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, June 2022, 45(2):202-212
DOI: https://doi.org/10.5398/tasj.2022.45.2.202
Available online at http://journal.ipb.ac.id/index.php/tasj

Growth, Blood, and Intestinal Indices of Broilers at High Density Pens Provided with Fermented *Averrhoa bilimbi* Fruit Filtrate

S. Sugiharto*, E. Widiastuti, T. Sartono, H. Wahyuni, A. Pratama, & T. Yudiarti
Department of Animal Science, Faculty of Animal and Agricultural Sciences, Diponegoro University
Jalan Prof. Soedarto, SH, Tembalang, Semarang, 1269, Indonesia
*Corresponding author: sgh_undip@yahoo.co.id
(Received 06-08-2021; Revised 01-10-2021; Accepted 05-10-2021)

ABSTRACT

The present study investigated the effect of fermented *Averrhoa bilimbi* fruit filtrate (FABFF) on growth, blood indices, and gut ecology of broilers raised at a high stocking density. Using 378 chicks (14 days of age), the study was arranged based on a factorial design with stocking densities (normal with 9 birds/m² or high with 18 birds/m²) and treatment with 2% FABFF from drinking water or not as the two factors. Live body weight and feed consumption were registered weekly, while blood and intestinal contents were collected at day 35. Broilers at high density receiving FABFF had the lowest (p<0.05) feed conversion ratio (FCR). Thymus was smaller (p<0.05) in high-stocked broilers receiving only drinking water. Birds administered FABFF had greater (p<0.05) *bursa of Fabricius* than birds given plain water. The FABFF elevated (p<0.05) serum superoxide dismutase (SOD) levels of broilers. The malondialdehyde levels were higher (p<0.05) in high-stocked broilers given drinking water compared to normal-stocked broilers given drinking water or high-stocked broilers receiving FABFF. Drinking FABFF elevated (p<0.05) the ratio of lactic acid bacteria/coliform in the ileum. Cecal coliform was less (p<0.05) in chicks receiving FABFF than those receiving only water. High-stocked broilers receiving FABFF showed lower (p<0.05) *Enterobacteriaceae* counts than the other birds. The ratio of lactic acid bacteria/coliform increased (p<0.05) in broilers receiving FABFF. In conclusion, FABFF was capable of maintaining the development of immune organs and improving FCR, antioxidative status, and intestinal microbial balance of broilers stocked at high-density pens.

Keywords: acid fruit; antioxidants; broilers; organic acids; overcrowding stress; probiotic

INTRODUCTION

Broiler-chicken farming is currently one of the most important industries to meet domestic animal protein needs in Indonesia. In broiler production, efficiency is the key to the success of the breeders. Since higher profits can be achieved by elevating the number of animals per unit of space, stocking density has important consequences for the poultry industry. However, overcrowding or raising broilers in a high-density environment can trigger stress, which can have a negative impact on the growth, physiological conditions, and the balance of microflora in the gut (Li *et al.*, 2019). In addition, the increased stress intensity is linked to the depression of immunity and, as a result, increased disease susceptibility in chickens (Li *et al.*, 2019; Haque *et al.*, 2020). Previously, antibiotic growth promoters (AGP) were employed to compensate for the retarded growth rate and compromised immune competencies of broilers under stress conditions (Altaf *et al.*, 2019; Haque *et al.*, 2020). However, long-term use of AGP may induce antibiotic resistance both in birds and humans as consumers. For this reason, the use of AGP is now prohibited in broiler production all over the world (Sugiharto, 2016). In the absence of AGP, raising broilers in high-density conditions has been reported to result in problems in the growth and health conditions of the birds (Haque *et al.*, 2020). For sustainable broiler production, finding a safe alternative for AGP is therefore crucial. Attempts have been made to use dietary approaches to address the growth and health concerns in broilers stocked at a high-density condition during the period of antibiotic prohibition (Altaf *et al.*, 2019; Chegini *et al.*, 2019; Li *et al.*, 2019; Rashidi *et al.*, 2019; Magnuson *et al.*, 2020).

Organic acids are commonly used in broiler production to increase the growth rate and wellbeing of the chicks. The acid materials have also been used as antioxidants to help broiler chickens cope with environmental stress's harmful effects (Pearlin *et al.*, 2020). At overcrowding conditions, the application of organic acids (a mixture of lactic acid, citric acid, formic acid, benzoic acid, and acetic acid) has been reported to increase immune competence and intestinal development without affecting the growth of broilers (Fascina *et al.*, 2017). In line with this, the application of ascorbic acid has been shown to boost the growth rate of broilers during

overcrowding conditions, as reported by Adeyemi *et al.* (2015). Likewise, the dietary administration of probiotics was demonstrated to improve the daily weight gain of broilers raised under a high-density condition (Rashidi *et al.,* 2019). Literature suggests that organic acids may be combined with probiotic bacteria to improve their efficacies as a replacement for AGP (Sugiharto, 2016). In this case, Abudabos *et al.* (2016) documented that a blend of organic acids and probiotic *Bacillus* sp. effectively replaced AGP in increasing growth rate, nutrient absorption, and intestinal ecology of broiler chickens.

The fruit of *Averrhoa bilimbi* L. is an acidic fruit containing many bioactive components that can function as antimicrobials, antioxidants, immunomodulators, and acidifiers, all of which are beneficial to the growth and health of broiler chickens. Organic acids, especially acetic acid and citric acid, ascorbic acid, as well as phenolic compounds that can act as antioxidants, can also be found in this underutilized fruit (Sugiharto, 2020). Owing to these properties, the fruit filtrate of *A. bilimbi* may be employed as the alternative to AGP to boost broilers' growth, physiological indices, and intestinal ecology (Pratama *et al.,* 2021). Shrimp paste is a popular food additive made from fermented shrimp with a distinct aroma. Like other fermented foods, shrimp paste can be a natural origin of lactic acid bacteria (LAB) and, therefore, a source of probiotics (Amalia *et al.,* 2018). In general, total colonies of LAB in Indonesian shrimp paste range from 4 to 6 log cfu/g, according to Kobayashi *et al.* (2003). *Lactobacillus plantarum*, *Lactococcus lactis*, *Vagococcus fluvialis,* and *Lactococcus garvieae* are among the LAB isolated from shrimp paste (Maeda *et al.,* 2014), with *L. plantarum* being the most prominent LAB species (Amalia *et al.,* 2018). In the previous investigation, Mareta *et al.* (2020) found the improved feed conversion ratio (FCR), physiological conditions, and intestinal microbial ecosystem of broilers after fermenting *A. bilimbi* fruit filtrate with Indonesian shrimp paste and providing it through drinking water. The high contents of organic acids and LAB in the fermented (using Indonesian shrimp paste as a fermentation starter) *Averrhoa bilimbi* fruit filtrate (FABFF) convincingly reduced intestinal pH and pathogenic coliform counts and improved physiological conditions, resulting in better growth performances of broilers (Mareta *et al.,* 2020). To our knowledge, there has never been any documentation of the use of FABFF for broilers under stress conditions. Therefore, we investigated the impact of FABFF on production traits, blood indices, and gut ecology of broilers reared under stress conditions induced by high stocking density. It was hypothesized that FABFF improved growth, blood parameters, and intestinal ecology of broilers stocked at a high stocking density.

