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Germinated papaya seed alone or in combination with chitosan on growth, health and meat quality of broilers during grower period

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ABSTRACT

The study investigated the effect of a mixture of germinated papaya seed flour and chitosan (GPS-CH) in comparison to the germinated papaya seed flour (GPS) alone on growth, blood profile, intestinal indices and meat quality of broilers. A total of 288 14-day old Cobb chicks were divided into three groups with 8 replications, including CONT (chicks receiving basal feed with no additive), GPS (chicks receiving basal feed supplemented with 0.5% GPS), and GPS-CH (chicks receiving basal feed supplemented with 0.5% GPS and 0.2% chitosan). Treatments had no effect ($p > 0.05$) on broiler growth. Spleen was lower ($p < 0.05$) in GPS and GPS-CH than in CONT. Thrombocytes were lower ($p < 0.05$) in GPS and GPS-CH than in CONT. Total triglyceride and protein were higher ($p < 0.05$) in GPS and GPS-CH than in CONT. Jejunal villi height (VH) and ileal VH to crypt depth ratio of GPS-CH were higher ($p < 0.05$) than that of CONT and GPS birds. Total fat in breast meat was lower ($p < 0.05$) in GPS-CH than in CONT and GPS. The highest ($p < 0.05$) pH was found in GPS-CH breast. The yellowness values were lower ($p < 0.05$) in GPS-CH than in CONT and GPS breast. GPS thigh had lowest ($p < 0.05$) moisture and highest ($p < 0.05$) fat. Ash was higher ($p < 0.05$) in GPS-CH than in CONT thigh. Water holding capacity (WHC) was higher ($p < 0.05$) in GPS-CH than in CONT and GPS thigh meats. Cooking loss was lower ($p < 0.05$) in GPS-CH than in GPS thigh meats. Compared to GPS, the pH values of thigh meats were higher ($p < 0.05$) in GPS-CH and CONT meats. The yellowness values were lower in GPS and GPS-CH than in CONT thigh. In conclusion, GPS-CH was beneficial in improving immune responses, nutrient bioavailability, intestinal morphology and meat quality of broilers during the grower period.

1. Introduction

The search for an alternative to antibiotic growth promoters (AGP) for broiler chickens is being greatly promoted in response to the prohibition on the use of AGP in broiler chicken feed. The search attempt is crucial considering that the prohibition of AGP in feed can interfere with growth performance and increase disease prevalence in broilers. Phytobiotics is one of the alternatives to AGP that exhibit antimicrobial and immune-boosting properties, anti-inflammation, growth promoter, antioxidant, and other properties that can have positive impacts on the physiological condition, health, and growth of broiler chickens (Kikusato, 2021). Besides being used for broiler chickens, phytobiotics are also widely used for human purposes. This makes phytobiotics have a fairly high economic value so that their use for chickens can increase production costs (Sugiharto, 2021). The latter condition may encourage poultry nutritionists to utilize waste-based phytobiotics for broilers so that their use does not compete with humans. Papaya seeds are agricultural waste containing many active ingredients that can function as antimicrobial, antioxidant, immunomodulatory, anti-inflammatory, and so on (Kolu, Olumide & Akintunde, 2021). Indeed, papaya seeds have been used as feed additives and functional feeds which are expected to improve the physiological conditions and growth of chickens (Dassidi et al., 2020). Yet, the use of papaya seed is often attributed to the pathological condition in the liver of chickens due to the presence of anti-nutrients in the seeds (Oloruntola, 2019). To deal with the latter issue, and to improve the effectiveness of the papaya seed as a feed additive or functional feed, papaya seed may be germinated so that its nutritive and functional values are enhanced (Sugiharto et al., 2022). In the previous study, feeding germinated papaya seed meal resulted in better immune responses, blood lipid profile, and bacterial balance in broiler chicken intestines than non-germinated papaya seed meal. However, its use in feed had no notable effect on production

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performance of broilers (Sugiharto et al., 2022; Sugiharto, Pratama & Yudiarti, 2021).

