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Effect of sprouted papaya seed meal on physiological conditions, intestinal bacterial populations and meat quality of broilers

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ABSTRACT

The study investigated the effect of sprouted papaya seed meal (SPSM) on physiological conditions, intestinal bacteria and meat quality of broilers. A 390 broiler chicks were distributed to T0 (control feed), T1 (feed with 2.5% papaya seed meal [PSM]), T2 (1% SPSM), T3 (2.5% SPSM), and T4 (5% SPSM). Blood, intestinal digesta and meat were obtained at day 36. Feeding 2.5% PSM lowered (P<0.05), but SPSM up to 5% had no effect on daily gain. PSM reduced (p<0.05) feed intake, but not SPSM. Feed efficiency was lower (P<0.05) in T4. Feeding 5% SPSM increased (P=0.06) *bursa of fabricius*. T1, T3 and T4 had lower (P=0.09) heterophils. Mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were lower (P<0.05) in T4. Cholesterol to high-density lipoprotein (HDL) ratio of PSM and SPSM was lower (P<0.05) than control. SPSM at 2.5% increased (P<0.05) serum HDL. PSM-fed birds had lower cholesterol (P=0.07), triglyceride (P=0.09) and low-density lipoprotein (P=0.09). PSM or SPSM decreased (P<0.05) serum total protein, albumin and globulin. PSM and SPSM reduced (P<0.05) creatinine. Alanine aminotransferase was reduced (P<0.05) with SPSM at 1 and 2.5%. Ileal lactic acid bacteria to coliform ratio in PSM and SPSM was greater (P<0.05) than in control. Ileal coliform was lower (P=0.08) in PSM and SPSM. PSM reduced (P=0.08) saturated fatty acids, while 1 and 2.5% SPSM increased (P=0.09) unsaturated fatty acids contents of meats. In conclusion, SPSM improved immune competence, blood lipid profile and gut bacterial population of broilers.

Keywords: antioxidant, broiler, germination, seed, stress

INTRODUCTION

Modern broiler strains have generally been attributed to fast growth rates, efficient feed utilization, and a short production time. Apart from its superiority, broiler chickens are very vulnerable to stress induced by intensive rearing management. Indeed, stress are associated with retarded growth rate, disrupted physiological conditions, imbalanced intestinal microbiology, as well as impaired meat quality (Sugiharto *et al*., 2017). Subtherapeutic antibiotics and synthetic antioxidants have been employed in broiler production to counteract the negative effects of stress. Yet, the long-term use of subtherapeutic antibiotics and/or synthetic antioxidants may leave residues on meats that are hazardous to consumers' health. This might lead to the use of subtherapeutic antibiotics being prohibited and synthetic antioxidants being restricted in broiler

farming. Following the removal and restriction respectively of antibiotics and synthetic antioxidants from broiler diets, functional feeds have recently received a lot of interest as feed ingredients. Sugiharto *et al*. (2018) suggested that functional feed may serve compounds that promote broiler health and growth.

Papaya (*Carica papaya* L.) seed has been incorporated in broiler diet either as a feed ingredient to reduce the proportion of soybean meal (Bolu *et al*., 2009) or as functional feed ingredient to exert health improvement in broilers (Adegbeye *et al*., 2020). Papaya seed contains 30.1% crude protein (Bolu *et al*., 2009), and also several bioactive compounds including alkaloid, tannin, phenol, saponin, and flavonoid, which can serve as antimicrobial, immune modulator, and antioxidants (Sugiharto, 2020a). Aside from its useful properties, the use of papaya seed in broiler diet has been limited by its high fibre (24.3%; Azevedo and Campagnol, 2014) and anti-nutritional components (Sugiharto, 2020a), which thereby impairs the digestibility of chicks. Germination or sprouting is one of the simple methods to increase protein and amino acids, reduce crude fibre, improve fibre fraction as well as reduce anti-nutritional components of seeds (Hooda and Jood, 2003; Nkhata *et al*., 2018). Sprouting has also been reported to improve the functional properties of seeds. In this regards, sprouting has been documented to enhance the antioxidant and antimicrobial activities of seeds (Sehrawat *et al*., 2020).

Several sprouted seeds have been included in broiler diets, for instances mung bean, red sorghum, barley, pearl millet, buckwheat, soybean, etc. (Sugiharto, 2021). Yet, the use of sprouted papaya seed in broiler feed has never been reported so far. Therefore, the present study aimed to investigate the effect of sprouted papaya seed meal (SPSM) on physiological conditions, intestinal bacterial populations and meat quality of broiler chickens.

