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Health Status of Broiler Chickens Fed Diets Containing Palm Kernel Cake with Enzyme Mixture Supplementation

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ABSTRACT

Palm kernel cake (PKC) utilization as poultry feed has limitations due to its high crude fiber and non-starch polysaccharides, which make it difficult for poultry to digest. This study aimed to determine the effect of enzyme supplementation on blood profile, gastrointestinal health, and intestine histomorphology of broiler chickens fed PKC. This study used 1,080 Indian River strain roosters and two kinds of enzyme mixtures. Enzyme 1 consisted of mannanase 182 g/ton, NSPase 200 g/ton, and protease 130 g/ton, and Enzyme 2 consisted of mannanase 182 g/ton, NSPase 400 g/ton, and protease 260 g/ton. This study used six treatments and six replicates, and each replicate consisted of 30 birds. Data were analyzed using a completely randomized design with a factorial pattern of 2 × 3 and 6 replications. The treatment of factor A consisted of 3 levels of enzyme addition (No enzyme, Enzyme 1, and Enzyme 2), and factor B consisted of 2 levels of PKC (10% and 20%). The data obtained was analyzed using analysis of variance (ANOVA), and the significant difference among treatments was further analyzed using Duncan's multiple range test. The experimental broilers fed PKC supplemented with enzyme had increased ($p<0.05$) villus height in the duodenum and ileum and decreased ($p<0.05$) relative weight percentage of the ventriculus (A2B1 and A3B1). The PKC supplementation increased ($p<0.05$) the relative weight percentage of proventriculus, duodenum, ileum, caecum, pH of ileum, crypta depth in the duodenum and ileum, but decreased ($p<0.05$) villus height in the jejunum. Enzyme supplementation increased ($p<0.05$) the plasma albumin, pH of duodenum, and villus width, but decreased crypta depth in the duodenum. This research concluded that Indian River strain roosters fed ration with 10% PKC supplemented with mannase, NPSase, and protease at doses of 182 g/ton, 200 g/ton, and 130 g/ton had the best health status.

Keywords: blood biochemistry; digestive tract; enzyme; histomorphology of small intestine; PKC

INTRODUCTION

Palm kernel cake (PKC) is a byproduct of palm kernel oil production (Olukomaiya *et al.*, 2019). PKC has high crude protein, high fiber (mannan), low digestibility (Sundu *et al.*, 2024), and low amino acid balance, especially the essential amino acids lysine and methionine (Azizi *et al.*, 2021). In addition, the chemical composition of PKC is 95.45%, 4.51% ash, 15.95% crude protein (CP), 8.36% extract ether, 21.21% crude fiber (CF), 0.55% lysine, 0.09% methionine, 47% lauric acid (Zubaidah *et al.*, 2024), 62.99% neutral detergent fiber, 42.21% acid detergent fiber, 20.78% hemicellulose (Pasaribu *et al.*, 2019), 11.53% mannose (Gomes-Osorio *et al.*, 2022), and 35.2% mannan (Fan *et al.*, 2014).

The high mannan content in PKC, one of which is β-mannan as an anti-nutritional component in monogastric feed that can increase digesta viscosity in the digestive tract and reduce nutrient digestibility. This affects the immunological response and bacterial proliferation in the digestive tract (Shastak *et al.*, 2019). Poultry cannot produce enzymes such as mannanase, cellulase, and xylanase; thus, exogenous enzymes must be added when feed ingredients contain PKC. Exogenous enzymes must be added to increase nutrient availability. The addition of enzymes is one alternative to increase nutrient digestibility in poultry. Koranteng *et al*. (2022) showed that 20% PKC with commercial multi-enzymes (xylanase, β-glucanase, cellulase, amylase, protease, and phytase enzymes) had higher weight in the digestive tract, especially gizzard weights compared to the addition of 10% PKC with the same multi-enzymes. Abdollahi *et al*. (2016) conducted the treatment of combination of multienzymes (β-mananase, xylanase, amylase, protease, cellulase, and β-glucanase) and supplementation of PKC at a level of 8% in feed showed that PKC supplementation at the level of 8% was able to reduce feed conversion ratio (FCR) compared with PKC supplementation at the levels of 16%-26%. Pasaribu *et al*. (2019) conducted PKC fermentation using a bacterial cocktail (mixed bacteria between *Bacillus amyloliquefaciens* and *Trichoderma harzanium*) incubated for 7 days showed that this fermentation was able to increase protein and

reduce crude fiber better than using a single bacteria (*B. amyloliquefaciens* or *T. harzianum*).