Chitosan, which is waste from the shells of marine animals such as shrimp, crab, and lobster, is another waste-based material that can be used as an alternative to AGP for broilers. Recent study by Ayman et al. (2022) documented that dietary supplementation of chitosan was capable of improving the growth performance, intestinal histomorphology and physiological conditions of broiler chickens. In line with this, Nuengjamnonga & Angkanaporn (2018) demonstrated that feeding chitosan improved feed conversion ratio (FCR), intestinal bacterial population and morphology of broilers. Studies demonstrate that when two or more ingredients are used together, there will be an interaction among them. These interactions enable a synergistic effect to arise, thus having a greater influence on the host. Shraddha, Visha and Nanjappan (2017) documented that the use of Neem leaf meal mixed with chitosan in broiler feed had a greater impact on abdominal fat reduction than either ingredient used alone. In another study, He et al. (2014) noticed that azithromycin and chitosan worked in concert to combat *Pseudomonas aeruginosa*. With regard particularly to chitosan, this component has been reported to have absorption-enhancing effect and the capacity to increase the bioavailability of active compounds for the host (Tiyaboonchai, 2003). Because of the latter properties of chitosan, it was expected that combining chitosan with GPS would increase the bioavailability of phytogenic compounds in GPS and thus improve the efficacy of GPS as an AGP alternative for broilers. Overall, the aim of this study was to investigate the effect of GPS-CH in comparison to the GPS alone on growth, blood profile, intestinal indices and meat quality of broilers.

2. Materials and methods

2.1. Animal ethics

The Animal Ethical Committee of the Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang, approved the *in vivo* study with approval number 58–04c/A-5/KEP-FPP.

2.2. In vivo study

The study was conducted on a total of 288 Cobb (unsex) broiler chicks and was set up based on a fully randomized design. The chicks were grown communally for their first 14 days on commercial prestarter feed containing (according to the feed label) of 22–24% crude protein, less than 5% crude fiber, 5% crude fat, and 7% ash. The chicks were individually weighed (average body weight of 356 ± 4.92 g) and divided into three treatment groups with 8 replications/pens beginning on day 15. The treatment groups included CONT (chicks receiving basal feed with no additive), GPS (chicks receiving basal feed supplemented with 0.5% GPS), GPS-CH (chicks receiving basal feed supplemented with 0.5% GPS and 0.2% chitosan).

Chicks were raised in broiler house with open sides and beds made of rice husks. Throughout the experiment, a constant lighting schedule was implemented. Grower feed (in mash form) was formulated according to the Indonesian National Standard for finisher broiler feed (Table 1) and given to chicks from day 15 to day 35. Chicks were vaccinated against Newcastle disease (ND) and infectious bronchitis (IB) immediately after hatching. At the age of 18, chicks were also vaccinated against ND vaccine. Feed intake and body weight were measured at the end of the study. Blood was obtained from one male chick's wing vein (one per pen/replicate; eight per treatment group). Blood was placed in a vacutainer with ethylenediaminetetraacetic acid (EDTA) and the remaining blood was placed in a vacutainer without an anticoagulant. Chickens whose blood samples were slaughtered, defeathered, eviscerated and immediately the intestines and internal organs were removed. The segments (*ca* 2 cm) of duodenum, jejunum, and ileum were placed in 10% buffered formalin (Leica Biosystems Richmond, Inc., Richmond, USA) for small intestinal morphology (villi height and crypt depth) evaluation. Digesta from the ileum and cecum was collected to count the population of selected bacteria in the intestine. Internal organs were weighed (empty condition) thereafter. Samples of breast and thigh meats were obtained and stored at −10 °C until meat qualities assessment.

Table 1
Feed compositions of broilers (days 15–35).

Items	%, unless otherwise noted
Yellow maize	58.5
Palm oil	2.96
Soybean meal	34.7
DL-methionine	01.9
Bentonite	0.75
Limestone	0.75
Monocalcium phosphate	1.30
Premix[1]	0.34
Chlorine chloride	0.07
Salt	0.40
Chemical compositions	
ME (kcal/kg)[2]	3000
Crude protein,%	20.0
Crude fiber,%	5.51
Ca,%	1.02
P (available),%	0.58

[1] *Per kg of feed contained 1100 mg Zn, 1000 mg Mn, 75 mg Cu, 850 mg Fe, 4 mg Se, 19 mg I, 6 mg Co, 1225 mg K, 1225 mg Mg, 1250,000 IU vit A, 250,000 IU vit D_3, 1350 g pantothenic acid, 1875 g vit E, 250 g vit K_3, 250 g vit B_1, 750 g vit B_2, 500 g vit B_6, 2500 mg vit B_{12}, 5000 g niacin, 125 g folic acid and 2500 mg biotin.*

[2] *ME (metabolizable energy) was calculated based on formula (Bolton, 1967): 40.81 {0.87 [crude protein + 2.25 crude fat + nitrogen-free extract] + 2.5}.*

2.3. Laboratory analysis

The Prima Fully-Auto Hematology Analyzer (PT. Prima Alkesindo Nusantara, Jakarta, Indonesia) was used to measure the complete blood counts, according to the manufacturer's description. The coagulated blood was left at room temperature for about 2 h before being centrifuged at 5000 rpm for 10 min to produce serum. The serum was stored in a freezer (at −10 °C) until it was analysed. The lipid profile (total triglycerides, total cholesterol, low-density lipoprotein, and high-density lipoprotein) and serum levels of uric acid and creatinine were determined on the basis of enzyme-based colorimetric techniques. Total protein, albumin, glucose, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) concentrations in broiler chicken serum were determined using spectrophotometric/photometric methods. To calculate the globulin concentration, the albumin value in serum was subtracted from the total protein value. All biochemical analyses on serum samples were performed in accordance with the manufacturer's instructions (DiaSys Diagnostic System GmbH, Holzheim, Germany).

