commercial proportions of the ICC were also not influenced (p>0.05) by the treatments (Table 9). The a* values of breast meats were lower (p<0.05) in HSD+FPLS than those particularly in HSD+mE and HSD+FPLS+mE chicks, but the values did not differ from LSD and HSD chicks. In thigh meat, LSD had greater (p<0.05) a* values than the other chicks. LSD chicks also had greater (p<0.05) b* values than HSD+mE and HSD+FPLS+mE chicks (Table 9).

DISCUSSION

Performances of the ICC

It was documented in this study that the ICC in LSD group consumed more feed and had greater FCR than those raised at high stocking density. In modern broiler strains, high stocking density has been confirmed

Table 7. Intestinal morphology of Indonesian crossbred chickens given dietary experimental up to 10 weeks of age

Items	Treatments					SEM	p value
	LSD	HSD	HSD+mE	HSD+FPLS	HSD+FPLS+mE		
Duodenum							
Villi height (μm)	1,077	1,128	983	1,105	970	33.4	0.46
Crypt depth (μm)	92.2	103	93.0	104	107	2.42	0.17
VH/CD	12.0	11.2	10.8	10.9	9.22	0.43	0.36
Jejunum							
Villi height (μm)	1,007	882	942	822	868	45.9	0.76
Crypt depth (μm)	130	118	111	128	128	3.67	0.40
VH/CD	7.91	7.62	8.63	6.34	6.89	0.35	0.27
Ileum							
Villi height (μm)	680	657	581	569	629	21.3	0.41
Crypt depth (μm)	110	98.6	94.7	99.4	98.7	2.71	0.47
VH/CD	6.19	6.77	6.39	5.85	6.47	0.22	0.77

Note: LSD= chicks fed conventional feed at low stocking density; HSD= chicks fed conventional feed at high stocking density; HSD+mE= chicks fed conventional feed plus multienzyme at high stocking density; HSD+FPLS= chicks fed FPLS at high stocking density; HSD+FPLS+mE= chicks fed FPLS plus multienzyme at high. stocking density; VH/CD= the ratio of villi height to crypt depth; SEM= standard error of means.

Table 8. Internal organ weight of Indonesian crossbred chickens given dietary experimental up to 10 weeks of age

Variables (% live BW)	Treatments					SEM	p value
	LSD	HSD	HSD+mE	HSD+FPLS	HSD+FPLS+mE		
Heart	0.51	0.49	0.48	0.53	0.52	0.01	0.54
Liver	2.19	2.11	2.19	2.19	2.18	0.03	0.46
Proventriculus	0.60	0.60	0.64	0.60	0.58	0.01	0.50
Gizzard	2.63	2.48	2.64	2.52	2.40	0.05	0.56
Pancreas	0.24	0.23	0.26	0.23	0.24	0.01	0.77
Abdominal fat	0.98	1.02	0.81	1.03	0.82	0.07	0.78
Duodenum	0.57	0.63	0.56	0.58	0.62	0.02	0.50
Jejunum	0.98	0.95	0.94	1.02	0.98	0.02	0.78
Ileum	0.58	0.70	0.66	0.65	0.60	0.02	0.19
Caeca	0.69	0.61	0.64	0.60	0.64	0.02	0.35
Spleen	0.28	0.25	0.24	0.26	0.23	0.01	0.86
Thymus	0.48	0.47	0.45	0.43	0.47	0.02	0.97
Bursa of Fabricius	0.08	0.08	0.09	0.08	0.08	<0.01	0.92

Note: LSD= chicks fed conventional feed at low stocking density; HSD= chicks fed conventional feed at high stocking density; HSD+mE= chicks fed conventional feed plus multienzyme at high stocking density; HSD+FPLS= chicks fed FPLS at high stocking density; HSD+FPLS+mE= chicks fed FPLS plus multienzyme at high stocking density; BW= body weight; SEM= standard error of means.