The supplementation of β-mannanase in feed is a strategy to improve the nutrient utilization of feed containing β-mannan. Feed supplemented with β-mannanase showed positive effects on the performance and nutrient digestibility of chickens (Shastak *et al.,* 2019). PKC supplemented with β-mannanase can lead to the formation of prebiotic manno-oligosaccharide (MOS) compounds, which are short-chain carbohydrates; thus, enzymatic hydrolysis of mannan turns into manobiose, manotriose, and manotetraose (Chacher *et al.,* 2017). Enzymes such as xylanase and mannanase can improve the use of NSP in feed and protease can boost protein utilization (Singh *et al.,* 2015). Some enzymes that have potential use in the feed industry are β-mannanase, cellulase, β-glucanase, phytase, xylanase, protease, galactosidase, and lipase (Sureshkumar *et al.,* 2023). PKC can be degraded using specialized enzymes, such as mannanase, xylanase, cellulase, α-galactosidase, and β-manosidase (Alshelmani *et al.,* 2014).

Increased crude fiber or manan in poultry feed can affect health status, including albumin, cholesterol, and glucose concentrations in the blood plasma as well as intestinal histomorphology. Saenphoom *et al*. (2013) reported that villi height in the duodenum, jejunum, and ileum did not show significant differences, but crypta depth decreased with feeding palm kernel meal supplemented with enzymes. Hakim *et al*. (2022) reported that fermented PKC can increase duodenal villi height, Jejunum crypta depth, and ileum compared to PKC feeding without fermentation. Rahim *et al*. (2007) reported that duodenal villus height, jejunum, and ileum decreased with the addition of fermented PKC with the increased levels of 0%, 9%, 18%, 27%, and 36%. Alshelmani *et al*. (2016) reported that feeding PKC without fermentation or with fermentation of 0%, 5%, 10%, and 15% did not show significant differences in villus height and depth of duodenal crypts, jejunum, and ileum. Factors that influence the microbiota of the digestive tract of poultry include feed additives such as phytobiotics, probiotics, prebiotics, enzymes, and organic acids, feed composition, and genetics. These feed additives will indirectly manipulate the gut microbiota and increase the intestinal villi, resulting in better nutrient absorption (Shehata *et al.,* 2022). As the villus height in the small intestine increases, the villus surface becomes wider, increasing the absorption area. The crypts of intestinal epithelial cells regenerate, and the number of goblet cells in the intestine increases with the addition of prebiotics (Bogucka *et al.,* 2017). The novelty of this research is the addition of 3 kinds of enzymes, namely mannanase, NSPase, and protease in broiler feed containing palm kernel cake. Therefore, this study aimed to determine the effect of enzyme supplementation on blood biochemistry, gastrointestinal health, and intestinal histomorphology of broiler chicken fed PKC.

MATERIALS AND METHODS

Ethical Clearance

The study was performed in a closed house cage in PT. Japfa Comfeed, Faculty of Animal Science Universitas Gadjah Mada (UGM), with ethical clearance number 048/EC-FKH/Eks/2022 issued by the Faculty of Veterinary Medicine UGM.

Enzymes

Commercial mannanase, NSPase (NDP-sugar pyrophosphorylases), and protease enzymes were used. The mannanase enzyme is a Hemicell®HT product derived from Elanco Animal Health (Indiana, USA) with enzyme activity of 10^6 U/kg based on COA. The NSPase enzyme is a product of Kemin Industries (Asia) Pte. Ltd. (Singapore) whose active ingredients consist of 270 U/g alpha-amylase, 2,500 U/g cellulase, 1,875 U/g xylanase, and 900 U/g protease based on COA, and the protease enzyme is a Poultrygrow 250™ product from Jefo Nutrition Inc. (Quebec, Canada).