Small intestinal segments were examined histologically in 5 μm sections of the duodenum, jejunum, or ileum stained with hematoxylin and eosin. The villi height and crypt depth in each segment were determined using an optical microscope equipped with a digital camera (Leica Microsystems GmbH, Wetzlar, Germany). Five measurements were employed to calculate the mean values of villous height and crypt depth for each sample. Bacterial populations in the ileal and cecal contents were determined based on total plate count procedure. Following a 24 h aerobic incubation at 38 °C, the number of coliforms and lactose-negative enterobacteria was counted as red and colourless colonies on MacConkey agar (Merck KGaA, Darmstadt, Germany). The numbers of *Enterobacteriaceae* were defined by the total number of coliforms and lactose-negative enterobacteria. After 48 h of anaerobic incubation at 38 °C, the number of lactic acid bacteria (LAB) was determined on de Man, Rogosa, and Sharpe (MRS; Merck KGaA) agar. The frozen meat was thawed at room temperature for about 30 min prior

to analysis. To determine the pH values of meats, 1 g of breast or thigh meat from each sample was homogenized in 9 mL of distilled water, and the pH of the resulting filtrate was measured with a digital pH meter (Hanna Instruments, Woonsocket, Rhode Island). The water holding capacity (WHC) of meats was determined based on the press method using filter paper (Grau and Hamm, 1953), while the chemical compositions of broiler breast and thigh meats were measured using standard proximate analysis (AOAC, 1995). Breast or thigh meat samples were placed in a plastic bag and cooked in boiling water at 80 °C for 1 h to determine cooking loss. After allowing the meat to cool at room temperature, it is weighed. Cooking loss was defined as the difference in weight between before and after cooking. The color of broiler meat was measured in Mac OS X with a digital color meter (set to CIE Lab). The L* (brightness), a* (redness), and b* (yellowness) values were used to represent the color of broiler meats. The color analysis was carried out in triplicate.

2.4. Statistical analysis

The obtained data were subjected to an analysis of variance (ANOVA, SPSS version 16.0). Duncan's multiple analysis was used when the treatments had a significant effect ($p < 0.05$).

3. Results

3.1. Production parameters of broiler chickens

There was no significant ($p > 0.05$) effect of treatments on final body weight, body weight gain, feed conversion ratio (FCR), feed cost per kg live body weight gain, income over feed cost and feed consumption of broilers (Table 2).

3.2. Organ weights of broiler chickens

Table 3 shows the data on the relative weight of internal organs in broilers. It is shown that the relative weight of spleen was lower ($p < 0.05$) in GPS and GPS-CH than in CONT birds. The relative weights of heart, liver, proventriculus, gizzard, pancreas, small intestine, caeca, abdominal fat, thymus and *Bursa of fabricius* did not vary ($p > 0.05$) across the treatment groups of broilers.

3.3. Blood profiles of broiler chickens

Table 4 presents the data on complete blood counts of broiler chickens. The levels of thrombocytes were lower ($p < 0.05$) in GPS and GPS-CH than that in CONT broilers. There was no substantial variation ($p > 0.05$) among chicks on the profiles of red blood cells and white blood cells.

Table 2
Production parameters of broiler chickens (days 15–35).

Items	CONT	GPS	GPS-CH	SEM	*p* value
Final body weight (g/bird)	1770	1841	1900	24.6	0.09
Body weight gain (g/bird)	1413	1487	1543	24.7	0.09
Feed consumption (g/bird)	2223	2198	2210	20.1	0.89
FCR	1.58	1.48	1.44	0.03	0.08
Feed cost per kg live BWG (IDR)[1]	12,008	11,250	10,958	198	0.08
Income over feed cost (IDR)[2]	13,198	14,593	15,506	420	0.07

[1] *Value was calculated as the cost of feed consumed to produce one kg of live weight gain at the time of the study.*

[2] *Value was calculated using total revenue minus total feed cost at the time of experiment CONT: basal feed with no additive, GPS: basal feed supplemented with 0.5% GPS, GPS-CH: basal feed supplemented with 0.5% GPS and 0.2% chitosan, FCR: feed conversion ratio, BWG: body weight gain, IDR: Indonesian rupiah (Indonesian currency), SEM: standard error of means.*

Table 3
Internal organ relative weights of broiler chickens.