MATERIALS AND METHODS

Production of SPSM

The ripe papaya seed was collected from street vendors around the university. The seed was washed with running water and then spread on tray at room temperature for about 24 hours. The papaya seeds were soaked for 24 hours, and subsequently put in perforated bucket. The seed was allowed to germinate for two weeks. Each day, the seed in bucket was sprinkled with water to maintain the seed wet during the sprouting process. After this period, the sprouted seed was collected, sun-dried, and finely ground. To produce papaya seed meal (PSM), the ripe papaya seed was directly sun-dried after being washed using running water. The seed was then finely ground and placed in room temperature until use for *in vivo* experiment. For proximate analysis, about 100 g sample of PSM and SPSM was obtained. Data on proximate composition of PSM and SPSM are presented in Table 1.

In vivo experiment

Three hundred and ninety day-old Lohmann broiler chicks were fed with commercial starter diet having 23.0% crude protein, 5.0% crude fibre, 5.0% crude fat, and 7.0% ash (based on feed label) from day 0 to 14. Starting from days 14, the birds were randomly distributed to T0 (chicks received basal feed), T1 (feed with 2.5% PSM), T2 (feed with 1% SPSM), T3 (feed with 2.5% SPSM), and T4 (feed with 5% SPSM). The study was conducted based on a completely randomized design (CRD), with five groups and six replicates. Each replicate contained 13 birds, and thus each dietary group had 78 birds. An open-sided chicken house was used to raise the birds during the experiment, with rice husk was used as bedding material. Throughout the rearing period, a constant lighting schedule was implemented. The feeds were prepared in mash form to be isocaloric and isonitrogenous (Table 2). The Indonesian National Standard for broiler finisher feed was used as a reference during feed formulation. The chicks were vaccinated with Newcastle disease vaccine on days 4 and 18, and Gumboro vaccine on day 12. Daily weight gain (DWG) was determined as = (final body weight – initial weight)/days of treatment. Daily feed intake (DFI) was defined as = (feed offered – leftover feed)/days of feeding, while feed efficiency was estimated by dividing the body weight gain by the feed intake during treatment, and then multiplying the result by 100%.

At the end of experiment (day 36), two male broilers from each replicate/pen were taken and sample of blood was collected from their wing vein using disposable 3 mL syringe. The blood was put in ethylenediaminetetraacetic acid (EDTA) containing vacutainer for the determination of complete blood counts, and the remaining blood was placed in vacutainer-free anticoagulant for the production of serum. The Prima Ful-

Table 1. Proximate Compositions of Papaya Seed and Sprouted Papaya Seed Meals

Compositions (% DM)	PSM	SPSM
Moisture	7.19	10.3
Crude protein	21.7	24.7
Crude fat	22.9	21.2
Crude fibre	38.7	36.9
Ash	10.8	6.67

DM: dry matter, PSM: papaya seed meal, SPSM: sprouted papaya seed meal

Table 2. Ingredients and Chemical Compositions of Feeds (Days 14-36)

Variables (%, unless otherwise noted)	T0	T1	T2	T3	T4
Yellow maize	58.5	57.0	58.1	57.3	56.1
Palm oil	3.00	3.00	2.90	2.90	2.80
SBM	34.7	33.7	34.2	33.5	32.3
PSM	-	2.50	-	-	-
SPSM	-	-	1.00	2.50	5.00
DL-methionine	0.19	0.19	0.19	0.19	0.19
Bentonite	0.75	0.75	0.75	0.75	0.75
Limestone	0.75	0.75	0.75	0.75	0.75
MCP	1.30	1.30	1.30	1.30	1.30
Premix	0.34	0.34	0.34	0.34	0.34
Chlorine chloride	0.07	0.07	0.07	0.07	0.07
Salt	0.40	0.40	0.40	0.40	0.40
Calculated chemical compositions:					
ME, (kcal/kg)[1]	3,000	3,000	3,000	3,000	3,000
Crude protein	20.0	20.0	20.0	20.0	20.0
Crude fibre	5.51	6.33	5.83	6.30	7.08
Analyzed chemical composition (on dry basis):					
Moisture	12.7	14.3	13.2	13.2	11.9
Crude protein	22.9	25.1	22.1	23.9	24.9
Crude fat	4.55	5.18	4.89	4.72	5.16
Crude fibre	8.18	8.82	9.37	9.92	9.96
Ash	6.85	6.32	5.79	5.90	9.38