to deleteriously influence the growth and feed efficiency of chicks, especially during the growing phase (Rashidi *et al*., 2019). The lacking impact of high stocking density on the crossbred chickens has actually been reported by Huo & Na-Lampang (2012; 2016). The latter investigators reported that Thai crossbred chicken could be safely raised as high density as 16 chicks per m^2 up to 12 weeks old (body weight of around 1,242 g). It was interesting to report in the current investigation that chicks reared at low stocking density resulted in poor FCR as compared to those reared at high stocking density. The reason for this difference was not well understood, but more space available for chickens could increase chicken activity (Patria *et al*., 2016) and thus assign more feed-derived energy to the activity rather than for growth. Notice that, unlike modern broiler strains (fast-growing chickens), ICC (slow-growing chickens) displayed higher physical activity (Tallentire *et al*., 2016). With regard to the higher feed consumption in LSD (than in chicks reared at high stocking density), the higher physical activity seemed to increase the energy need and thus increase the feed consumption of chickens. Also, less competition among chicks in LSD group may increase the access to feed and thereby increase feed intake of ICC. In this study, the higher feed intake and FCR in LSD may consequently increase the feed cost per weight gain of chickens. It was apparent in the current work that HSD+FPLS+mE had higher income over feed cost than those particularly of LSD and HSD chicks. The improved FCR and the cheaper price of feed containing FPLS (than that of conventional feed) may be attributed to the higher income over feed cost in HSD+FPLS+mE chicks.

Table 9. Eviscerated carcass and commercial proportions of Indonesian crossbred chickens given dietary experimental up to 10 weeks of age

Variables	Treatments					SEM	p value
	LSD	HSD	HSD+mE	HSD+FPLS	HSD+FPLS+mE		
Eviscerated carcass (% live BW)	60.7	60.7	60.3	60.2	60.2	0.22	0.90
			% eviscerated carcass				
Breast	25.5	25.4	25.5	25.5	25.0	0.15	0.77
Wings	14.6	14.6	14.6	14.7	14.5	0.10	0.99
Thigh	17.4	17.7	17.0	17.3	17.1	0.13	0.51
Drumstick	17.2	16.7	16.7	17.2	17.3	0.12	0.33
Back	25.3	25.5	26.2	25.7	26.1	0.15	0.25
			Meat color				
Breast meat							
L*	47.0	47.7	47.2	47.7	46.6	0.49	0.95
a*	4.79^{ab}	5.71^{ab}	6.17^{a}	4.00^{b}	6.11^{a}	0.27	0.04
b*	10.6	11.9	10.4	12.1	11.9	0.25	0.10
Thigh meat							
L*	49.3	50.7	51.0	50.9	50.3	0.25	0.19
a*	11.2^{a}	7.89^{b}	6.44^{b}	7.51^{b}	6.93^{b}	0.34	<0.01
b*	10.8^{a}	9.91^{ab}	9.15^{bc}	10.3^{ab}	8.19^{c}	0.23	<0.01

Note: LSD= chicks fed conventional feed at low stocking density; HSD= chicks fed conventional feed at high stocking density; HSD+mE= chicks fed conventional feed plus multienzyme at high stocking density; HSD+FPLS= chicks fed FPLS at high stocking density; HSD+FPLS+mE= chicks fed FPLS plus multienzyme at high stocking density; BW= body weight; L*= values of lightness; a*= values of redness; b*= values of yellowness; SEM= standard error of means.

Complete Blood Counts of the ICC

Full blood counts were investigated in the present study, and we found that platelet distribution widths (PDW) were lower in HSD, HSD+FPLS, and HSD+FPLS+mE when compared with LSD chicks. Santimone *et al*. (2011) previously reported that during inflammation, the numbers of platelets (thrombocytes) increased while PDW decreased because platelets become smaller and more monotypic. Note that high stocking density is often attributable to the inflammatory conditions in chickens (Magnuson *et al*., 2020). With regard particularly to HSD+mE, the ICC in this group had no significant difference in PDW when compared with that in LSD group. This may suggest that the HSD+mE chicks experienced less stress conditions due to high stocking density. An earlier study by Müller Fernandes *et al*. (2015) showed that dietary enzymes (i.e., protease, amylase, xylanase, and galactosidase) could alleviate the stress condition due to high stocking density in broiler chickens. Likewise, Lee *et al*. (2019) noticed that phytase might serve as a stress alleviator in broiler chickens due to hot temperature. In this study, the numbers of lymphocytes tended to be lower in HSD as compared particularly to HSD+mE chicks. High stocking density has usually been associated with the stress condition, which may implicate in the decreased lymphocyte value in chickens (Qaid *et al*., 2016). Indeed, the higher lymphocyte value in the HSD+mE ICC chicks seemed to be contributed by the role of multienzyme in ameliorating the stress condition due to high stocking density (Müller Fernandes *et al*., 2015; Lee *et al*., 2019).