MATERIALS AND METHODS

Preparation of FABFF Using Shrimp Paste

The ripe *A. bilimbi* fruit was obtained from gardens surrounding the campus, while shrimp paste was purchased from the District of Rembang, Central Java Province, Indonesia. The shrimp paste contained LAB of 14.15±0.33 log cfu/g, based on the standard plate count procedure using de Man Rogosa and Sharpe agar (MRS; Merck KGaA, Darmstadt, Germany) cultured anaerobically at 38 °C for 48 hours. The preparation of FABFF using shrimp paste was conducted as described by Mareta *et al.* (2020). In brief, after being washed and draining, the *A. bilimbi* fruit was crushed using an electronic blender and filtered with a cheesecloth. The shrimp paste was then inoculated (g:L) into the prepared fruit filtrate (pH 1.45±0.06, measured using portable pH meter, OHAUS ST300) in an anaerobic jar and cultured anaerobically at room temperature. After 4 days of incubation, the FABFF contained LAB of 30.37±0.16 log cfu/mL and a pH value of 1.30±0.08. The FABFF was stored at -10 °C until used for *in vivo* experiments.

Broiler Chicken Experiment

The Animal Ethics Committee of the Faculty of Animal and Agricultural Sciences, Universitas Diponegoro (No. 57-04/A3/KEP/FPP) approved the broiler study. A number of 400 day-old Lohmann broiler chicks with the initial body weight of 36.6±0.10 g (means ± standard deviation) were raised until day 14 with commercial starter feed comprising 14% moisture, 20% crude protein, 5% crude fat, 5% crude fiber, and 8% crude ash (according to feed label). On day 14, the chicks were individually weighed, and 378 chicks (average body weight of 370±9.25 g) were selected and subsequently used for the present study. The experiment was arranged based on the factorial design with stocking densities (normal density consisting of 9 birds per m^2 or high density consisting of 18 birds per m^2) and treatment with 2% FABFF in drinking water or not as the two factors. Half of the chicks were provided with the FABFF through drinking water within each density group, and the other half received only water without FABFF. Hence, there were 4 different treatment groups each with 7 replicates/pen. From day 14 onward, the chicks were provided with formulated feed (Table 1) in mash form. In this study, the pH value of plain water was 7.49±0.01, while the drinking water containing 2% FABFF the pH value was 5.59±0.05.

On the 4^{th} and 18^{th} days, the experimental birds received the Newcastle disease vaccine via eye drops and drinking water. On day 12, chickens were given the Gumboro vaccine via drinking water. The experimental chicks were reared in a broiler house using rice husk mats during the experiment. Throughout the experiment, the birds were kept in a constant light environment. During the experiment, light bulbs and plastic curtains were used to regulate temperature and humidity in the broiler house.

Data Collection and Analysis

Broiler weight, feed intake, and feed conversion ratio (FCR) were all reported weekly during the experiment. On day 35, blood samples were taken from male

Table 1. Ingredients and chemical constituents of feed for broiler chickens (days 14-35)

Items	(%, Unless otherwise noted)
Yellow maize	58.50
Palm oil	3.00
Soybean meal (crude protein of 44.15%)	34.70
DL-methionine, 990 g	0.19
Bentonite	0.75
Limestone	0.75
Monocalcium phosphate	1.30
Premix[1]	0.34
Chlorine chloride	0.07
Salt	0.40
Calculated chemical constituents:	
ME (kcal/kg)[2]	3,000
Crude protein	20.00
Crude fiber	5.51
Ca	1.02
P (available)	0.58

Note: [1]Provided per kg of feed: 1.100 mg Zn, 1.000 mg Mn, 75 mg Cu, 850 mg Fe, 4 mg Se, 19 mg I, 6 mg Co, 1.225 mg K, 1.225 mg Mg, 1.250,000 IU vit A, 250.000 IU vit D3, 1.350 g pantothenic acid, 1,875 g vit E, 250 g vit K3, 250 g vit B1, 750 g vit B2, 500 g vit B6, 2.500 mg vit B12, 5.000 g niacin, 125 g folic acid, and 2.500 mg biotin.
[2]ME (metabolizable energy) was calculated according to formula (Bolton, 1967): 40.81 {0.87 [crude protein + 2.25 crude fat + nitrogen-free extract] + 2.5}.

chicks reflecting the average body weight of each pen through the brachial vein on the wings. Blood sampling was conducted on one bird from each replicate (seven birds per treatment group). To avoid physiological differences related to sex and body weight, male chicks with bodyweight reflecting the bodyweight of each house were used for sampling. The collected blood sample was placed in an ethylenediaminetetraacetic acid (EDTA)-containing tube to determine the complete blood profile. The remaining blood was placed in an anti-coagulant-free tube for serum processing. To make serum, the blood was allowed to sit at room temperature for 2 hours before being centrifuged for 10 minutes at 5.000 rpm. The serum was kept frozen (at -10 °C) until it was analyzed. The same experimental chicks having been collected their blood samples were killed, de-feathered, and dissected on day 35. The internal organs of experimental chickens were collected and measured using an analytical balance (empty condition). The ileum and caecum digesta were collected for pH measurements and bacterial counts in the intestine. An automated pH meter (portable pH meter, OHAUS ST300) was used to determine the pH of intestine digesta.

Following the procedure described in Sugiharto *et al.* (2018), a complete blood profile analysis was performed using a hematology analyzer (Prima Fully-auto Hematology Analyzer, PT. Prima Alkesindo Nusantara, Jakarta, Indonesia). The enzyme-based colorimetric assays were used to measure the serum concentrations of lipid, uric acid, and creatinine. Total serum protein, albumin, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were measured using spectrophotometric/photometric tests. Total protein in the serum was subtracted from albumin in the serum to get the globulin concentration. The serum biochemistry assays were performed as directed by the manufacturer (DiaSys Diagnostic System GmbH, Holzheim, Germany).

The measurements of superoxide dismutase (SOD) enzyme and malondialdehyde (MDA) activities were conducted in accordance with the methods described by Agusetyaningsih *et al.* (2021). The ability of the samples to decrease pyrogallol auto-oxidation was used to investigate the activity of SOD enzyme. The sample was mixed with 50 mM Tris-HCl (pH 8.2) and 1 mM pentaacetic acid diethylenetriamine. Pyrogallol (final concentration, 0.2 mM) was used to start the reaction, and the absorbance was estimated kinetically. The SOD levels are presented in U/mL. Malondialdehyde activity was detected using a thiobarbituric acid (TBA) reactive material test. Each sample was vortexed, treated with 8.1% sodium dodecyl, and left at room temperature for 10 minutes; controls received the same treatment. Following the incubation period, the samples were treated with 20% acetic acid and 0.6% TBA before being immersed in a water bath for 1 hour at a temperature of 90-95 °C. The supernatant was then treated with a 15:1 combination of butanol and pyridine, which was vortexed and centrifuged. The MDA levels were measured in nmol/mL.