[1]ME (metabolizable energy) was estimated based on formula (Bolton, 1967): 40.81 {0.87 [crude protein + 2.25 crude fat + nitrogen-free extract] + 2.5}
T0: chicks received basal feed, T1: feed with 2.5% PSM, T2: feed with 1% SPSM, T3: feed with 2.5% SPSM, T4: feed with 5% SPSM, PSM: papaya seed meal, SPSM: sprouted papaya seed meal, SBM: soybean meal, MCP: monocalcium phosphate

ly-Auto Hematology Analyzer (PT. Prima Alkesindo Nusantara, Jakarta, Indonesia) was employed to measure the complete blood counts based on the manufacturer's description. For the production of serum, the collected blood was let at room temperature for about 2 hours. To separate the serum and blood clot, the blood was centrifuged for 10 minutes at 5,000 rpm. The serum was then kept in the freezer (at -10°C) until analysis. After the blood was taken, one of the two birds was slaughtered. The internal organs were obtained and weighed (in empty condition). For the enumeration of selected bacterial population, the digesta was collected from ileum and caecum of broilers immediately after slaughter. The sample of breast meat was also collected from broiler, and kept frozen (at -10°C) until the analysis of fatty acid profile and meat colour.

The serum lipid, uric acid, and creatinine levels were measured using enzyme-based colorimetric techniques. Spectrophotometric/ photometric assays were used to assess total se-

rum protein, albumin, glucose, alanine aminotransferase (ALT), and aspartate aminotransferase (AST). To calculate the globulin concentration, total protein in serum was deducted from albumin in serum. The serum biochemistry tests were carried out according to the manufacturer's guidance (DiaSys Diagnostic System GmbH, Holzheim, Germany). Coliform and lactose-negative *Enterobacteriaceae* were enumerated as red and colourless colonies on MacConkey agar (Merck KGaA, Darmstadt, Germany) after 24 hours of aerobic incubation at 38°C. The sum of coliform and lactose-negative *Enterobacteriaceae* was regarded as *Enterobacteriaceae*. Following anaerobic incubation at 38°C for 48 hours on MRS agar (Merck KGaA), the numbers of lactic acid bacteria (LAB) were counted.

A digital colour meter running on Mac OS X was used to verify the colour of the meat (set to CIE Lab). L* (lightness), a* (redness), and b* (yellowness) values were used to indicate the colour. A traditional gas chromatography method was used to evaluate the fatty acid content of breast meats. The presence of fatty acids was evaluated by comparing the retention periods of each sample to the standard retention times. The area percentage was normalized and adjusted to g per 100 g of edible part for fatty acid measurement using a lipid conversion factor. To determine total saturated fatty acids (SFA) and unsaturated fatty acids (UFA), each component of SFA and UFA was added individually.

Statistical analysis

The data were statistically treated according to CRD using analysis of variance (ANOVA, SPSS 16.0 version). The Duncan multi-range test was employed when dietary treatments showed a significant effect ($P<0.05$).The $0.05 \leq P<0.10$ was regarded as a trend.

RESULTS AND DISCUSSION

The incorporation of 2.5% PSM in feed resulted in lower ($P<0.05$) daily weight gain of broilers in the current investigation (Table 3). Different from PSM, the inclusion of SPSM up to 5% in diets had no meaningful effect ($P>0.05$) on the daily gain of broilers. This seemed to be accounted to the improved nutrient quality of SPSM due to germination, with regards particularly to its increased protein and decreased fibre contents (Table 1). Likewise, the reduced content of anti-nutritional components in SPSM with sprouting process may increase feed digestibility and thereby nutrient availability for growth (Sugiharto, 2020a). There was an absence effect ($P>0.05$) of feeding SPSM up to 5% on daily feed intake of broiler, when compared with the birds fed on control diet (Table 3). Conversely, feeding PSM reduced ($P<0.05$) daily feed intake of broilers. This may be attributed to the presences of anti-nutritional factors (Sugiharto, 2020a) and low-digested fibre (lignin, cellulose and hemicellulose; Adesuyi and Ipinmoroti, 2011) in PSM that can impair feed digestibility and intake. In this study, feed efficiency was lower ($P<0.05$) in broiler fed 5% SPSM, as compared to other birds. It was most likely that the higher fibre content of the respective feed (Table 2) may compromise feed digestibility and thus weight gain of broilers.