Serum SOD, MDA, Biochemical Indices, and Antibody Titers against NDV of the ICC

The SOD is an enzyme that plays a key role in animals' first adaptation to different stress environments (Surai *et al*., 2019). Compared to LSD, the ICC in HSD group had higher concentrations of SOD, indicating that stress due to high stocking density enhanced the level of SOD in the blood of chickens. This current result was in contrast to the report of Li *et al*. (2019), documenting the decreased SOD concentration in the serum of poultry at high stocking density. The increased production of SOD is, in fact, an adaptive mechanism of poultry during stress conditions to mitigate free radical formation and avoid oxidative stress. The SOD is decreased only when the stress condition becomes more severe (Surai *et al*., 2019). In this study, the rearing of 16 chicks per square meter seemed to exert mild stress, not severe stress (as there was no noticeable increase in MDA level). As a result, the increased SOD level in HSD was most likely the adaptive response of the ICC to stress-related high stocking density. With regard particularly to HSD+mE chicks, their SOD levels were lower compared to the other chicks. The SOD is an inducible enzyme, and that free radical is the prominent factor inducing the production of SOD in poultry (Surai, 2016). Taken together, the capacity of multienzyme in reducing the production of free radicals (Attia *et al*., 2020) could therefore be attributed to the lower need of SOD for neutralizing the free radicals. Compared to LSD, the chicks in HSD+FPLS and HSD+FPLS+mE groups showed higher SOD levels. In this study, FPLS seemed to contribute to the increase SOD level of the ICC. This assumption was supported by our previous study showing the increased SOD con-

centration in broilers fed with fermented cassava pulp and *Moringa oleifera* leaf powder (Sugiharto *et al*., 2020b).

Our result showed that HDL level was lower in HSD+mE than those in LSD, HSD, and HSD+FPLS. Irrespective of the stocking density effect, dietary administration of multienzyme seemed to reduce the serum HDL concentration of the ICC. The explanation for such a latter circumstance was not definitely known, but multienzyme may increase the fiber digestibility (Attia *et al*., 2012), thus decrease bile acid secretion in the small intestine and consequently decrease HDL in serum (Aghili *et al*., 2019). In support of our result, Amer *et al*. (2020) pointed out that dietary supplementation of AlphaGal (galactosidase; EC 3.2.1.22) also decreased the blood concentration of HDL in broiler chickens. In contrast, however, Attia *et al*. (2020) pointed out that multienzyme treatment increased HDL level in the serum without affecting the apparent digestibility of crude fiber of broilers. The variations in chicken breeds, enzymes (types and doses), feeds, and other trial circumstances may be accounted to the various results above. Irrespective of stocking density, feeding FPLS resulted in a lower albumin concentration in the serum of the ICC. It was most likely that the high fiber content in the feed containing FPLS may be attributed to the reduced protein digestibility (Sobayo *et al*., 2012), thus reduced the total protein and albumin values in the serum of poultry (Law *et al*., 2018). With regard to HSD+FPLS+mE, the high fiber content in the feed may be compensated by multienzyme in improving the digestibility of fiber and protein (Attia *et al*., 2012; 2020) that eventually increase total protein and albumin in the serum of broilers. There was an obvious tendency that high stocking density decreased the serum creatinine level of the ICC. This finding was in contrary to that pointed out by Silas *et al*. (2014) that stocking density had no noticeable impact on the serum creatinine level of broiler chickens. In general, serum creatinine is derived from creatine in the muscle (Patel *et al*., 2013). Given that high stocking density may induce stress, which can attenuate the creatine biosynthesis (Gonzalez-Esquerra & Leeson, 2006), the lower serum creatinine levels in HSD than that in LSD could therefore be understood. Moreover, multienzyme and FPLS seemed to alleviate the stress-related high stocking density and therefore could maintain the levels of serum creatinine in the ICC.