Feeding Trials

The feed composition was formulated based on NRC (1994). This study used 10% and 20% PKC. Two kinds of mixed enzymes were used. Enzyme 1 consisted of mannanase at a dose of 182 g/ton, NSPase at a dose of 200 g/ton, and protease at a dose of 130 g/ton, whereas Enzyme 2 consisted of mannanase at a dose of 182 g/ton, NSPase at a dose of 400 g/ton, and protease at a dose of 260 g/ton. The treatment of Factor A consisted of 3 levels of enzyme addition (A1 without enzyme supplementation, A2 with Enzyme 1 supplementation, and A3 with Enzyme 2 supplementation), Factor B consisted of 2 levels, i.e., B1 with 10% PKC and B2 with 20% PKC. The treatments were A1B1 (feed with 10% PKC without enzyme supplementation); A1B2 (feed with 20% PKC without enzyme supplementation); A2B1 (feed with 10% PKC added with Enzyme 1 (NSPase 200 g/ton, protease 130 g/ton, and mannanase 182 g/ton)), A2B2 (feed with 20% PKC, added with Enzyme 1 (NSPase 200 g/ton, protease 130 g/ton, and mannanase 182 g/ton)); A3B1 (feed with 10% PKC added with Enzyme 2 (NSPase 400 g/ton, protease 260 g/ton, and mannanase 182 g/ton)); A3B2 (feed with 20% PKC added with Enzyme 2 (NSPase 400 g/ton, protease 260 g/ton, and mannanase 182 g/ton)). Table 1 shows the formulation and nutrient composition of the research feed.

Chicken Rearing

This study used 1,260 male Indian River broiler chickens (± 40–45 g) with seven treatments and six replicates. Each replicate used 30 chickens with a cage size per flock of 2 × 1.25 m^2 using a closed house cage at the Faculty of Animal Science, Universitas Gadjah Mada. The experimental chickens were reared for 35 days and provided feeding and watering *ad libitum*. Before slaughtering, the experimental chickens were fasted for

Table 1. Formulation and nutrient content of the experimental feed

Items	Type of feed (%)					
	A1B1	A1B2	A2B1	A2B2	A3B1	A3B2
Feed ingredients						
Corn	55.93	49.93	55.93	49.93	55.93	49.93
Palm kernel cake	10.00	20.00	10.00	20.00	10.00	20.00
Soya bean meal	20.00	16.00	20.00	16.00	20.00	16.00
Meat bone meal	10.00	10.00	10.00	10.00	10.00	10.00
Soybean oil	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin premix	0.03	0.03	0.03	0.03	0.03	0.03
Mineral premix	0.03	0.03	0.03	0.03	0.03	0.03
NaCl	0.06	0.06	0.06	0.06	0.06	0.06
CaCO3	1.00	1.00	1.00	1.00	1.00	1.00
L-Lysine HCl	0.15	0.15	0.15	0.15	0.15	0.15
DL-Methionine	0.20	0.20	0.20	0.20	0.20	0.20
Mono-calcium phosphate	0.60	0.60	0.60	0.60	0.60	0.60
Total	100	100	100	100	100	100
Nutrient composition						
Dry matter (%)	83.28	84.73	84.28	84.73	84.28	84.73
Ether extract (%)	6.23	6.86	6.23	6.86	6.23	6.86
Crude fiber (%)	3.65	4.91	3.65	4.91	3.65	4.91
Ash (%)	10.37	10.76	10.37	10.76	10.37	10.76
Metabolizable energy (kcal/kg)	3,013	3,002	3,013	3,002	3,013	3,002
Crude protein (%)	20.99	20.66	20.99	20.66	20.99	20.66
Calsium (%)	0.82	0.84	0.82	0.84	0.82	0.84
Available phosphorus (%)	0.50	0.50	0.50	0.52	0.50	0.52
Lysine (%)	1.11	1.05	1.11	1.05	1.11	1.05
Methionine (%)	0.49	0.49	0.49	0.49	0.49	0.49
Methionine + cystine (%)	1.19	1.08	1.03	1.08	1.03	1.08