Items (% live BW)	CONT	GPS	GPS-CH	SEM	*p* value
Heart	0.48	0.51	0.49	0.01	0.47
Liver	2.81	2.70	2.56	0.09	0.51
Proventriculus	0.63	0.58	0.62	0.02	0.28
Gizzard	1.67	1.65	1.69	0.03	0.86
Pancreas	0.30	0.27	0.34	0.02	0.14
Small intestine	2.94	2.53	2.88	0.10	0.20
Caeca	0.44	0.41	0.46	0.01	0.30
Abdominal fat	1.43	1.03	1.21	0.07	0.08
Spleen	0.22[a]	0.14[b]	0.12[b]	0.02	0.04
Thymus	0.26	0.23	0.24	0.01	0.66
Bursa of fabricius	0.06	0.05	0.06	<0.01	0.88

[a,b] Means with different superscripts within the same row differ significantly ($p < 0.05$) CONT: basal feed with no additive, GPS: basal feed supplemented with 0.5% GPS, GPS-CH: basal feed supplemented with 0.5% GPS and 0.2% chitosan, BW: body weight, SEM: standard error of means.

Table 4
Complete blood counts of broiler chickens.

Items	CONT	GPS	GPS-CH	SEM	*p* value
erythrocytes (10^{12}/l)	2.78	2.83	2.65	0.09	0.75
hemoglobin (g/dl)	10.1	10.6	10.3	0.31	0.77
Haematocrits (%)	33.5	34.1	31.8	1.14	0.72
MCV (fl)	121	122	121	0.46	0.81
MCH (pg)	36.2	37.6	39.9	1.05	0.35
MCHC (g/dL)	30.1	31.2	33.4	0.92	0.34
RDW-SD (fl)	51.8	52.0	52.5	1.36	0.98
RDW-CV (%)	11.3	10.2	11.5	0.52	0.56
MPV (fl)	9.59	10.3	9.23	0.43	0.62
PDW (%)	13.4	12.2	10.2	0.68	0.17
Leukocytes (10^9/L)	105	91.3	87.8	4.23	0.21
Heterophils (10^9/L)	10.5	8.06	8.75	0.64	0.28
Lymphocytes (10^9/L)	94.8	83.3	79.1	4.01	0.26
Thrombocytes (10^9/L)	30.1[a]	17.0[b]	14.8[b]	2.73	0.04

[a,b] Means with different superscripts within the same row differ significantly ($p < 0.05$) CONT: basal feed with no additive, GPS: basal feed supplemented with 0.5% GPS, GPS-CH: basal feed supplemented with 0.5% GPS and 0.2% chitosan, 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, SEM: standard error of means.

3.4. Serum biochemical parameters of broilers

Total triglyceride and total protein were higher ($p < 0.05$) in GPS and GPS-CH than in CONT birds. There was no substantial effect of treatments on total cholesterol, LDL, HDL, albumin, globulin, creatinine, uric acid, AST and ALT in the serum of broilers (Table 5).

3.5. Intestinal bacterial counts of broiler chickens

Table 6 shows the data on the selected bacterial counts in the ileum and cecum of broilers. Both in ileum and cecum, the counts of lactic acid bacteria, coliform, lactose negative *Enterobacteriaceae* and *Enterobacteriaceae* did not vary ($p > 0.05$) across the treatment groups of broilers.

3.6. Intestinal morphology of broiler chickens

Data on the intestinal morphology of broilers are listed in Table 7. Observation in duodenum showed there was no substantial effect ($p > 0.05$) of dietary treatments on villi height, crypt depth and villi height to crypt depth ratio of broilers. In jejunum, villi height was higher ($p < 0.02$) in GPS-CH than that in CONT and GPS birds. In ileum, the villi height to crypt depth ratio of GPS-CH birds was higher ($p < 0.05$) than

Table 5
Serum biochemistry of broiler chickens.