When comparing broilers fed a diet containing 5% SPSM to those fed a control diet, there was an apparent trend ($P=0.06$) for the relative weight of *bursa of fabricius* to be greater (Table 4). Numerous variables influence the development of the *bursa of fabricius*, one of which is stress. In the study of Tarek *et al*. (2013), the decrease in *bursa of fabricius* weight has been attributed to stressful environment leading to oxidative stress. In our case, the increased antioxidative compounds in sprouting seeds (Sehrawat *et al*., 2020) appeared to offset the detrimental effect of stress on *bursa of fabricius* development. Overall, it was therefore worth noting that antioxidant compounds found in SPSM are critical for reducing the detrimental effects of stress on broiler's immune competences (Sugiharto, 2020a).

Table 5 shows complete blood counts of broiler chickens at day 36. In this study, it was discovered that giving 2.5% PSM or SPSM at levels of 2.5% and 5% of feeds resulted in a decrease ($P=0.09$) in heterophils values. Modern broiler strains have typically been raised in a stressful environment with restricted access to natural behaviour, inadequate cleanliness, high stocking density, and a high ambient temperature. In poultry, the rise in heterophils levels is usually attributed to the stressful environment (Maxwell and Robertson, 1998; Kontecka *et al*., 1999) as well as inflammation owing to pathogenic bacterial invasion (Harmon, 1998). Given these circumstances, it was possible that feeding PSM or SPSM might help to relieve the stressful condition, therefore avoiding the excessive increase in heterophils production. As compared to those in other birds, MCH and MCHC levels in broilers given SPSM were lower ($P<0.05$) especially when administered at 5% in feed. The MCH and MCHC levels were often greater in chickens exposed to stressful conditions (Kontecka *et al*., 1999; Aengwanich, 2007). This might be a stress-related physiological reaction, in which birds increase their oxygen-carrying capacity to assist their energy-producing metabolism. It should be noted that stressed birds require more energy than non-stressed birds. Overall, because of the increased concentration of antioxidative compounds in SPSM ingested, it was possible that broilers given a diet containing 5%

SPSM experienced less oxidative stress.

The cholesterol to HDL ratio in the serum of broilers fed PSM or SPSM was lower (P<0.05) in the current research than in the serum of those fed a control diet (Table 6). The impaired immune competences of broilers is often associated with a greater cholesterol to HDL ratio (dyslipidemia) (Sugiharto, 2020b). In this case, giving PSM or SPSM to broiler chickens therefore appeared to be advantageous to their health and well-being. In this study, feeding SPSM at a rate of 2.5% resulted in a higher (P<0.05) serum HDL level than either the control or PSM diet. It was most likely that the flavonoids content in SPSM was responsible for the increased HDL-cholesterol level of broilers as reported by Ouyang *et al*. (2016). In this study, the birds fed on PSM tended to have lower total cholesterol (P=0.07), triglyceride (P=0.09) and LDL-cholesterol (P=0.09) when compared with control birds. In a prior study, Sarikhan *et al*. (2009) found that consuming insoluble fibre in the diet reduced cholesterol, triglyceride, and LDL-cholesterol levels in broiler blood. Insoluble fibre may enhance cholesterol and bile acid excretion in the faeces, according to the researchers. It was shown in this study that the reduction in serum total cholesterol, triglyceride, and LDL-cholesterol levels seemed to be more pronounced in PSM fed than SPSM fed broilers. As suggested previously that germination may improve the fibre fractions (i.e., increase soluble fibre content) of seeds (Hooda and Jood, 2003; Nkhata *et al*., 2018). In the earlier study, Razdan *et al*. (1997) compared between chitosan (considered as insoluble fibre) and pectin (soluble fibre) with regard to their capacity in lowering total plasma cholesterol of broilers. They noticed that chitosan was more effective in reducing plasma cholesterol concentration than that of pectin. One possible reason for these differences is that birds on the pectin diet had greater bile acid resorption than those on the chitosan diet, which is likely because pectin may only bind superficially to bile acids, but chitosan may sequester bile acids more firmly. On this background, it could be understood that PSM was more prominent than SPSM in lowering cholesterol, triglycerides, and LDL.