Note: A1B1, feed with 10% palm kernel cake (PKC) without enzyme supplementation; A1B2, feed with 20% PKC without enzyme supplementation; A2B1, feed with 10% PKC supplemented with NSPase at a dosage of 200 g/ton, protease at a dose of 130 g/ton, mannanase at a dose of 182 g/ton, A2B2, basal feed with 20% PKC supplemented with NSPase at a dose of 200 g/ton, protease at a dose of 130 g/ton, and mannanase at a dose of 182 g/ton; A3B1, feed with 10% PKC added with enzyme NSPase at a dose of 400 g/ton, protease at a dose of 260 g/ton, and mannanase at a dose of 182 g/ton; A3B2: feed with 20% PKC added with NSPase at a dose of 400 g/ton, protease at a dose of 260 g/ton, and mannanase at a dose of 182 g/ton. Vitamin premix used contained 4,500 IU of vitamin A, 1,620 IU of vitamin D3, 31.5 mg of vitamin E, 1.35 mg of vitamin K, 1.35 mg of thiamine (B1), 3.30 mg of riboflavin (B2), niacin (B3) at the level of 18 mg, pantotheanic acid (B5) at the level of 5.40 mg, pyridoxine (B6) at the level of 2.10 mg, folic acid (B10) at the level of 0.69 mg, vitamin at the level of 12 0.01 mg, biotin at the level of 0.09 mg. The minerals premix used contained 4.8 mg/kg of copper, 0.38 mg/kg of iodine, 7.5 mg/kg of iron, 36 mg/kg of manganese, 0.06 mg/kg of selenium, 30 mg/kg of zinc, and 0.03 mg/kg of cobalt.

10 h. After rearing, two chicks were chosen randomly from each flock and weighed. Before slaughtering, 3 mL of blood was drawn by syringe from the brachial vein and transferred to a tube containing the anticoagulant ethylene diamine tetraacetic acid. The experimental chickens were halal slaughtered (Farouk *et al.*, 2014) and examined for gastrointestinal tract abnormalities. The structures of the gastrointestinal tract were then cut and separated. The height and weight of the internal organs of each broiler were measured using a measuring tape and a digital scale. The proventriculus, gizzard (ventriculus), liver, pancreas, and cecum were also measured, as well as the pH and weight of the duodenum, jejunum, and ileum. The relative weight percentage of each gastrointestinal organ was measured. Moreover, 2-cm sections of the duodenum, jejunum, and ileum were taken to make histomorphological preparations of the intestine using hematoxylin and eosin staining.

$$\text{Relative organ weight (\%)} = \frac{\text{Organ weight}}{\text{Live weight}} \times 100$$

Blood Analysis

Blood was taken from 42 hens via the brachial vein. The blood samples were put into tubes with coagulant and then centrifuged for 10 min at 3000 rpm to obtain the plasma. The plasma was analyzed for the levels of glucose, albumin, and total cholesterol using a semi-automatic biochemistry analyzer (ND-201; Caretium Medical Instruments, China). Stanbio reagents were used (Stanbio Laboratory, Boerne, USA).

Intestinal Histomorphology

Chickens were slaughtered at the age of 35 days, and 2 cm of the duodenum, jejunum, and ileum were taken for intestinal histomorphology preparations and stained with hematoxylin and eosin. The intestinal samples were fixed using 10% formalin and dehydrated using graded alcohol from 70% to 100%. Subsequently, alcohol was cleared from the tissue using Xylol I, II, and III, and the tissue was infiltrated using liquid paraffin.