Items	CONT	GPS	GPS-CH	SEM	*p* value
Total cholesterol (mg/dL)	82.8	99.0	108	5.22	0.14
Total triglyceride (mg/dL)	57.8[b]	91.4[a]	89.9[a]	4.82	<0.01
LDL (mg/dL)	21.1	32.1	36.6	3.24	0.13
HDL (mg/dL)	50.1	48.9	52.4	1.97	0.78
Total protein (g/dL)	2.61[b]	3.07[a]	3.53[a]	0.14	0.02
Albumin (g/dL)	1.00	1.24	1.08	0.06	0.28
Globulin (g/dL)	1.61	1.83	2.45	0.15	0.07
Uric acid (mg/dL)	5.53	7.58	7.60	0.41	0.05
Creatinine (mg/dL)	0.04	0.04	0.04	<0.01	1.00
AST (U/L)	208	230	226	8.04	0.50
ALT (U/L)	1.70	1.68	1.53	0.11	0.82

[a,b] Means with different superscripts within the same row differ significantly ($p < 0.05$) CONT: basal feed with no additive, GPS: basal feed supplemented with 0.5% GPS, GPS-CH: basal feed supplemented with 0.5% GPS and 0.2% chitosan, 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 means.

Table 6
Selected intestinal bacterial counts of broiler chickens.

Items (log cfu/g)	CONT	GPS	GPS-CH	SEM	*p* value
Ileum					
LAB	3.14	4.07	3.24	0.44	0.66
Coliform	2.39	2.00	2.00	0.13	0.39
LNE	2.00	2.00	2.00	<0.01	1.00
Enterobacteriaceae	2.65	2.30	2.30	0.12	0.38
LAB/coliform	1.33	2.03	1.62	0.21	0.41
Cecum					
LAB	7.53	7.33	7.42	0.12	0.80
Coliform	3.22	3.42	3.42	0.27	0.95
LNE	2.76	2.30	2.30	0.11	0.12
Enterobacteriaceae	3.62	3.61	3.57	0.24	0.99
LAB/coliform	2.64	2.55	2.43	0.18	0.90

CONT: basal feed with no additive, GPS: basal feed supplemented with 0.5% GPS, GPS-CH: basal feed supplemented with 0.5% GPS and 0.2% chitosan, LAB: lactic acid bacteria, LNE: lactose negative Enterobacteriaceae, SEM: standard error of means.

Table 7
Intestinal morphology of broiler chickens.

Items	CONT	GPS	GPS-CH	SEM	*p* value
Duodenum					
Villi height (μm)	1005	1290	1228	55.8	0.08
Crypt depth (μm)	195	181	189	9.11	0.83
VH/CD	5.19	7.17	7.14	0.44	0.09
Jejunum					
Villi height (μm)	979[b]	817[b]	1401[a]	89.8	0.02
Crypt depth (μm)	188	153	190	8.04	0.10
VH/CD	5.30	5.31	7.51	0.45	0.07
Ileum					
Villi height (μm)	594	620	804	51.8	0.19
Crypt depth (μm)	168	165	163	9.36	0.98
VH/CD	3.76[b]	3.67[b]	4.94[a]	0.24	0.04

[a,b] Means with different superscripts within the same row differ significantly ($p < 0.05$) CONT: basal feed with no additive, GPS: basal feed supplemented with 0.5% GPS, GPS-CH: basal feed supplemented with 0.5% GPS and 0.2% chitosan, VH: villi height, CD: crypt depth, SEM: standard error of means.

the other birds.

3.7. Carcass proportions and meat qualities of broiler chickens

The proportion of drumstick was higher ($p < 0.05$) in GPS than in GPS-CH, but did not differ from that of CONT birds. Eviscerated carcass (relative to live body weight) and the proportions (relative to eviscerated carcass) of breast, wings, thigh, back and edible giblets did not vary ($p > 0.05$) across the treatment groups (Table 8).

The chemical and physical traits of broiler breast and thigh meats are presented in Table 9. Total fat in breast meat was lower ($p < 0.05$) in GPS-CH than that in CONT and GPS. The highest ($p < 0.05$) pH values were found in GPS-CH breast meat, followed by CONT and GPS. The b* values were lower ($p < 0.05$) in GPS-CH as compared to that in CONT and GPS breast meats. Observation on thigh meats showed that GPS had lower ($p < 0.05$) moisture content and higher ($p < 0.05$) total fat contents than other groups. Ash content was higher ($p < 0.05$) in GPS-CH than in CONT, but did not differ from that of GPS. WHC was higher ($p < 0.05$) in GPS-CH than in CONT and GPS thigh meats. Cooking loss was lower ($p < 0.05$) in GPS-CH than in GPS, but did not differ from that of CONT. Compared to GPS, the pH values of thigh meats was higher ($p < 0.05$) in GPS-CH and CONT meats. The b* values were lower in GPS and GPS-CH compared to that in CONT thigh meats.