It was reported in the current study that dietary inclusion of PSM or SPSM decreased (P<0.05) serum

Table 3. Growth Performance of Broilers (Days 14-36)

Variables	T0	T1	T2	T3	T4	SEM	P value
DWG, g/d	61.4[a]	54.3[b]	60.9[a]	57.9[ab]	58.1[ab]	0.78	0.02
DFI, g/d	102[a]	94.4[b]	105[a]	102[a]	109[a]	1.36	<0.01
FE, %	60.0[a]	57.7[a]	57.9[a]	56.8[ab]	53.3[b]	0.66	0.02

[a,b]Within the same row, the means with different superscript characters varied substantially (P<0.05)

T0: chicks received basal feed, T1: feed with 2.5% PSM, T2: feed with 1% SPSM, T3: feed with 2.5% SPSM, T4: feed with 5% SPSM, DWG: daily weight gain, DFI: daily feed intake, FE: feed efficiency, SEM: standard error of the mean

Table 4. Relative Internal Organ Weight of Broilers at Day 36

Variables (% live BW)	Variables	T0	T1	T2	T3	SEM	P value
Heart	0.48	0.52	0.47	0.55	0.54	0.01	0.35
Liver	2.42	2.25	2.15	2.04	2.22	0.07	0.62
Proventriculus	0.51	0.54	0.56	0.53	0.57	0.01	0.37
Gizzard	1.57	1.74	1.70	1.63	1.88	0.04	0.21
Pancreas	0.25	0.26	0.28	0.26	0.26	0.01	0.88
Duodenum	0.57	0.60	0.61	0.47	0.60	0.02	0.23
Jejunum	1.38	1.22	1.29	1.16	1.12	0.06	0.66
Ileum	0.74	0.76	0.79	0.85	0.81	0.03	0.68
Caeca	0.59	0.57	0.50	0.56	0.52	0.02	0.62
Abdominal fat	0.83	0.88	0.74	0.88	0.90	0.06	0.92
Spleen	0.13	0.11	0.11	0.11	0.11	0.01	0.85
Thymus	0.28	0.38	0.34	0.33	0.26	0.02	0.48
Bursa of fabricius	0.17	0.21	0.16	0.22	0.27	0.01	0.06

T0: chicks received basal feed, T1: feed with 2.5% PSM, T2: feed with 1% SPSM, T3: feed with 2.5% SPSM, T4: feed with 5% SPSM, BW: body weight, SEM: standard error of the mean

Table 5. Complete Blood Indices of Broilers at Day 36

Variables	T0	T1	T2	T3	T4	SEM	P value
Erythrocytes (10^{12}/L)	3.24	3.00	3.05	3.11	2.74	1.70	0.93
Haemoglobin (g/dL)	20.1	11.3	11.3	8.67	9.92	1.68	0.21
Haematocrits (%)	43.4	39.4	40.3	34.7	36.5	1.88	0.64
MCV (fl)	134	132	135	134	134	0.59	0.48
MCH (pg)	37.7^{a}	37.7^{a}	36.1^{ab}	36.2^{ab}	35.5^{b}	0.26	0.02
MCHC (g/dL)	28.2^{ab}	28.8^{a}	27.5^{bc}	27.3^{bc}	26.7^{c}	0.20	0.01
RDW-SD (10^{-15} L)	51.6	50.3	51.6	54.1	53.4	0.58	0.25
RDW-CV (%)	10.1	10.1	10.3	10.7	10.5	0.11	0.41
MPV (10^{-15} L)	8.68	8.17	8.54	8.51	8.86	0.09	0.15
PDW (%)	8.25	6.15	7.21	7.86	7.24	0.27	0.13
Leukocytes (10^{9}/L)	126	112	138	99.4	106	6.86	0.39
Heterophils (10^{9}/L)	22.1	10.3	19.7	11.4	10.5	1.80	0.09
Lymphocytes (10^{9}/L)	112	102	119	88.0	95.6	6.47	0.58
Thrombocytes (10^{9}/L)	12.9	11.5	12.7	12.7	13.1	0.44	0.82

[a,b]Within the same row, the means with different superscript characters varied substantially (P<0.05)
T0: chicks received basal feed, T1: feed with 2.5% PSM, T2: feed with 1% SPSM, T3: feed with 2.5% SPSM, T4: feed with 5% SPSM, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin 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, SEM: standard error of the mean