The bacterial populations in the intestine were counted as defined previously by Sugiharto *et al.* (2018). Following aerobic incubation at 38 °C for 24 hours, the number of coliforms and lactose-negative *Enterobacteriaceae* was counted as red and colorless colonies on MacConkey agar (Merck KGaA, Darmstadt, Germany). The coliform and lactose-negative *Enterobacteriaceae* were grouped as *Enterobacteriaceae*. After anaerobic culture at 38 °C for 48 hours, the counting of LAB was counted on MRS agar (Merck KGaA).

Data were analyzed based on a 2 (normal and high stocking densities) x 2 (treatment with FABFF or not) factorial arrangement using the General Linear Models Procedure in SAS (SAS Inst. Inc., Cary, NC, USA). Interactions between the main effects were included in the model, but interactions that were not statistically significant were eliminated. The statistical analysis results are provided as least squares means (LSMEANS) and standard error (SE) for treatments. Duncan's Multiple Range Test was used to distinguish statistically different means ($p<0.05$).

RESULTS

Growth Performance of Broilers

Table 2 presents the performance of broilers raised at various density rates and given either FABFF or not. Broiler chicks raised at a normal density gained a higher weight ($p<0.05$) than those raised at high density. The impact of administering FABFF was not noticed

(p>0.05). There was no meaningful interaction (p>0.05) between the two factors on the final live weight and weight gain. In terms of feed intake and FCR of broilers, there was an interaction (p<0.05) between density and FABFF using shrimp paste. The lowest feed intake was found in the experimental birds raised at high stocking density and given FABFF through drinking water. The highest feed intake was found in the birds raised at normal stocking density and given FABFF through drinking water. Compared to chicks grown at normal density and given FABFF or chicks kept at a high density and given only drinking water, the lowest FCR was found in birds stocked at high density and given FABFF.

Relative Weights of Internal Organs of Experimental Broilers

The relative weights of the internal organ of experimental broilers are presented in Table 3. In terms of the abdominal fat content of the experimental broiler, there was a substantial interaction (p<0.05) between the two factors, with broilers kept at normal density and offered with FABFF having less (p<0.05) abdominal fat content than broilers in the other groups. A significant interaction was also found in the relative weight of the thymus. In comparison to the other birds, the relative thymus weight was smaller (p<0.05) in chicks kept at high stocking density and offered only drinking water without FABFF. The *bursa of Fabricius* was greater (p<0.05) in broilers given FABFF in their drinking water than in birds given only drinking water without FABFF. No interaction (p>0.05) effect was found between the stocking density and supplementation of FABFF on the weight of *bursa of Fabricius*.

Complete Blood Counts of Broilers

The data on the complete blood counts of experimental birds are described in Table 4. There was an interaction effect (p<0.05) between the stocking density and FABFF addition in the drinking water on the values of mean platelet volume (MPV) of experimental broilers. In this respect, broilers kept at high density and offered FABFF in drinking water had higher MPV values (p<0.05) than broilers housed at normal density and offered FABFF. There was no substantial difference in the other blood count parameters of broilers due to different treatments applied.

Serum Biochemistry of Broilers

Table 5 presents the data on serum biochemistry indices of experimental broilers. In general, there was no meaningful interaction effect (p>0.05) between the stock-

Table 2. Performances of broiler chickens at high density pens provided with fermented *Averrhoa bilimbi* fruit filtrate (days 14-35)

Items	Normal density		High density		SE	p value		
	–	+	–	+		D	FAB	D*FAB
Final BW (g)	1843[a]	1881[a]	1711[b]	1727[b]	30.3	<0.01	0.37	0.72
Weight gain (g)	1464[a]	1502[a]	1350[b]	1366[b]	30.3	<0.01	0.37	0.72
Feed intake (g)	2331[b]	2540[a]	2191[c]	2061[d]	40.6	<0.01	0.34	<0.01
FCR	1.60[ab]	1.69[a]	1.63[a]	1.51[b]	0.04	0.05	0.78	<0.01

Note: [a,b]Mean in the same row with different superscripts differ significantly (p<0.05). BW= body weight, FCR: feed conversion ratio, SE= standard error, D= density, FAB= FABFF using shrimp paste, D*FAB= interaction between stocking density and FABFF using shrimp paste, "–": chicks receiving only water, "+"= chicks provided with 2% FABFF through drinking water.

Table 3. Relative weights of internal organs of broiler chickens at high density pens provided with fermented *Averrhoa bilimbi* fruit filtrate

Items (% live BW)	Normal density		High density		SE	p value		
	–	+	–	+		D	FAB	D*FAB
Heart	0.43	0.47	0.46	0.43	0.02	0.78	0.78	0.05
Liver	2.40	2.55	2.66	2.66	0.12	0.14	0.56	0.54
Proventriculus	0.55	0.57	0.49	0.53	0.03	0.08	0.27	0.87
Gizzard	1.38	1.5	1.55	1.49	0.06	0.19	0.66	0.12
Pancreas	0.30	0.33	0.31	0.33	0.02	0.64	0.31	0.83
Duodenum	0.61	0.64	0.77	0.64	0.07	0.26	0.51	0.26
Jejunum	1.39	1.46	1.30	1.30	0.11	0.26	0.75	0.75
Ileum	0.62	0.59	0.68	0.55	0.08	0.89	0.34	0.55
Caeca	0.56	0.59	0.55	0.55	0.03	0.33	0.73	0.66
Abdominal fat	1.24[a]	0.59[b]	1.10[a]	1.15[a]	0.16	0.20	0.07	0.04
Spleen	0.09	0.16	0.13	0.27	0.08	0.34	0.20	0.71
Thymus	0.29[a]	0.23[ab]	0.19[b]	0.26[a]	0.03	0.22	0.85	0.02
Bursa of fabricius	0.15[b]	0.18[a]	0.14[b]	0.17[a]	0.02	0.49	0.04	0.96

Note: [a,b]Mean in the same row with different superscripts differ significantly (p<0.05). BW= body weight, SE= standard error, D= density, FAB= FABFF using shrimp paste, D*FAB= interaction between stocking density and FABFF using shrimp paste, "–": chicks receiving only water, "+"= chicks provided with 2% FABFF through drinking water.

Table 4. Complete blood counts of broiler chickens at high density pens provided with fermented *Averrhoa bilimbi* fruit filtrate

Items	Normal density		High density		SE	p value		
	–	+	–	+		D	FAB	D*FAB
Erythrocytes (10^{12}/L)	4.34	2.94	2.96	3.98	0.90	0.85	0.83	0.19
Hemoglobin (g/dL)	14.4	11.0	11.4	14.1	2.62	0.98	0.91	0.25
Hematocrits (%)	39.2	36.8	35.8	40.2	3.42	1.00	0.77	0.33
MCV (fl)	111	126	110	124	8.36	0.85	0.09	0.98
MCH (pg)	35.4	37.5	37.3	37.2	0.93	0.42	0.31	0.26
MCHC (g/dL)	31.2	30.0	33.1	30.2	1.41	0.43	0.15	0.55
RDW-SD (10^{-15} L)	45.4	50.7	45.1	49.9	4.59	0.91	0.27	0.95
RDW-CV (%)	11.6	10.7	11.7	10.6	0.54	0.97	0.07	0.93
MPV (10^{-15} L)	8.99^{ab}	8.44^{b}	8.83^{ab}	9.20^{a}	0.19	0.12	0.64	0.02
PDW (%)	8.94	8.77	9.29	8.03	0.78	0.80	0.37	0.50
Leukocytes (10^{9}/L)	107	119	114	112	11.90	0.98	0.70	0.56
Heterophils (10^{9}/L)	12.4	11.8	12.8	15.3	3.52	0.26	0.60	0.34
Lymphocytes (10^{9}/L)	94.9	107	101	97.2	10.8	0.87	0.71	0.46
H/L ratio	0.13	0.11	0.12	0.26	0.06	0.22	0.29	0.16
Thrombocytes (10^{9}/L)	14.7	17.4	16.7	13.9	1.79	0.67	0.97	0.13