Selected Bacterial Counts and Intestinal Morphology of ICC

The counts of *Enterobacteriaceae* were reduced in the cecal content of HSD+FPLS compared to that of LSD and HSD chicks. Regardless of the effect of stocking density, the antibacterial activity of papaya seed (Sugiharto, 2020) and leaves (Lonkala & Reddy, 2019) in FPLS may be responsible for the reduced *Enterobacteriaceae* population in the cecum of ICC in the present study. Indeed, the fermented feed component itself may also lead to the reduced count of *Enterobacteriaceae* in the cecal content of ICC, as was previously indicated by Olukomaiya *et al*. (2019) in broiler chickens. In this current trial, treatment posed no impact on the intestinal morphology of the ICC. This finding was in contrast to the result reported by Kridtayopas *et al*. (2019), showing the decreased villous height and the ratio of villous height to crypt depth in broilers at high stocking density. In general, there was a close connection between intestinal morphology and intestinal bacterial population in poultry. Kridtayopas *et al*. (2019) reported that broiler chickens bred under high stocking density exhibited higher pathogenic bacteria, lower lactic acid bacteria, lower intestinal villi, and a lower ratio of villi height to crypt depth in comparison to normal stocking density. In the current study, the bacterial populations in the small intestine (particularly in the ileum) were not different among treatment groups. For this reason, the absent impact of treatments on the small intestinal morphology of the ICC could therefore be understood. The differences in the type of chickens, the number of chicks per square meter, and the condition during the trial are among the arguments explaining the divergent impacts of particularly stocking density on poultry.

Internal-organ Weights and Carcass Traits of the ICC

In this study, the effect of stocking density was not substantial on the internal-organ weight of the ICC. In accordance with this finding, Sekeroglu *et al*. (2011) found no effect of stocking density on the relative weight of broiler chickens' internal organs. Irrespective of the effect of stocking density, feeding FPLS resulted in lower a* values in the breast meat when compared, especially to the multienzyme treatment. It was most likely that high fiber content in diets impaired protein digestibility (Sobayo *et al*., 2012) and thus reduced protein availability and deposition in the breast meats. Note that there is a positive relationship between protein content and a* values of chicken meat (Sugiharto *et al*., 2019c). With regard to multienzyme, such treatment may improve fiber and protein digestibility (Attia *et al*., 2012; 2020), and thereby increase the muscle protein deposition as was indicated by the higher a* values. In thigh meats, high stocking density decreased a* values of meat, regardless of the dietary treatments. Perhaps, stress conditions due to high stocking density increased myoglobin oxidation and thereby reduce a* values of chicken meats (Pan *et al*., 2018). Irrespective of the effect of stocking density, the b* values were lower in HSD +mE and HSD+FPLS+mE as compared to control. It has been thought that b* values may reflect the oxidative meat stability, in which higher b* values are associated with the improved oxidative stability of chicken meats (Szymczyk *et al*., 2007). In general, chicken meats, especially thigh meat, are vulnerable to fat oxidation as it is rich in polyunsaturated fatty acids (Botsoglou *et al*., 2002). In respect to the action of complex enzymes in increasing the polyunsaturated fatty acids in broiler meats (Disetlhe *et al*., 2019), the more sensitive thigh meats in HSD +mE and HSD+FPLS+mE chicks to lipid oxidation could therefore be understood.