4. Discussion

The findings of this study demonstrated that dietary supplementation with GPS had no significant impact on broiler growth performance and FCR. This finding was consistent with that of Sugiharto et al. (2022) in which feeding GPS as a functional feed ingredient had no discernible effect on broiler growth rate and FCR. Our finding further revealed no substantial improvement on growth performance, FCR, feed cost per kg live body weight gain and income over feed cost when combining GPS and chitosan. In this respect, the synergistic effect of GPS and chitosan or the bioavailability-enhancing effect of chitosan on active compounds of GPS did not appear to be improving the growth performance of broilers. With regard particularly to chitosan, this present study contradicted to Nuengjamnonga & Angkanaporn (2018) showing the growth-promoting effect of chitosan on broiler chickens. However, our results were consistent with Jasim and Nafea (2021) reporting no beneficial effect of chitosan on growth performance of broilers. They suggested that chitosan may bind fat, especially in the digestive system, and in turn reduce fat contents in the body and eventually reduce body weight of chickens. Such conditions seemed to alleviate the growth-promoting effect of chitosan on broiler chickens in the present study. The latter inference was actually supported by the fact in the present study that the total fat content in meats was lower in the GPS-CH group compared to other chicken groups.

Data in our study documented that feeding GPS or GPS-CH was associated with the lower weight of spleen of broilers. The control chickens, on the other hand, had spleen enlargement. In general, the enlargement of spleen is attributed to the infections in broiler chickens (Kumari, Mishra & Lather, 2013; Wani, Darzi, Mir, Sheikh & Irfan, 2017). As a major secondary organ in the immune system, the spleen works hard in response to incoming antigens in the chicken body. This

Table 8
Carcass proportions of broiler chickens.

Items	CONT	GPS	GPS-CH	SEM	*p* value
Eviscerated carcass (% live BW)	65.4	65.3	67.0	0.53	0.38
	(% eviscerated carcass)				
Breast	34.9	34.5	35.5	0.36	0.54
Wings	11.4	11.1	11.3	0.22	0.82
Thigh	15.7	16.3	15.7	0.25	0.61
Drumstick	15.3[ab]	15.8[a]	14.6[b]	0.20	0.04
Back	22.6	22.5	22.9	0.33	0.87
Edible giblets[1]	7.61	7.44	7.10	0.17	0.47

[a,b] Means with different superscripts within the same row differ significantly ($p < 0.05$).

[1] Edible giblets: cumulative weight of heart, liver and gizzard CONT: basal feed with no additive, GPS: basal feed supplemented with 0.5% GPS, GPS-CH: basal feed supplemented with 0.5% GPS and 0.2% chitosan, BW: body weight, SEM: standard error of means.

Table 9
Chemical and physical characteristics of broiler meats.

Items	CONT	GPS	GPS-CH	SEM	*p* value
Breast					
Moisture (%)	74.6	75.0	75.1	0.11	0.22
Total protein (%)	21.3	20.9	21.1	0.10	0.41
Total fat (%)	1.12[a]	1.11[a]	0.68[b]	0.05	<0.01
Ash (%)	1.18	1.16	1.12	0.02	0.50
WHC (%)	40.3	38.7	39.0	0.31	0.07
Cooking loss (%)	27.0	26.7	26.4	0.17	0.35
pH	6.31[b]	6.24[c]	6.42[a]	0.02	<0.01
L*	52.8	52.6	51.5	0.26	0.09
a*	3.44	2.36	4.20	0.68	0.56
b*	9.67[a]	8.75[a]	7.15[b]	0.22	<0.01
Thigh					
Moisture (%)	76.0[a]	75.5[b]	76.2[a]	0.08	<0.01
Total protein (%)	19.1	19.2	19.0	0.11	0.67
Total fat (%)	2.17[b]	2.50[a]	2.04[b]	0.07	0.01
Ash (%)	0.92[b]	1.01[ab]	1.06[a]	0.02	0.04
WHC (%)	31.4[b]	31.10[b]	35.1[a]	0.32	<0.01
Cooking loss (%)	32.0[ab]	32.7[a]	31.4[b]	0.21	0.02
pH	6.64[a]	6.43[b]	6.59[a]	0.02	<0.01
L*	51.8	52.1	51.7	0.21	0.79
a*	6.69	5.08	5.79	0.34	0.19
b*	9.25[a]	7.39[b]	7.77[b]	0.25	<0.01

[a,b,c] Means with different superscripts within the same row differ significantly ($p < 0.05$) CONT: basal feed with no additive, GPS: basal feed supplemented with 0.5% GPS, GPS-CH: basal feed supplemented with 0.5% GPS and 0.2% chitosan, WHC: water holding capacity, L*: lightness value, a*: redness value, b*: yellowness value, SEM: standard error of means.

may therefore increase the weight of spleen. Given the lower spleen weight in the treated broilers, it was possible to assume that the antigen load in the treated broilers was lower than in the control broilers. In this respect, the antimicrobial properties of GPS (Masfufatun, N & Putri, 2019) as well as chitosan (Yan et al., 2021) appeared to protect chickens from pathogenic antigens.