Note: ^{a,b}Mean in the same row with different superscripts differ significantly ($p<0.05$). SE= standard error, D= density, FAB= FABFF using shrimp paste, D*FAB= interaction between stocking density and FABFF using shrimp paste, "–"= chicks receiving only water, "+"= chicks provided with 2% FABFF through drinking water, MCV= mean corpuscular volume, MCH= mean corpuscular hemoglobin, MCHC= mean corpuscular hemoglobin concentration, RDW-SD= red blood cell distribution width-standard deviation, RDW-CV= red blood cell distribution width-coefficient variation, MPV= mean platelet volume, PDW= platelet distribution width, H/L ratio= heterophils to lymphocytes ratio, SE= standard error, SE= standard error, D= density, FAB= FABFF using shrimp paste, D*FAB= interaction between stocking density and FABFF using shrimp paste.

Table 5. Serum biochemical indices of broiler chickens at high density pens provided with fermented *Averrhoa bilimbi* fruit filtrate

Items	Normal density		High density		SE	p value		
	–	+	–	+		D	FAB	D*FAB
Total cholesterol (mg/dL)	59.0^{b}	72.3^{b}	113^{a}	92.0^{a}	11.4	<0.01	0.76	0.15
Total triglyceride (mg/dL)	40.1^{b}	50.3^{b}	66.3^{a}	70.0^{a}	9.81	0.03	0.47	0.74
LDL (mg/dL)	56.4^{b}	69.4^{b}	110^{a}	88.8^{a}	11.4	<0.01	0.74	0.15
HDL (mg/dL)	45.4	61.6	53.1	45.1	6.65	0.54	0.56	0.08
Cholesterol/HDL ratio	1.40^{b}	1.30^{b}	2.37^{a}	2.37^{a}	0.38	0.01	0.89	0.90
Total protein (g/dL)	1.90^{b}	1.98^{b}	3.26^{a}	2.86^{a}	0.35	<0.01	0.65	0.50
Albumin (g/dL)	0.78^{b}	0.91^{b}	1.33^{a}	1.13^{a}	0.14	0.01	0.79	0.24
Globulin (g/dL)	1.12^{b}	1.07^{b}	1.93^{a}	1.73^{a}	0.23	<0.01	0.58	0.75
A/G ratio	0.74	0.91	0.69	0.68	0.09	0.14	0.40	0.31
Uric acid (mg/dL)	2.88^{b}	3.26^{b}	5.01^{a}	4.86^{a}	0.64	0.01	0.86	0.68
Creatinine (mg/dL)	0.03^{b}	0.03^{b}	0.06^{a}	0.06^{a}	0.01	<0.01	0.73	0.86
AST (U/L)	174	239	337	279	57.3	0.09	0.95	0.29
ALT (U/L)	1.01	1.72	2.56	1.74	0.59	0.20	0.93	0.20

Note: ^{a,b}Mean in the same row with different superscripts differ significantly ($p<0.05$). BW= body weight, SE= standard error, D= density, FAB= FABFF using shrimp paste, D*FAB= interaction between stocking density and FABFF using shrimp paste, "–"= chicks receiving only water, "+"= chicks provided with 2% FABFF through drinking water, LDL= low-density lipoprotein, HDL= high-density lipoprotein, A/G ratio= albumin to globulin ratio, AST= aspartate aminotransferase, ALT= alanine aminotransferase, SEM= standard error of the means.

ing density and FABFF supplementation through drinking water in terms of parameters measured. High stocking density was associated with the increase ($p<0.05$) in total cholesterol, total triglyceride, LDL-cholesterol, total cholesterol/HDL ratio, total protein, albumin, globulin, uric acid, and creatinine, but had no impact on HDL-cholesterol, AST, and ALT concentrations in the serum of experimental broilers. Supplementation with FABFF through drinking water showed no effect ($p>0.05$) on the serum biochemical parameters determined in experimental broilers.

Serum Concentrations of Superoxide Dismutase and Malondialdehyde and Antibody Titers of Experimental Broilers

Table 6 presents the serum concentrations of SOD and MDA of experimental broilers. The serum SOD concentrations differed ($p<0.05$) between densities, with broilers housed at high densities having greater serum SOD concentrations than those housed at normal densities. Drinking FABFF also affected serum SOD concentrations ($p<0.05$). The elevated serum SOD con-

centrations were attributed to the addition of FABFF in drinking water. In terms of serum SOD concentration, there was no interaction effect (p>0.05) between stocking density and FABFF addition in drinking water. In terms of MDA serum concentrations, there was a substantial interaction effect (p<0.05) between stocking density and FABFF addition in drinking water. The MDA levels were higher in high-stocked broilers given drinking water compared to normal-density broilers given drinking water or high-stocked broilers given FABFF. Overcrowding was attributed to the increased (p<0.05) antibody titers toward NDV, whereas FABFF had no significant effect on the antibody titers to NDV. There was an interaction effect (p>0.05) was observed between stocking density and FABFF supplementation on the antibody titers toward NDV in the experimental broilers.

pH and Selected Bacterial Counts in the Intestine of Experimental Broilers

The pH values in the guts of experimental broilers are presented in Table 7. Overall, there was no significant interaction between density rates and whether or not broilers were given FABFF. The effect of each factor on the pH values of intestine segments of the experimental broiler was also not significant.

The significant interaction between stocking density and FABFF supplementation in the drinking water was not found on the selected bacterial counts in the ileal contents of experimental broilers (Table 8). Regardless of stocking density, the addition of FABFF in drinking water was attributed to the elevated (p<0.05) LAB/coliform ratio in the ileum of experimental broilers. In the caecum, the counts of coliform were lower (p<0.05) in experimental birds provided with FABFF than that of experimental broilers given drinking water without FABFF. The significant interaction effect was observed in the caecum of the experimental broiler, particularly in the numbers of *Enterobacteriaceae*. In the latter case, broilers housed at high stocking density and provided with FABFF showed the lower (p<0.05) numbers of *Enterobacteriaceae* compared to the other groups of experimental birds. Irrespective of stocking density, the LAB/coliform ratio in the experimental broilers increased (p<0.05) with the provision of FABFF through drinking water.