CONCLUSION

High stocking density resulted in mild stress conditions, as was demonstrated by the increased SOD (as an adaptive mechanism to avoid oxidative stress) and decreased PDW and redness values of the ICC thigh meat. Combination of FPLS and multienzyme ameliorated the adverse effect of high stocking density-induced stress by increasing the production of SOD to scavenge or neutralize the excess free radicals. The improved antioxidative status due to feeding FPLS and multienzyme was attributed to the health improvement (demonstrated by the increased numbers of lymphocytes and reduced cecal Enterobacteriaceae count) in chicks. The improvement in health seemed to increase energy allocation for growth and hence improved income over feed cost of the ICC. Overall, the combination of FPLS and multienzyme was beneficial to alleviate the negative effect of high stocking density on the ICC.

CONFLICT OF INTEREST

The authors declare that they don't have a competing interest.

ACKNOWLEDGEMENT

The study was financially supported by Universitas Diponegoro (Contract No. 329- 88/UN7.6.1/PP/2020, 20 April 2020).

REFERENCES

Aghili, A. H., M. Toghyani, & S. A. Tabeidian. 2019. Effect of incremental levels of apple pomace and multi enzyme on performance, immune response, gut development and blood biochemical parameters of broiler chickens. Int. J. Recycl. Org. Waste Agric. 8:321-334. https://doi.org/10.1007/s40093-019-00305-8

Amer, S.A., M. A. F. Naser, A. A. A. Abdel-Wareth, A. A. Saleh, S. A. M. Elsayed, D. M. A. Fattah, & A. E. Metwally. 2020. Effect of dietary supplementation of alpha-galactosidase on the growth performance, ileal digestibility, intestinal morphology, and biochemical parameters in broiler chickens. BMC Vet. Res. 16:144. https://doi.org/10.1186/s12917-020-02359-7

AOAC. 1995. Official Methods of Analysis. 16th Ed. Association of Official Analytical Chemists, Washington DC.

Asghar, N., S. A. R. Naqvi, Z. Hussain, N. Rasool, Z. A. Khan, S. A. Shahzad, T. A. Sherazi, M. R. S. A. Janjua, S. A. Nagra, M. Zia-Ul-Haq, & H. Ze Jaafar. 2016. Compositional difference in antioxidant and antibacterial activity of all parts of the *Carica papaya* using different solvents. Chem. Cent. J. 10:5. https://doi.org/10.1186/s13065-016-0149-0

Attia, Y. A., M. A. Al-Harthi, & A. S. El-Shafey. 2020. Influence of different time and frequency of multienzyme application on the efficiency of broiler chicken rearing and some selected metabolic indicators. Animals. 10:450. https://doi.org/10.3390/ani10030450

Attia, Y. A., W. S. El-Tahawy, A. E. E. A. El-Hamid, S. S. Hassan, A. Nizza, & M. I. El-Kelaway. 2012. Effect of phytase with or without multienzyme supplementation on performance and nutrient digestibility of young broiler chicks fed mash or crumble diets. Ital. J. Anim. Sci. 11:3. https://doi.org/10.4081/ijas.2012.e56

Bolton, W. 1967. Poultry Nutrition. MAFF Bulletin No.174. HMSO, London.

Botsoglou, N. A., E. Christaki, D. J. Fletouris, P. Florou-Paneri, & A. B. Spais. 2002. The effect of dietary oregano essential oil on lipid oxidation in raw and cooked chicken during refrigerated storage. Meat Sci. 62:259-265. https://doi.org/10.1016/s0309-1740(01)00256-x

Chen, Y., Qiao, Y., Xiao, Y., Chen, H., Zhao, L., Huang, M., & Zhou G. 2016. Differences in physicochemical and nutritional properties of breast and thigh meat from crossbred chickens, commercial Broilers, and spent hens. Asian-Australas. J. Anim. Sci. 29:855-64. https://doi.org/10.5713/ajas.15.0840

Disetlhe, A. R. P., U. Marume, V. Mlambo, & A. Hugo. 2019. Effects of dietary humic acid and enzymes on meat quality and fatty acid profiles of broiler chickens fed canola-based diets. Asian-Australas. J. Anim. Sci. 32:711-720. https://doi.org/10.5713/ajas.18.0408