Table 6. Serum Biochemical Indices of Broilers at Day 36

Variables	T0	T1	T2	T3	T4	SEM	P value
Total cholesterol (mg/dL)	150	116	124	131	140	4.16	0.07
Total triglyceride (mg/dL)	81.2	47.2	51.4	66.1	65.1	4.30	0.09
LDL (mg/dL)	146	112	119	124	132	4.13	0.09
HDL (mg/dL)	65.0^{bc}	62.6^{c}	78.3^{ab}	79.8^{a}	67.8^{abc}	2.25	0.04
Cholesterol/HDL ratio	2.80^{a}	1.92^{b}	1.60^{b}	1.65^{b}	2.22^{ab}	0.13	0.02
Glucose (mg/dL)	297	230	206	285	254	13.4	0.18
Total protein (g/dL)	3.59^{a}	2.70^{bc}	2.89^{bc}	2.52^{c}	3.15^{ab}	0.10	<0.01
Albumin (g/dL)	1.53^{a}	1.19^{bc}	1.26^{bc}	1.18^{c}	1.41^{ab}	0.04	<0.01
Globulin (g/dL)	2.06^{a}	1.51^{bc}	1.63^{bc}	1.34^{c}	1.74^{ab}	0.06	<0.01
Uric acid (mg/dL)	5.60	3.19	4.50	4.33	4.23	0.32	0.23
Creatinine (mg/dL)	0.07^{a}	0.05^{b}	0.05^{b}	0.03^{b}	0.05^{b}	<0.01	<0.01
AST (U/L)	294	322	253	238	268	12.5	0.22
ALT (U/L)	2.35^{a}	1.59^{ab}	1.01^{b}	1.10^{b}	1.46^{ab}	0.14	0.02

[a,b]Within the same row, the means with different superscript characters varied substantially (P<0.05)
T0: chicks received basal feed, T1: feed with 2.5% PSM, T2: feed with 1% SPSM, T3: feed with 2.5% SPSM, T4: feed with 5% SPSM, 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 mean

concentrations of total protein, albumin and globulin of broilers (Table 6). To date, the definite reason for such conditions is not known. Typically, blood protein concentration may reflect the degree of protein synthesis in the liver. Apata (2011) documented that the reduction in serum total protein indicated a decrease in protein synthesis, which might be due to a low protein intake or low digestibility. Higher fibre content has usually been attributed to the reduction in protein digestibility in broilers (Apata, 2011). Taking a consideration that diets containing PSM or SPSM contained greater fibre content that that of control

Table 7. Selected Intestinal Bacterial Populations of Broilers at Day 36

Variables (log cfu/g)	T0	T1	T2	T3	T4	SEM	P value
Ileum							
Coliform	7.73	6.46	6.30	5.63	5.91	0.26	0.08
LNE	5.54	6.87	5.63	5.62	5.26	0.21	0.12
Enterobacteriaceae	7.74	7.35	6.30	5.99	5.91	0.28	0.12
LAB	8.01	9.67	8.87	9.15	9.94	0.30	0.28
LAB/coliform ratio	1.04[b]	1.52[a]	1.48[a]	1.67[a]	1.74[a]	0.08	0.02
Cecum							
Coliform	8.58	7.65	8.86	8.12	8.05	0.22	0.45
LNE	6.85	6.15	7.35	6.46	6.41	0.27	0.68
Enterobacteriaceae	8.65	7.74	8.98	8.17	8.08	0.22	0.43
LAB	11.7	11.4	11.6	11.6	11.4	0.07	0.42
LAB/coliform ratio	1.37	1.53	1.32	1.49	1.46	0.05	0.64

[a,b]Within the same row, the means with different superscript characters varied substantially (P<0.05)
T0: chicks received basal feed, T1: feed with 2.5% PSM, T2: feed with 1% SPSM, T3: feed with 2.5% SPSM, T4: feed with 5% SPSM, LNE:lactose negative *Enterobacteriaceae*, LAB: lactic acid bacteria, cfu: colony forming unit

Table 8. Fatty Acid Composition and Colour of Broiler Meats at Day 36

Variables	T0	T1	T2	T3	T4	SEM	p value
Fatty acids							
Total SFA (g/100 g)	0.22	0.16	0.34	0.31	0.24	0.02	0.08
Total UFA (g/100 g)	0.41	0.30	0.63	0.60	0.48	0.04	0.09
n-3 PUFA (mg/100 g)	8.83	4.46	8.98	6.46	7.77	0.67	0.17
n-6 PUFA (mg/100 g)	136	96.8	192	177	141	12.4	0.11
Meat colour							
L* (lightness)	49.1[bc]	50.6[abc]	51.8[a]	51.0[ab]	48.9[c]	0.30	0.01
a* (redness)	4.41	4.99	4.11	3.97	4.61	0.17	0.35
b* (yellowness)	8.99[c]	10.2[b]	9.60[bc]	11.3[a]	8.95[c]	0.18	<0.01