DISCUSSION

Our findings revealed that stocking broiler chickens at a high density reduced final body weights, body weight gains, and feed intakes, consistent with results reported by Li *et al.* (2019) and Rashidi *et al.* (2019). The current investigation found that administering FABFF via drinking water improved the FCR of the experimental broilers stocked at a high-density condition. This result implied that the administration of broiler chickens with FABFF improved the digestive performance in the intestine, which eventually improved their nutrient utilizations. In this case, the FABFF may serve as an acidifier and a probiotic source that eventually improves the health and functions of gastrointestinal tracts (Mareta *et al.*, 2020; Pratama *et al.*, 2021). In this study, the effect of FABFF on FCR in the experimental broilers housed at a normal density rate was not as apparent as in the high stocking density condition. The cause of the non-significant effect of FABFF treatment on the FCR of experimental broiler chickens housed in a normal density

Table 6. Serum superoxide dismutase, malondialdehyde, and antibody titer against Newcastle disease of broiler chickens at high density pens provided with fermented *Averrhoa bilimbi* fruit filtrate

Items	Normal density		High density		SE	p value		
	–	+	–	+		D	FAB	D*FAB
SOD (U/mL)	33.9^{by}	35.2^{bx}	39.3^{ay}	41.6^{ax}	0.80	<0.01	0.03	0.50
MDA (nanomol/mL)	2.35^{b}	2.58^{ab}	2.61^{a}	2.35^{b}	0.12	0.86	0.88	0.04
Antibody titers toward NDV (Log_2 GMT)	1.57^{b}	1.29^{b}	2.43^{a}	2.57^{a}	0.22	0.02	0.86	0.61

Note: ^{a,b}Means of SOD denoted by different superscript letters differed significantly (p<0.05) between densities.
^{x,y}Means of SOD denoted by different superscript letters differed significantly (p<0.05) between treatment with FABFF or not.
^{a,b}Means of MDA denoted by different superscript letters in the same row differed significantly (p<0.05).
SE= standard error, D= density, FAB= FABFF using shrimp paste, D*FAB= interaction between stocking density and FABFF using shrimp paste, "–"= chicks receiving only water, "+"= chicks provided with 2% FABFF through drinking water, SOD= superoxide dismutase, MDA= malondialdehyde, NDV= Newcastle disease, GMT= geometric mean titer.

Table 7. pH values of intestinal segments of broilers at high density pens provided with fermented *Averrhoa bilimbi* fruit filtrate

Items	Normal density		High density		SE	p value		
	–	+	–	+		D	FAB	D*FAB
Duodenum	6.79	6.54	6.80	6.80	0.15	0.37	0.42	0.41
Jejunum	6.10	5.59	5.97	5.62	0.36	0.90	0.23	0.82
Ileum	5.96	5.37	5.23	5.29	0.21	0.07	0.24	0.14
Cecum	6.78	7.14	6.76	6.68	0.19	0.23	0.48	0.25

Note: ^{a,b}Mean in the same row with different superscripts differ significantly (p<0.05). BW= body weight, SE= standard error, D= density, FAB= FABFF using shrimp paste, D*FAB= interaction between stocking density and FABFF using shrimp paste, "–"= chicks receiving only water, "+"= chicks provided with 2% FABFF through drinking water.

Table 8. The numbers of selected bacteria in the intestine of broilers at high density pens provided with fermented *Averrhoa bilimbi* fruit filtrate

Items (log cfu/g)	Normal density		High density		SE	p value		
	–	+	–	+		D	FAB	D*FAB
Ileum								
Coliform	6.14	5.63	6.28	5.90	0.47	0.66	0.34	0.89
LNE	6.38	6.74	6.55	6.14	0.59	0.72	0.96	0.52
Enterobacteriaceae	6.40	6.74	7.09	6.27	0.61	0.85	0.70	0.35
LAB	9.88	10.7	10.1	10.4	0.34	0.97	0.13	0.48
LAB/coliform ratio	1.66[b]	2.00[a]	1.66[b]	1.80[a]	0.10	0.34	0.02	0.35
Cecum								
Coliform	8.92[a]	8.50[b]	8.65[a]	8.11[b]	0.18	0.07	0.01	0.75
LNE	7.24	8.04	7.37	6.35	0.49	0.14	0.83	0.07
Enterobacteriaceae	8.73[a]	8.90[a]	8.72[a]	8.29[b]	0.14	0.04	0.36	0.04
LAB	9.65	9.74	9.74	9.74	0.02	0.07	0.07	0.05
LAB/coliform ratio	1.08[b]	1.15[a]	1.13[b]	1.21[a]	0.03	0.05	0.01	0.80

Note: [a,b]Mean in the same row with different superscripts differ significantly ($p<0.05$). LNE= Lactose negative *Enterobacteriaceae*, LAB= lactic acid bacteria; BW= body weight, SE= standard error, D= density, FAB= FABFF using shrimp paste, D*FAB= interaction between stocking density and FABFF using shrimp paste, "–"= chicks receiving only water, "+"= chicks provided with 2% FABFF through drinking water.

remains unknown. However, extra space available for experimental chickens housed in a normal density may have increased their activities (Patria *et al.*, 2016), causing them to use more feed-derived energy for physical activity rather than for growth. In terms of feed intake, increased physical activity appeared to increase energy demand, which in turn increased feed consumption in the experimental broiler chickens. Also, because there was less competition among chicks in a normal density cage, access to feed may be increased, resulting in increased feed intake.

Our findings showed that broilers raised at a normal density and offered with FABFF had lower abdominal fat content than broilers in the other treatment groups. This implied the fat-lowering effect of the FABFF on modern broiler chickens. Owing to its acid content, the FABFF may inhibit *de novo* fatty acid synthesis (Peng *et al.*, 2016), resulting in the reduced deposition of fat in the abdomen of experimental broilers. Following this, the LAB content in the experimental broiler treated with FABFF may suppress the *de novo* synthesis of fatty acid in the liver and increase β-oxidation of fatty acids, which may reduce the abdominal fat pad of experimental broilers (Wang *et al.*, 2017). However, it should be noted that the fat-lowering effect of the FABFF was not apparent when broilers were stocked at high-density conditions. It was most likely that the stress induced by high stocking density counteracted the potentials of the FABFF in lowering the fat deposition in broilers. Note that high stocking density is generally ascribed to the higher abdominal fat deposition due to experimental broilers' less activity (locomotion) (Beg *et al.*, 2011). In this study, the relative weight of the thymus was smaller in the experimental broilers stocked at high density and offered simply drinking water without FABFF. Interestingly, administration of the FABFF through drinking water was capable of maintaining the thymus weight, comparable to the experimental broilers raised at normal density pens. Apart from the effect of stocking density, the FABFF administration increased the relative weight of *bursa of fabricius* of experimental broilers. In this case, some bioactive components in the *A. bilimbi* fruit filtrate, such as organic acids and LAB (Sugiharto, 2020), as well as LAB in the shrimp paste (Amalia *et al.*, 2018), may be responsible for supporting the development of immune organs in the experimental broilers. In support of our result, Ghazalah *et al*. (2011) documented that feeding formic acid increased the relative weight of the main immune organs of broilers, i.e., spleen, thymus, and bursa *Fabricius*. Likewise, LAB-based probiotic treatment was associated with the heavier weights of immune organs in the broiler (Alkhalf *et al.*, 2010).