Gonzalez-Esquerra, R., & S. Leeson. 2006. Physiological and metabolic responses of broilers to heat stress - Implications for protein and amino acid nutrition. World's Poult. Sci. J. 62:282-295. https://doi.org/ 10.1079/WPS200597

Hosseini, S. M. & M. Afshar. 2017. Effect of diet form and enzyme supplementation on stress indicators and bone mineralisation in heat-challenged broilers fed wheat-soybean diet. Ital. J. Anim. Sci. 16:616-623. https://doi.org/10.1080/1828051X.2017.1321973

Huo, X. & P. Na-Lampang. 2012. Thai crossbred chickens can be raised in a high stocking density. Asian J. Poult. Sci. 6:146-151. https://doi.org/10.3923/ajpsaj.2012.146.151

Huo, X. & P. Na-Lampang. 2016. Effects of stocking density on feather pecking and aggressive behaviour in Thai crossbred chickens. Agric. Nat. Res. 50:396-399. https://doi.org/10.1016/j.anres.2016.04.006

Jeong, S.-B., Y. B. Kim, J.-W. Lee, D.-H. Kim, B.-H. Moon, H.-H. Chang, Y.-H. Choi, & K.-W. Lee. 2020. Role of dietary gamma-aminobutyric acid in broiler chickens raised under high stocking density. Anim. Nutr. 6:293-304. https://doi.org/10.1016/j.aninu.2020.03.008

Jobe, M. C., C. N. Ncobela, N. W. Kunene, & A. R. Opoku. 2019. Effects of *Cassia abbreviata* extract and stocking density on growth performance, oxidative stress and liver function of indigenous chickens. Trop. Anim. Health Prod. 51:2567-2574. https://doi.org/10.1007/s11250-019-01979-y

Kridtayopas, C., C. Rakangtong, C. Bunchasak, & W. Loongyai. 2019. Effect of prebiotic and synbiotic supplementation in diet on growth performance, small intestinal morphology, stress, and bacterial population under high stocking density condition of broiler chickens. Poult. Sci. 98:4595-4605. https://doi.org/10.3382/ps/pez152

Law, F. L., I. Zulkifli, A. F. Soleimani, J. B. Liang, & E. A. Awad. 2018. The effects of low-protein diets and protease supplementation on broiler chickens in a hot and humid tropical environment. Asian-Australas. J. Anim. Sci. 31:1291-1300. https://doi.org/10.5713/ajas.17.0581

Lee, S. A., D. Nagalakshmi, M. V. L. N. Raju, S. V. R. Rao, M. R. Bedford, & C. L. Walk. 2019. Phytase as an alleviator of high-temperature stress in broilers fed adequate and low dietary calcium. Poult. Sci. 98:2122-2132. https://doi.org/10.3382/ps/pey566

Li, W., F. Wei, B. Xu, Q. Sun, W. Deng, H. Ma, J. Bai, & S. Li. 2019. Effect of stocking density and alpha-lipoic acid on the growth performance, physiological and oxidative stress and immune response of broilers. Asian-Australas. J. Anim. Sci. 32:1914-1922. https://doi.org/10.5713/ajas.18.0939

Lonkala, S. & A. R. N. Reddy. 2019. Total phenolic, antibacterial activity of carica papaya leaves and *Allium sativum* cloves alone and in combination against multiple strains. Pharmacogn. J. 11:600-602.

Madrid, J., P. Catalá-Gregori, V. García, & F. Hernández. 2010.

Effect of a multienzyme complex in wheat-soybean powder diet on digestibility of broiler chickens under different rearing conditions. Ital. J. Anim. Sci. 9:e1. https://doi.org/10.4081/ijas.2010.e1

Magnuson, A.D., G. Liu, T. Sun, S. A. Tolba, L. Xi, R. Whelan, & X. G. Lei. 2020. Supplemental methionine and stocking density affect antioxidant status, fatty acid profiles, and growth performance of broiler chickens. J. Anim. Sci. 98:skaa092. https://doi.org/10.1093/jas/skaa092

Müller Fernandes, J. I., C. Bortoluzzi, A. M. Burin Junior, A. Rorig, R. Perini, A. Rorig, R. Perini, & A. B. de Cristo. 2015. Effect of different enzymatic supplements in diets of broilers raised at high stocking density. J. Vet. Med. Res. 2:1016.