As previously suggested, GPS and GPS-CH treated broilers had lower antigen load than control broilers. The fact that broilers supplemented with GPS or GPS-CH had lower thrombocyte levels in the blood supported the above suggestion. In most cases, thrombocytes in chicks may indicate an inflammatory condition caused by infection (Ferdous, Maurice & Scott, 2008). Lower thrombocytes were therefore associated with lower antigen load and thus lower inflammatory response and thrombocyte levels in broiler blood. Data in our current experiments showed that total triglyceride and total protein in the serum were higher in broilers supplemented with GPS or GPS-CH. The increase in serum triglyceride and protein concentrations seemed to be contributed by the high contents of triglyceride and protein in the GPS included in the diets (Sugiharto et al., 2022). Furthermore, the ability of papaya seed (Muazu & Aliyu-Paiko, 2020) and chitosan (Osho & Adeola, 2020) to increase nutrient digestibility appeared to be responsible for the increased bioavailability of nutrients as indicated by increased levels of triglyceride and protein in blood. With regard particularly to total protein, the higher total protein in serum was in line with the relatively higher final body weight of broilers in the respective chickens. This data was in accordance with Zhu et al. (2022) showing a linear relationship between the higher total protein level in blood and the higher final body weight of broilers. In the latter case, the higher total protein may contribute to the muscle tissue formation and hence increased growth rate of broilers.

Data in our current experiments did not show any effect of GPS and GPS-CH on the populations of lactic acid bacteria, coliform and *Enterobacteriaceae* in the intestine of broilers. As a result, this finding failed to demonstrate the antimicrobial activity of GPS and chitosan, as suggested by Kolu et al. (2021) and Menconi et al. (2014), respectively. The lack of antimicrobial activity of these two materials in this study was unknown, but it was very likely that the hygienic conditions of broiler house at the time of the study had an impact on the bacterial population, particularly pathogenic bacteria, which were underdeveloped. Hence, additives like GPS and chitosan become less effective.

Current observations on the jejunum and ileum revealed that villi height and the ratio of villi height to crypt depth, respectively, were higher in broilers supplemented with GPS-CH compared to that of CONT- and GPS-supplemented groups. In general, higher villi height and villi height to crypt depth ratio were expected because these conditions were associated with broilers' improved ability to absorb nutrients derived from the digestion process (Awad, Ghareeb & Böhm, 2008). The latter authors further pointed out that the improved intestinal morphology resulted in improved nutrient absorption and thus broiler growth performance. In this study, chitosan possibly exhibited the bioavailability-enhancing effect on active compounds of GPS, resulting in improved broiler intestinal morphology. Yet, this assumption must be treated with caution as there was no significant effect of GPS on the villi height and the ratio of villi height to crypt depth of the jejunum and ileum, respectively. Another possibility was that chitosan alone may play a substantial role in improving the morphology of the jejunum and ileum of broiler. In such case, chitosan may alleviate the stress condition in broiler chickens due to tropical environmental conditions (average temperature and humidity during study were 32±1.5 °C and 78±4%). Study by Yoo et al. (2016) confirmed that high temperature in the tropics was associated with the impaired intestinal morphology of broilers, and that dietary antioxidant supplementation was capable of ameliorating such negative effect of tropical condition on intestinal morphology. Given its antioxidant potentials (Muthu et al., 2021), chitosan may therefore act in alleviating the stress condition in broilers raised under tropical conditions. Also, the antibacterial activity of chitosan (Menconi et al., 2014; Yan et al., 2021) appeared to contribute to improved intestine morphology in broilers. This inference was supported by the fact that improved microbial balance may be associated with improved antioxidant capacity and inflammatory responses, resulting in less intestinal inflammation and deterioration of intestinal morphology (Chuang et al., 2021). Moreover, the absence or less of pathogenic bacteria in the intestine may be associated with an increase in epithelial cell proliferation, which consequently raises the height of the intestinal villi (Ayman et al., 2022). However, the latter inference should be interpreted with caution because no difference in selected bacterial counts was observed in broiler intestine in the current study.