Broilers kept in high-density pens and provided FABFF showed higher MPV values than those kept in normal density pens and provided FABFF. Park *et al.* (2018) found that stress caused by high stocking density lowered the MPV level of broiler ducks in a previous study. However, it seemed not the case in our present study since stocking density had no substantial impact on the MPV levels of experimental broilers chickens. In human studies, researchers discovered an inverse relationship between MPV and plasma bicarbonate (HCO^{3-}) (Hornsey *et al.*, 2006; Nasri & Baradaran, 2006). High stocking density, which is associated with the environmental stress due to high ambient temperature (Abudabos *et al.*, 2016), has been attributed to the increased respiratory rate (as part of the thermoregulatory mechanism to release the excessive heat load from the body), resulting in the increased CO_2 release to the environment. The latter condition results in the decreased bicarbonate concentration in the plasma and, consequently, increased MPV levels in the broilers. Yet, this assumption should be interpreted with caution as there was no significant difference in MPV values between birds housed at high and normal stocking densities in the present study.

High stocking density was attributed to the increase in the serum concentrations of total cholesterol, triglyceride, LDL-cholesterol, as well as the ratio of total

cholesterol to HDL-cholesterol. In agreement with this finding, Qaid *et al.* (2016) noticed the increased serum level of cholesterol in broilers reared at high-density pens. Also, Gholami *et al.* (2020) pointed out that high stocking density is implied in the increased plasma triglycerides and very-low-density lipoprotein (VLDL) concentrations of broilers. Similar to broiler chickens, Park *et al.* (2018) also reported the increased total cholesterol, triglycerides, and LDL-cholesterol in the blood of ducks raised under high-density conditions. The changes in serum lipid profiles of high-stocked broilers in this study indicated stress conditions in the experimental broiler chicks, which is consistent with the observation of Qaid *et al.* (2016). Stress condition may activate corticosterone, which in turn enhance the expression of genes synthesizing cholesterol and fatty acid (Zaytsoff *et al.*, 2019). Total protein, albumin, and globulin concentrations increased with stocking broilers at high-density pens. This result was in agreement with the observation of Gholami *et al.* (2020), in which increasing density of birds from 10 to 17 chicks/m^2 increased the levels of total protein and globulin in the plasma. The alterations in the concentrations of circulatory protein (and its fractions) in the experimental birds seem to be the response of birds toward the increased needs of protein (for physiological and immunological needs) during stress conditions, but on the other hand, the supply of protein decrease due to the decrease in feed intake. The decreased feed intake may also reduce exogenous energy supply, and hence the birds may use body energy reserves, including muscle protein. In this regard, protein catabolism may increase circulatory protein levels as well as the levels of uric acid and creatinine (Qaid & Al-Garadi, 2021). Indeed, the latter condition was also observed in the present study. Different from the above-mentioned reports, Mahmoud & El-Rayes (2016) showed that increasing the density of stocking rate from 10 to 14 chicks/m^2 reduced total protein, albumin, and globulin values in the plasma of broilers. The exact reason for such divergent results remains unclear; however, the variations in the number of birds per m^2, nutrient compositions of feeds, feed intake, ages of broilers, and the conditions during the experiment (especially ambient temperature) may determine the effect of stocking density on the protein turnover in broilers.

Data in our present study showed that broilers housed at high densities had greater serum SOD concentrations than those housed at normal densities. Our present finding was consistent with those reported in broiler chickens by Jang *et al.* (2014) and in French Pekin ducks by Abo Ghanima *et al.* (2020). These investigators pointed out that high stocking density resulted in the increased SOD levels of poultry. However, our findings contrasted with those reported by Li *et al.* (2019), who found that SOD levels were lower in broilers raised at high stocking density. SOD levels in birds raised under overcrowding conditions can be attributable to a number of reasons, one of which is the intensity of stress experienced by the birds. In the study of Li *et al.* (2019), although the birds were raised in the environmentally controlled house (possibly the heat stress due to overcrowding was not the major factor inducing stress), the stocking density of 45.0 kg body weight/m^2 seemed to reduce the space per individual chick and induce the intense competition to get feed and water resulting in severe stress for broilers. In our current investigation, the birds were reared in an opened house with a density of 30.9 kg/m^2, which implied more space available and less competition (less stress) to reach the feeder and drinker. Indeed, severe oxidative stress was suggested to suppress the SOD activity of broilers (Li *et al.*, 2019). In our case, the increased SOD concentrations seemed to be the response of experimental chickens to the mild stress induced by high stocking density. This inference was supported by Abo Ghanima *et al.* (2020), who stated that enhanced antioxidant enzyme activities were a natural response of birds during overcrowding stress to protect living cells from oxidative damage. Regarding the ratio of heterophils to lymphocytes (H/L ratio), the elevated H/L ratio has been attributed to the high-stress levels in the experimental chickens (Qaid *et al.*, 2016). Indeed, our current investigation did not show any effect of density rate on the H/L ratio of birds. This may suggest that stocking at a high-density pen did not induce severe stress in broilers during our current trial. In this study, drinking of FABFF increased the SOD levels of broilers. Previous studies reported that the acids (He *et al.*, 2020), probiotics (Bai *et al.*, 2018), and antioxidants (Delles *et al.*, 2014) were attributed to the increased SOD levels of birds. In such a case, these active components may increase the antioxidant capacity of birds, primarily by activating the relative gene expression of antioxidants (Bai *et al.*, 2018). The SOD is an antioxidant enzyme that reacts with reactive oxygen species directly (Delles *et al.*, 2014). Drinking water containing FABFF would beneficial in alleviating the oxidative stress and improving the physiological conditions and well-being of broilers. Stress-induced free radical overproduction has been linked to higher MDA levels and thus tissue damage (Bai *et al.*, 2018). In this work, supplementing broilers with FABFF reduced the elevated MDA levels caused by high-density stress. Cengiz *et al.* (2012) found similar findings, in which a dietary organic-acids blend reduced the increased MDA concentration caused by delayed feed access. Similarly, Bai *et al.* (2018) found that probiotic bacteria (*Bacillus subtilis*) reduced oxidative stress in birds, as was seen by a drop in MDA levels. In terms of antioxidant activity, Chen *et al.* (2020) discovered that feeding pterostilbene (polyphenol) reduced MDA levels in the livers of broiler chickens. Overall, enhanced antioxidant enzymes like SOD appeared to reduce the overproduction of free radicals and consequently lipid peroxidation and MDA levels of broilers (Chen *et al.*, 2020).

Antibody titers against NDV were found to be greater in broilers housed in high-density pens in this investigation. This increase was in contrast to the result reported by Law *et al.* (2019), who found that high stocking density reduced antibody titers against NDV, and Uzum & Toplu (2013), who reported that stocking density had no significant effect on antibody titers against NDV. The cause of these disparities has remained

a mystery up to now. Antibody titers against NDV (Messaï *et al.,* 2019) and infectious bursal disease (IBD) (Salhi *et al.,* 2020) were found to be higher in herds with poor cleanliness compared to herds with good hygiene in previous investigations. In this case, a high stocking density condition was linked to greater excreta (poor hygiene) and thus higher antibody titers. However, this inference should be treated with caution because in most cases, poor cleanliness has been linked to a greater broiler mortality rate (Messaï *et al.,* 2019; Salhi *et al.,* 2020). In this study, no mortality was found during the period of study.