Oloruntola, O. D., S. O. Ayodele, S. A. Adeyeye, M. H. Ogunsipe, O. T. Daramola, & E. S. Ayedun. 2018. Effect of pawpaw leaf powder and multienzyme supplementation in the diet on performance, digestibility, and oxidative enzyme status of rabbits. J. Basic Appl. Zool. 79:26. https://doi.org/10.1186/s41936-018-0039-1

Olukomaiya, O., C. Fernando, R. Mereddy, X. Li, & Y. Sultanbawa. 2019. Solid-state fermented plant protein sources in the diets of broiler chickens: A review. Anim. Nutr. 5:319-330. https://doi.org/10.1016/j.aninu.2019.05.005

Pan, L., P. F. Zhao, X. K. Ma, Q. H. Shang, S. F. Long, Y. Wu, W. Wang, & X. S. Piao. 2019. *Forsythia suspensa* extract protects broilers against breast muscle oxidative injury induced by corticosterone mimicked pre-slaughter acute stress. Poult. Sci. 97:2095-2105. https://doi.org/10.3382/ps/pey046

Patel, S. S., M. Z. Molnar, J. A. Tayek, J. H. Ix, N. Noori, D. Benner, S. Heymsfield, J. D. Kopple, C. P. Kovesdy, & K. Kalantar-Zadeh. 2013. Serum creatinine as a marker of muscle mass in chronic kidney disease: results of a cross-sectional study and review of literature. J. Cachexia Sarcopenia Muscle. 4:19-29. https://doi.org/10.1007/s13539-012-0079-1

Patria, C. A., R. Afnan, & I. I. Arief. 2016. Physical and microbiological qualities of kampong-broiler crossbred chickens meat raised in different stocking densities. Med. Pet. 39:141-147. https://doi.org/10.5398/medpet.2016.39.3.141

Qaid, M., H. Albatshan, T. Shafey, E. Hussein, & A. M. Abudabos. 2016. Effect of stocking density on the performance and immunity of 1- to 14-d- old broiler chicks. Rev. Bras. Cienc. Avic. 18:683-692. https://doi.org/10.1590/1806-9061-2016-0289

Rashidi, N., M. R. Ghorbani, A. Tatar, & S. Salari. 2019. Response of broiler chickens reared at high density to dietary supplementation with licorice extract and probiotic. J. Anim. Physiol. Anim. Nutr. (Berl). 103:100-107. https://doi.org/10.1111/jpn.13007

Santimone, I., A. Di Castelnuovo, A. De Curtis, M. Spinelli, D. Cugino, F. Gianfagna, F. Zito, M. B. Donati, C. Cerletti, G. de Gaetano, & L. Lacoviello. 2011. White blood cell count, sex and age are major determinants of heterogeneity of platelet indices in an adult general population: Results from the MOLI-SANI project. Haematologica. 96: 1180-1188. https://doi.org/10.3324/haematol.2011.043042

Sekeroglu, A., M. Sarica, M. S. Gulay, & M. Duman. 2011. Effect of stocking density on chick performance, internal organ weights and blood parameters in broilers. J. Anim. Vet. Adv. 10:246-250. https://doi.org/10.3923/javaa.2011.246.250

Silas, A. F. A., O. A. Adeyemi, D. Eruvbetine, O. M. Sogunle, O. B. Oluwole, & G. N. Elemo. 2014. Effect of stocking density and quantitative feed restriction on growth performance, digestibility, haematological characteristics and cost of starting broiler chicks. J. Anim. Health Prod. 2:60-64. https://doi.org/10.14737/journal.jahp/2014/2.4.60.64

Sobayo, R. A., A. O. Oso, O. A. Adeyemi, A. O. Fafiolu, A. V. Jegede, O. M. O. Idowu, O. U. Dairo, R. B. Iyerimah, O. A. Ayoola, & R. A. Awosanya. 2012. Changes in growth, digestibility and gut anatomy by broilers fed diets containing ethanoltreated castor oil seed (*Ricinus communis* L.) meal. Rev. Cient. UDO Agríc. 12:660-667.