The block was cooled with ice cubes. After the paraffin-treated tissue had solidified, it was cut using a 5-µm thick microtome, the cut block was placed in a water-bath with a temperature of 50 °C. The pieces were taken using an object glass, incubated at 50 °C for 15 min, and then stained with hematoxylin–eosin. Histological preparations in slides were observed under a light microscope equipped with computer-assisted OptiLab. Under ocular lens 10× magnification and objective lens 4× magnification, the preparation was photographed for the measurement of the villus height and width and crypt depth (Prakatur *et al.*, 2019).

Statistical Analysis

Data were analyzed using a completely randomized design with a factorial pattern of 2 × 3 and 6 replications. The treatment of Factor A consisted of 3 levels of enzyme addition (No enzyme, Enzyme 1, and Enzyme 2), and Factor B consisted of 2 levels of PKC (10% and 20%) followed by Duncan's multiple-range test. Enzyme 1 consisted of mannanase at a dose of 182 g/ton, NSPase at a dose of 200 g/ton, and protease at a dose of 130 g/ton feed, and Enzyme 2 consisted of mannanase at a dose of 182 g/ton, NSPase at a dose of 400 g/ton, and protease at a dose of 260 g/ton. Differences between treatments were tested using Duncan's multiple-range test (Steel & Torrie, 1981). Analysis of variance was used as the statistical model:

$$Yijk = \mu + \alpha_i + \beta j + \alpha\beta_{ij} + \varepsilon_{ijk}$$

where *i= 1, 2, …,a*; *j= 1, 2, …,b*; *k*= 1, 2…., n. α_i is the treatment effects for treatment factor *A*, βj indicates for treatment factor B, $\alpha\beta_{ij}$ is the interaction effects, Y_{ijk} represents the observation value of each individual, µ is overall mean; and ε_{ijk} is error.

RESULTS

Blood Profile

The group of experimental chickens fed with PKC supplemented with enzymes did not affect blood plasma glucose and cholesterol levels, but the addition of Enzyme 2 (A3) to PKC could increase blood plasma albumin levels ($p<0.05$). Blood biochemistry can be observed in Table 2. The glucose levels in this treatment diet ranged from 146 to 155.67 mg/dL, with no significant difference. Albumin levels increased when fed PKC supplemented with Enzyme 2 (A3) with the addition of mannanase at a dose of 182 g/ton, protease at a dose of 260 g/ton, and NSPase at a dose of 400 g/ton ($p<0.05$). There was an increase of about 61.87%-63.54% compared to A1 and A2. Total cholesterol levels did not differ between feed treatments, data obtained were within the standard range, and were not negatively affected by the treatments.

Gastrointestinal Health

The effects of PKC supplemented with enzyme on the relative percent of organ weight in broiler chickens are shown in Table 3. The groups fed with PKC supplemented with enzyme, (A2B1 and A3B1) could significantly reduce the relative weight of gizzard ($p<0.05$) compared to the other treatments. The group of experimental chicken fed ration containing 20% PKC supplemented with Enzyme 2 (A3B2) had the highest relative weight percentage of the gizzard (1.68%). Feed with 20% PKC could increase the relative weight percentage of proventriculus (15.09%), duodenum (15.35%), ileum (13.89%), and caecum (12.82%), while the liver, pancreas, and jejunum had no significant effect.

Intestinal pH

Feeding experimental chickens with ration containing PKC and supplemented with enzyme increased the intestinal pH in the duodenum ($p<0.05$) but had no significant effect on the jejunum and ileum. The addition of 20% PKC could increase the ileum pH compared with the addition of 10% PKC, while the duodenum and jejunum pH were not affected by the PKC level. Table 4 shows the pH levels of the duodenum, ileum, and jejunum.