It was apparent in this study that drinking FABFF increased the ratio of LAB/coliform in the ileum and cecum of broilers. In the intestine of broilers, the increasing ratio of LAB to coliform indicates that LAB was more dominant than coliform. Indeed, the better resilience of broilers to intestinal diseases has been attributed to the increased ratio of LAB/coliform (Dell'Anno *et al.,* 2021). Furthermore, the enhanced ratio of LAB/coliform may be useful in strengthening broiler immunological capabilities, according to the later authors. The higher ratio of intestinal LAB/coliform caused by FABFF administration was consistent with the better immunological competencies in broilers, as was evidenced by the heavier *bursa of Fabricius* in this investigation. The use of FABFF in drinking water reduced the number of coliform bacteria in the cecum. This condition appeared to be caused by the antibacterial activities of the FABFF (Mareta *et al.,* 2020; Pratama *et al.,* 2021). In terms of *Enterobacteriaceae* content in the cecum of broilers housed at high stocking density pens, the antibacterial activity of the FABFF was more evident than in those housed at standard density pens. The cause of the latter condition was unknown, but it was thought that, aside from the antibacterial activity of the FABFF, less feed intake in the high-stocked broilers limited *Enterobacteriaceae*'s access to substrate required for bacterial growth in the cecum.

CONCLUSION

The fermented *Averrhoa bilimbi* fruit filtrate (FABFF) improved the development of the immune organ, FCR, antioxidative status, and intestinal microbial balance of broilers stocked at high-density pens. However, FABFF failed to promote the growth rate of experimental broilers.

CONFLICT OF INTEREST

The authors declared that they had no conflict of interest

ACKNOWLEDGEMENT

The experiment was financially supported by Directorate of Research and Community Services, Directorate General of Higher Education, Ministry of Education, Culture, Research, and Technology, Republic of Indonesia, the year 2021.

REFERENCES

Abo Ghanima, M. M., M. E. Abd El-Hack, A. E. Taha, V. Tufarelli, V. Laudadio, & M. A. E. Naiel. 2020. Assessment of stocking rate and housing system on performance, carcass traits, blood indices, and meat quality of french pekin ducks. Agriculture 10:273. https://doi.org/10.3390/agriculture10070273

Abudabos, A. M., M. A. Murshed, M. M. Qaid, & A. G. Abdelrahman. 2016. Effect of probiotics on serum biochemical and blood constituents in chicken challenged with *Salmonella enterica* Subsp Typhimurium. Trop. J. Pharm. Res. 15:461. https://doi.org/10.4314/tjpr.v15i3.5

Adeyemi, O. A., E. O. Adedoyin, O. O. Olaleye, P. C. Njoku, & K. A. Sanwo. 2015. Effects of ascorbic acid supplementation on broiler chickens stocked at two different densities in a humid tropical environment. Mal. J. Anim. Sci. 18:89-101.

Agusetyaningsih, I., E. Widiastuti, H. I. Wahyuni, T. Yudiarti, R. Murwani, T. A. Sartono, & S. Sugiharto. 2021. Effect of encapsulated *Cosmos caudatus* leaf extract on the physiological conditions, immune competency, and antioxidative status of broilers at high stocking density. Annals Animal of Science https://doi.org/10.2478/aoas-2021-0043

Alkhalf, A., M. Alhaj, & I. Al-Homidan. 2010. Influence of probiotic supplementation on immune response of broiler chicks. Egypt. Poult. Sci. 30:271-280.

Altaf, M. T., A. Mahmud, S. Mehmood, & Saima. 2019. Effects of supplemented growth promoters on performance and intestinal morphology in broilers reared under different stocking densities. Rev. Bras. Cienc. Avic. 21:eRBCA-2019-1073. https://doi.org/10.1590/1806-9061-2019-1073

Amalia, U., Sumardianto, & T. W. Agustini. 2018. Characterization of lactic acid bacteria (LAB) isolated from Indonesian shrimp paste (terasi). IOP. Conf. Ser. Earth. Environ. Sci. 116:012049. https://doi.org/10.1088/1755-1315/116/1/012049

Bai, K., C. Feng, L. Jiang, L. Zhang, J. Zhang, L. Zhang, & T. Wang. 2018. Dietary effects of *Bacillus subtilis* fmbj on growth performance, small intestinal morphology, and its antioxidant capacity of broilers. Poult. Sci. 97:2312-2321. https://doi.org/10.3382/ps/pey116

Beg, M. A. H., M. A. Baqui, N. R. Sarker, & M. M. Hossain. 2011. Effect of stocking density and feeding regime on performance of broiler chicken in summer season. Int. J. Poult. Sci. 10:365-375. https://doi.org/10.3923/ijps.2011.365.375

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

Cengiz, O., B. H. Koksal, O. Tatli, O. Sevim, H. Avci, T. Epikmen, D. Beyaz, S. Buyukyoruk, M. Boyacioglu, A. Uner, & A. G. Onol. 2012. Influence of dietary organic acid blend supplementation and interaction with delayed feed access after hatch on broiler growth performance and intestinal health. Vet. Med. (Praha). 57:515–528. https://doi.org/10.17221/6363-VETMED

Chegini, S., A. Kiani, B. P. Kavan, & H. Rokni. 2019. Effects of propolis and stocking density on growth performance, nutrient digestibility, and immune system of heat-stressed broilers. Ital. J. Anim. Sci. 18:868-876. https://doi.org/10.1080/1828051X.2018.1483750

Chen, Y., Y. Chen, H. Zhang, & T. Wang. 2020. Pterostilbene as a protective antioxidant attenuates diquat-induced liver injury and oxidative stress in 21-day-old broiler chickens. Poult. Sci. 99:3158-3167. https://doi.org/10.1016/j.psj.2020.01.021

Dell'Anno, M., M. L. Callegari, S. Reggi, V. Caprarulo, C. Giromini, A. Spalletta, S. Coranelli, C. A. S. Rossi, & L.

Rossi. 2021. *Lactobacillus plantarum* and *Lactobacillus reuteri* as functional feed additives to prevent diarrhoea in weaned piglets. Animals 11:1766. https://doi.org/10.3390/ani11061766

Delles, R. M., Y. L. Xiong, A. D. True, T. Ao, & K. A. Dawson. 2014. Dietary antioxidant supplementation enhances lipid and protein oxidative stability of chicken broiler meat through promotion of antioxidant enzyme activity. Poult. Sci. 93:1561-70. https://doi.org/10.3382/ps.2013-03682

Fascina, V. B., G. A. M. Pasquali, F. B. Carvalho, E. M. Muro, F. Vercese, M. M. Aoyagi, A. C. Pezzato, E. Gonzales, & J. R. Sartori. 2017. Effects of phytogenic additives and organic acids, alone or in combination, on the performance, intestinal quality and immune responses of broiler chickens. Rev. Bras. Cienc. Avic. 19:497-508. https://doi.org/10.1590/1806-9061-2016-0422

Ghazalah, I., A. M. Attal, K. M. outElkloub, R. El moustafa, & F. H. Shata. 2011. Effect of supplementation of organic acids on performance, nutrients digestibility and health of broiler chicks. Int. J. Poult. Sci. 10:176-184. https://doi.org/10.3923/ijps.2011.176.184