Although the treatment had no substantial effect on broiler eviscerated carcass, the proportion of drumsticks was lower in broilers fed GPS-CH when compared to broilers fed only papaya seeds. It appears that chitosan had a negative impact on the proportion of broiler drumsticks in this study. However, this was not the case because chicken drumsticks given GPS-CH performed the same as the control. Overall, the data presented above differs from that of Tufan and Arslan (2020), who revealed that chitosan supplementation increased the proportion of dressing as well as the proportion of breast, leg, and broiler wings.

Our study found that broiler meats had less total fat when supplemented with GPS-CH. On the other hand, broiler meat's fat content was unaffected by GPS. Considering the absence of effect of GPS on the fat content in meats, the reduced fat level of broiler meats appears to be a result of chitosan. According to Wang et al. (2022), dietary chitosan administration was able to promote liver fat catabolism, which led to decreased fat deposition in the meat of native yellow-feathered chickens. Additionally, they suggested that chitosan changed the gene expression of adipose tissue in the liver, and hence reducing hepatic lipogenesis. In this study, the lower content of total fat in GPS-CH meats was in line with the higher WHC in the respective meats. Taking into account the hydrophobicity of fat, the low-fat content of GPS-CH meat leads to an increase in moisture bound by the meat tissue (Mir, Rafiq, Kumar, Singh & Shukla, 2017). It was shown in this study that the quality of chicken meat was not improved by the use of GPS alone. In this respect, chitosan seemed to play a significant role in improving the quality of broiler meats. In fact, chicken meat given GPS had higher fat content, lower moisture, and higher cooking loss than meats from broilers receiving GPS-CH. Research on the impact of the use of chitosan

on the quality of broiler meat is still very limited, but studies by Wang et al. (2022) demonstrated that chitosan was capable of reducing drip loss in indigenous yellow-feathered chickens in their study. In this respect, chitosan can reduce the fat content in meat so that it has an impact on the moisture content and WHC of the meat (Wang et al., 2022).

WHC is a function of various factors, one of which is meat pH (Mir et al., 2017). Compared to chicken meat given only GPS, the data in this study showed that meat of broilers given GPS-CH showed a higher pH value. In most cases, the decrease in pH causes a reduction in the reactive groups available for water binding to muscle proteins (Mir et al., 2017). Hence, increasing the pH value of broiler chicken that was given GPS-CH can have a positive impact on increasing WHC and decreasing cooking loss in this study. The thigh meat of broilers given GPS-CH had more ash than the control. However, there was no difference in the ash content between the meats of broilers given only GPS and the control group. By taking into account the absence of effect of GPS on meat ash content, this investigation may therefore point to the involvement of chitosan in enhancing the mineral retention by the meats. Indeed, Ahmed, Roohi & Roohi, 2021 revealed that chitosan may improve mineral digestibility of chickens and hence increase mineral retention by broiler meats.

The high value of lightness and yellowness are indicator of a decrease in the quality of chicken meat, which is commonly referred to as pale-soft-exudative (PSE) meat (Adzitey & Nurul, 2011; Mir et al., 2017). It was apparent in this study that dietary administration of GPS-CH resulted in lower yellowness values in breast and thigh meat of broilers. The rationale for the low yellowness values of the GPS-CH meats remains unclear, but Abdurrahman, Pramono and Suthama (2016) showed the relationship between low-fat content and low yellowness values of chicken meats. In agreement, Zhou et al. (2009) pointed out that less fat content in muscle was associated with lower yellowness values of broiler meats.

5. Conclusions

Dietary administration of 0.5% GPS or combination of 0.5% GPS and 0.2% chitosan had no beneficial effect on growth performance of broilers. Yet, GPS and GPS-CH was beneficial in improving immune responses of broilers against pathogenic antigens as indicated by the lower spleen relative weight and thrombocyte counts in the blood. GPS and GPS-CH also improved the nutrient bioavailability as reflected by the higher total triglyceride and total protein in serum. Treatment with GPS-CH improved intestinal morphology by enhancing jejunal villi height and ileal villi height to crypt depth ratio of broilers. GPS-CH improved the quality of broiler meats by lowering the fat content, yellowness values and cooking loss, and increasing moisture content, ash content, WHC and pH values of meats. Overall, GPS-CH was beneficial in improving immune response, nutrient bioavailability, intestinal morphology and meat quality of broilers during the grower period. Future research with simulated stress challenge and molecular approach is needed to elucidate the impact of GPS and GPS-CH on intestinal microbial diversity, antioxidative status and immune responses of broilers.

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Animal ethics statement

The Animal Ethical Committee of the Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang, approved the *in vivo* study with approval number 58–04c/A-5/KEP-FPP. The handling of the birds adhered to standards of best practice that are accepted both nationally and globally.

Declaration of Competing Interest

The authors state that they did not have conflict of interest.

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