Sugiharto, S. 2020. The potentials of two underutilized acidic fruits (*Averrhoa bilimbi* L. and *Phyllanthus acidus* L.) as phytobiotics for broiler chickens. J. Adv. Vet. Res. 10:179-185.

Sugiharto, S., T. Yudiarti, I. Isroli, E. Widiastuti, & F. D. Putra. 2017. Effect of dietary supplementation with *Rhizopus oryzae* or *Chrysonilia crassa* on growth performance, blood profile, intestinal microbial population, and carcass traits in broilers exposed to heat stress. Arch. Anim. Breed. 60:347-56. https://doi.org/10.5194/aab-60-347-2017

Sugiharto, S., T. Yudiarti, I. Isroli, E. Widiastuti, H. I. Wahyuni, & T. A. Sartono. 2019a. Fermented feed as a potential source of natural antioxidants for broiler chickens - A mini review. Agric. Conspec. Sci. 84:313-318.

Sugiharto, S., T. Yudiarti, & I. Isroli. 2019b. Growth performance, haematological parameters, intestinal microbiology, and carcass characteristics of broiler chickens fed two-stage fermented cassava pulp during finishing phase. Trop. Anim. Sci. J. 42:13-120. https://doi.org/10.5398/tasj.2019.42.2.113

Sugiharto, S., T. Yudiarti, I. Isroli, E. Widiastuti, H. I. Wahyuni, T. A. Sartono, A. N. Al-Baarri, & N. Nurwantoro. 2019c. Breast muscle characteristics of avian pathogenic *Escherichia coli* infected broilers fed with antibiotics or probiotic. Poult. Sci. J. 7:131-140. https://doi.org/10.22069/PSJ.2019.16572.1446

Sugiharto, S., E. Widiastuti, I. Isroli, H. I. Wahyuni, & T. Yudiarti. 2020a. Effect of a fermented mixture of papaya leaf and seed powder on production traits and intestinal ecology of the Indonesian indigenous crossbred chickens. Acta Univ. Agric. Silvic. Mendelianae Brun. 68:707-718. https://doi.org/10.11118/actaun202068040707

Sugiharto, S., E. Widiastuti, I. Isroli, T. Yudiarti, T. A. Sartono, & H. I. Wahyuni. 2020b. Effect of feeding fermented mixture of cassava pulp and *Moringa oleifera* leaf powder on immune responses, antioxidative status, biochemistry indices, and intestinal ecology of broilers. Vet. World. 13:392-399. https://doi.org/10.14202/vetworld.2020.392-399

Surai, P. F. 2016. Antioxidant systems in poultry biology: Superoxide dismutase. J. Anim. Res. Nutr. 1:1-17. https://doi.org/10.21767/2572-5459.100008

Surai, P. F., I. I. Kochish, V. I. Fisinin, & M. T. Kidd. 2019. Antioxidant defence systems and oxidative stress in poultry biology: An update. Antioxidants. 8:235. https://doi.org/10.3390/antiox8070235

Szymczyk, B., P. Hanczakowski, W. Szczurek, & M. Frys-Żurek. 2007. Effect of naked oat and enzymes in diets for broiler chickens on quality, fatty acid profile and oxidative stability of breast muscle. Pol. J. Food Nutr. Sci. 57:541-545.

Tallentire, C. W., I. Leinonen, & I. Kyriazakis. 2016. Breeding for efficiency in the broiler chicken: A review. Agron. Sustain. Dev. 36:66. https://doi.org/10.1007/s13593-016-0398-2

Villegas, P. 1987. Avian Virus Diseases Laboratory Manual. College of Veterinary Medicine. University of Georgia, Athens, Georgia, USA.

Welde, Y. & A. Worku. 2018. Identification and extraction of papain enzyme from papaya leaf in Adigrat Towen, Northern Ethiopia. J. Med. Plants Stud. 6:127-130.