Gholami, M., M. Chamani, A. Seidavi, A. A. Sadeghi, & M. Aminafschar. 2020. Effects of stocking density and climate region on performance, immunity, carcass characteristics, blood constitutes, and economical parameters of broiler chickens. Revista Brasileira de Zootecnia 49:e20190049. https://doi.org/10.37496/rbz4920190049

Haque, M. H., S. Sarker, M. S. Islam, M. A. Islam, M. R. Karim, M. Kayesh, M. Shiddiky, & M. S. Anwer. 2020. Sustainable antibiotic-free broiler meat production: current trends, challenges, and possibilities in a developing country perspective. Biology 9:411. https://doi.org/10.3390/biology9110411

He, S., Q. Yin, Y. Xiong, D. Liu, & H. Hu. 2020. Effects of dietary fumaric acid on the growth performance, immune response, relative weight and antioxidant status of immune organs in broilers exposed to chronic heat stress. Czech. J. Anim. Sci. 65:104-113. https://doi.org/10.17221/13/2020-CJAS

Hornsey, V. S., K. McColl, O. Drummond, L. McMillan, A. Morrison, L. Morrison, I. R. MacGregor, & C. V. Prowse. 2006. Extended storage of platelets in SSP platelet additive solution. Vox. Sang. 91:41-6. https://doi.org/10.1111/j.1423-0410.2006.00771.x

Jang, I.-S., S.-H. Yun, Y.-H. Ko, S.-Y. Kim, M.-H. Song, J.-S. Kim, S.-H. Sohn, & Y.-S. Moon. 2014. The effect of stocking density and strain on the performance and physiological adaptive responses in broiler chickens. Korean Journal of Poultry Science 41:205-15. https://doi.org/10.5536/KJPS.2014.41.3.205

Kobayashi, T., M. Kajiwara, M. Wahyuni, T. Kitakado, N. HamadaSato, C. Imada, & E. Watanabe. 2003. Isolation and characterization of halophilic lactic acid bacteria isolated from "terasi" shrimp paste: A traditional fermented seafood product in Indonesia. J. Gen. Appl. Microbiol. 49:279-286. https://doi.org/10.2323/jgam.49.279

Law, F. L., I. Zulkifli, A. F. Soleimani, J. B. Liang, & E. A. Awad. 2019. Effects of reduced-protein diets supplemented with protease in broiler chickens under high stocking density. Anim. Prod. Sci. 59:2212-2221. https://doi.org/10.1071/AN18477

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

Maeda, M., A. Shibata, G. Biswas, H. Korenaga, T. Kono, T. Itami, & M. Sakai. 2014. Isolation of lactic acid bacteria from Kuruma shrimp (*Marsupenaeus japonicus*) intestine and assessment of immunomodulatory role of a selected strain as probiotic. Mar. Biotechnol. 16:181-192. https://doi.org/10.1007/s10126-013-9532-1

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

Mahmoud, R. M. & T. K. El-Rayes. 2016. Effect of stocking density and probiotic supplementation on broiler performance. Journal of Animal and Poultry Production 7:491-497. https://doi.org/10.21608/jappmu.2016.48814

Mareta, I., G. Nathaniel, T. Yudiarti, E. Widiastuti, H. I. Wahyuni, & S. Sugiharto. 2020. Effect of *Averrhoa bilimbi* fruit filtrate and shrimp paste mixture on performance, gut microbes and blood profile of broilers. Jurnal Ilmu Ternak dan Veteriner 25:182-189.

Messaï, C. R., O. Salhi, D. Khelef, A. Lounas, A. Mohamed-Cherif, R. Kaidi, & K. Aït-Oudhia. 2019. Serological, clinical, and risk factors of the Newcastle disease on broilers flocks in Algeria. Vet. World. 12:938-944. https://doi.org/10.14202/vetworld.2019.938-944

Nasri, H. & A. Baradaran. 2006. Platelet count and mean volume (MPV) in association with plasma HCO_3 - in regular hemodialysis patients. Rev. Bras. Hematol. Hemoter. 28:127-130. https://doi.org/10.1590/S1516-84842006000200012

Park, B. S., K. H. Um, S. O. Park, & V. A. Zammit. 2018. Effect of stocking density on behavioral traits, blood biochemical parameters and immune responses in meat ducks exposed to heat stress. Arch. Anim. Breed. 61:425-432. https://doi.org/10.5194/aab-61-425-2018

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

Pearlin, B. V., S. Muthuvel, P. Govidasamy, M. Villavan, M. Alagawany, M. Ragab Farag, K. Dhama, & M. Gopi. 2020. Role of acidifiers in livestock nutrition and health: A review. J. Anim. Physiol. Anim. Nutr. (Berl). 104:558-569. https://doi.org/10.1111/jpn.13282

Peng, M., J. Han, L. Li, & H. Ma. 2016. Suppression of fat deposition in broiler chickens by (-)-hydroxycitric acid supplementation: a proteomics perspective. Sci. Rep. 6:32580. https://doi.org/10.1038/srep32580

Pratama, A., I. Mareta, T. Yudiarti, H. I. Wahyuni, E. Widiastuti, & S. Sugiharto. 2021. Administration of fermented *Averrhoa bilimbi* L. fruit filtrate on growth, hematological, intestinal, and carcass indices of broilers. Trop. Anim. Sci. J. 44:79-89. https://doi.org/10.5398/tasj.2021.44.1.79

Qaid, M. M. & M. A. Al-Garadi. 2021. Protein and amino acid metabolism in poultry during and after heat stress: a review. Animals 11:1167. https://doi.org/10.3390/ani11041167

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

Salhi, O., C.-R. Messai, K. Ait-Oudhia, A. Lounas, A. Abdelli, R. Kaidi, & D. Khelef. 2020. Epidemiological, serological, clinical and risk factors of infectious bursal disease (IBD), in broilers flocks in Algeria. Agriculture 1-2:113-114.

Sugiharto, S. 2016. Role of nutraceuticals in gut health and

growth performance of poultry. Journal of the Saudi Society of Agricultural Sciences. 15: 99-111. https://doi.org/10.1016/j.jssas.2014.06.001

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.

Uzum, M. H., & H. D. O. Toplu. 2013. Effects of stocking density and feed restriction on performance, carcass, meat quality characteristics and some stress parameters in broilers under heat stress. Revue. Méd. Vét. (Toulouse). 164:546-554.

Wang, H., X. Ni, X. Qing, D. Zeng, M. Luo, L. Liu, G. Li, K. Pan, & B. Jing. 2017. Live probiotic *Lactobacillus johnsonii* BS15 promotes growth performance and lowers fat deposition by improving lipid metabolism, intestinal development, and gut microflora in broilers. Front. Microbiol. 8:1073. https://doi.org/10.3389/fmicb.2017.01073

Zaytsoff, S. J. M., C. L. J. Brown, T. Montina, G. A. S. Metz, D. W. Abbott, R. Richard, E. Uwiera & G. D. Inglis. 2019. Corticosterone-mediated physiological stress modulates hepatic lipid metabolism, metabolite profiles, and systemic responses in chickens. Sci. Rep. 9:19225. https://doi.org/10.1038/s41598-019-52267-6