[a,b]Within the same row, the means with different superscript characters varied substantially (P<0.05)
T0: chicks received basal feed, T1: feed with 2.5% PSM, T2: feed with 1% SPSM, T3: feed with 2.5% SPSM, T4: feed with 5% SPSM, SFA: saturated fatty acids, UFA: unsaturated fatty acids, PUFA: polyunsaturated fatty acids, SEM: standard error of the mean

diet, the reduced concentrations of total protein, albumin and globulin in the PSM or SPSM fed birds may be due to the reduced protein digestibility in these respective birds. Creatinine has traditionally been used as a marker for protein metabolism. Increased muscle protein catabolism and consequently greater amounts of creatinine in broiler circulation are generally associated with increased corticosterone levels during stressful situations (Sugiharto, 2020b). In this investigation, feeding PSM or SPSM to broilers resulted in reduced (P<0.05) levels of creatinine in their serum when compared to control. As a result, our findings showed that PSM and SPSM might help broilers cope with stressful conditions. Similar to creatinine, the ALT levels often rise in broilers during stressful circumstances, indicating oxidative stress and injury to internal organs such as the liver and kidney (Sugiharto, 2020b). The blood levels of ALT was lower (P<0.05) in broilers fed SPSM at 1 and 2.5% of feed in this study (Table 6). Owing to this, the antioxidative properties of SPSM seemed to alleviate the oxidative stress in broilers during rearing, resulting in lower serum ALT level.

In our current investigation, the LAB to coliform ratio in the ileum of broilers given PSM or SPSM was greater (P<0.05) than in those fed a control diet (Table 7). In accordance with this, coliform counts in the ileum of broilers given PSM or SPSM tended (P=0.08) to be lower than those fed a control diet. The reduced coliform levels in the ileum of broilers were due to the antibacterial action of some antibacterial agents such as alkaloids, steroids, flavonoids, saponins, papain, and terpenoids in PSM or SPSM (Sugiharto, 2020a). However, the dietary treatment had no meaningful effect on the bacterial population in the caecum of broilers.

Table 8 describes fatty acid composition and colour of broiler meats collected at day 36. When comparing meat from broilers given a PSM-diet to meat from other broilers, total SFA was found to be re-

duced (P=0.08). This pattern actually corresponded to the amount of triglycerides in serum of broiler as described above. Higher insoluble fibre consumption appeared to decrease triglyceride synthesis in the liver (Sarikhan *et al*., 2009), leading to lower SFA deposition in meats. In terms of total UFA content, meats taken from broilers fed 1 or 2.5% SPSM tended (P=0.09) to have a greater total UFA content. Herchi *et al*. (2015) reported that germination lowered total fat and SFA contents, while increased PUFA content of flaxseed. Owing to this fact, feeding SPSM may therefore result in increased PUFA deposition in broiler meats. Yet, because the fatty acid composition of SPSM and broiler diets was not determined, the latter assumption should be treated with caution. The colour of broiler meat is one of the most important physical characteristics since it influences consumer choice. The pale-soft-exudative (PSE) and dark-firm-dry (DFD) condition of meats has traditionally been determined on the basis of the lightness (L*) values. There was no definite trend in the L* values of broiler breast meats in this study. Nonetheless, all meats from each treatment group appeared to be within the normal range, as Kralik *et al*. (2014) noticed that normal broiler meats had L* values ranging from 44 to 53. There was likewise no specific trend in the b* values of broiler meats. Kralik *et al*. (2014) categorized the b* values for PSE as 12.76, normal 9.63, and DFD 7.89, indicating that the results appeared to be within the normal range.

CONCLUSION

Dietary incorporation of SPSM improved immune competence, blood lipid profile and gut bacterial population of broilers. Feeding SPSM up to 5% had no detrimental effect on daily weight gain and feed consumption, but it impaired feed efficiency when included at 5% of feed.

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CONFLICT OF INTEREST

The authors had no competing interest.

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