Intestinal Histomorphology

The group of experimental chickens fed with PKC and supplemented with enzymes had an increase in

Table 2. Blood biochemistry of 35-day-old broiler chickens fed diets containing palm kernel cake supplemented with enzyme

Variables	PKC	Enzymes			Average
		A1	A2	A3	
Glucose (mg/dL)	B1	155.67 ± 9.89	146.17 ± 11.81	149.00 ± 11.92	150.28 ± 4.88
	B2	146.00 ± 11.71	152.67 ± 7.50	151.83 ± 8.84	150.17 ± 3.63
	Average	150.84 ± 6.84	149.42 ± 4.60	150.42 ± 2.00	
Albumin (mg/dL)	B1	1.87 ± 0.30	1.75 ± 0.27	5.03 ± 0.59	2.88 ± 1.86
	B2	1.78 ± 0.62	1.75 ± 0.27	4.57 ± 0.39	2.70 ± 1.62
	Average	1.83 ± 0.06^{b}	1.75 ± 0.00^{b}	4.80 ± 0.33^{a}	
Total cholesterol (mg/dL)	B1	142.89 ± 8.57	148.93 ± 28.41	155.11 ± 17.01	149.98 ± 6.11
	B2	146.82 ± 26.62	141.24 ± 6.61	138.73 ± 10.55	142.26 ± 4.14
	Average	144.86 ± 2.78	145.09 ± 5.44	146.92 ± 11.58	

Note: ^{abc}Different superscripts in the same raw indicate significant differences ($p<0.05$). PKC= palm kernel cake, A1= without supplementation of enzyme, A2= supplementation with NSPase at a dose of 200 g/ton, protease at a dose of 130 g/ton, and mannanase at a dose 182 g/ton (Enzyme 1), A3= supplementation with NSPase at a dose of 400 g/ton, protease at a dose of 260 g/ton, mannanase at a dose of 182 g/ton (Enzyme 2), B1= 10% PKC, B2= 20% PKC.

Table 3. Percent of relative organ weight of broiler chickens fed ration contained palm kernel cake supplemented with enzyme

Variables (% ROW)	PKC	Enzymes			Average
		A1	A2	A3	
Proventriculus	B1	0.46 ± 0.08	0.46 ± 0.04	0.44 ± 0.03	0.45 ± 0.01[b]
	B2	0.53 ± 0.05	0.54 ± 0.05	0.52 ± 0.05	0.53 ± 0.01[a]
	Average	0.50 ± 0.05	0.50 ± 0.06	0.48 ± 0.06	
Gizzard/ventriculus	B1	1.38 ± 0.09[b]	1.10 ± 0.09[c]	1.18 ± 0.10[c]	1.22 ± 0.15[b]
	B2	1.45 ± 0.09[b]	1.42 ± 0.18[b]	1.68 ± 0.21[a]	1.52 ± 0.20[a]
	Average	1.42 ± 0.09[a]	1.26 ± 0.21[b]	1.43 ± 0.31[a]	
Liver	B1	1.93 ± 0.27	2.01 ± 0.13	1.97 ± 0.49	1.97 ± 0.04
	B2	1.95 ± 0.18	2.24 ± 0.31	2.20 ± 0.15	2.13 ± 0.16
	Average	1.94 ± 0.01	2.13 ± 0.16	2.09 ± 0.16	
Pancreas	B1	0.28 ± 0.04	0.27 ± 0.04	0.29 ± 0.06	0.28 ± 0.01
	B2	0.29 ± 0.03	0.30 ± 0.04	0.30 ± 0.04	0.30 ± 0.01
	Average	0.29 ± 0.01	0.29 ± 0.01	0.30 ± 0.10	
Duodenum	B1	0.55 ± 0.03	0.59 ± 0.13	0.52 ± 0.05	0.55 ± 0.04[b]
	B2	0.58 ± 0.09	0.66 ± 0.17	0.70 ± 0.09	0.65 ± 0.06[a]
	Average	0.57 ± 0.02	0.63 ± 0.05	0.61± 0.13	
Jejunum	B1	1.17 ± 0.21	1.22 ± 0.09	1.13 ± 0.11	1.17 ± 0.05
	B2	1.12 ± 0.25	1.38 ± 0.11	1.33 ± 0.11	1.28 ± 0.14
	Average	1.15 ± 0.04	1.30 ± 0.11	1.23 ± 0.14	
Ileum	B1	0.94 ± 0.11	0.96 ± 0.12	0.90 ± 0.15	0.93 ± 0.03[b]
	B2	1.02 ± 0.15	1.12 ± 0.17	1.11 ± 0.25	1.08 ± 0.06[a]
	Average	0.98 ± 0.06	1.05 ± 0.11	1.01 ± 0.15	
Caecum	B1	0.36 ± 0.05	0.35 ± 0.07	0.31 ± 0.05	0.34 ± 0.03[b]
	B2	0.38 ± 0.10	0.41 ± 0.06	0.39 ± 0.05	0.39 ± 0.02[a]
	Average	0.37 ± 0.01	0.38 ± 0.04	0.35 ± 0.06	

Note: [abc] Different superscripts in the same raw/column indicate significant differences (p<0.05). PKC= palm kernel cake, A1= no supplementation of enzyme, A2= supplementation with NSPase at a dose of 200 g/ton, protease at a dose of 130 g/ton, and mannanase at a dose of 182 g/ton (Enzyme 1), A3= supplementation with NSPase at a dose of 400 g/ton, protease at a dose of 260 g/ton, mannanase at a dose of 182 g/ton (Enzyme 2), B1= 10% PKC, B2= 20% PKC, ROW= relative organ weight.

Table 4. pH of the duodenum, jejunum, and ileum of chicken fed with ration supplemented with palm kernel cake and enzymes

Variables	PKC	Enzymes			Average
		A1	A2	A3	
pH of the duodenum	B1	5.84 ± 0.21	5.96 ± 0.15	5.91 ± 0.12	5.90 ± 0.06
	B2	5.76 ± 0.14	5.97 ± 0.09	6.06 ± 0.08	5.93 ± 0.15
	Average	5.80 ± 0.06[b]	5.97 ± 0.01[a]	5.99 ± 0.11[a]	
pH of the jejunum	B1	6.19 ± 0.44	6.11 ± 0.17	5.85 ± 0.42	6.05 ± 0.18
	B2	6.21 ± 0.34	6.27 ± 0.42	5.97 ± 0.25	6.15 ± 0.16
	Average	6.20 ± 0.01	6.19 ± 0.11	5.91 ± 0.08	
pH of the ileum	B1	6.83 ± 0.33	6.66 ± 0.56	6.46 ± 0.51	6.65 ± 0.19[b]
	B2	6.98 ± 0.29	6.83 ± 0.49	7.02 ± 0.23	6.94 ± 0.10[a]
	Average	6.91 ± 0.11	6.75 ± 0.12	6.74 ± 0.40	

Note: [abc] Different superscripts in the same raw/column indicate significant differences (p<0.05). PKC= palm kernel cake, A1= no supplementation of enzyme, A2= supplementation with NSPase at a dose of 200 g/ton, protease at a dose of 130 g/ton, and mannanase at a dose of 182 g/ton (Enzyme 1), A3= supplementation with NSPase at a dose of 400 g/ton, protease at a dose of 260 g/ton, mannanase at a dose of 182 g/ton (Enzyme 2), B1= 10% PKC, B2= 20% PKC.

the height of the duodenal and ileal villi. Experimental chickens fed ration containing 10% PKC supplemented with Enzyme 1 (A2B1) showed the longest duodenal villi (2,054.16 μm) and ileal villi (1,196.96 μm) and experimental chickens fed ration contained 20% PKC supplemented with Enzyme 2 (A3B2) showed the lowest duodenal villi (1,479.18 μm) and ileal villi (754.38 μm). Enzyme addition could increase the width of duodenal and ileal villi, but decrease the depth of duodenal crypts. Increasing the level of PKC supplementation in the ration increased the depth of duodenal and ileal crypts, but decreased the height of jejenal villi. Histomorphology of the duodenum, jejunum, and ileum, including the villus height and width and crypt depth can be observed in Table 5. Figure 1 shows the histomorphology of the duodenum, jejunum, and ileum.

DISCUSSION

Blood Profile

Greenacre & Moroshita (2021) reported that blood glucose levels were below the normal range for non-fasted chickens, at 227–300 mg/dL because before slaughtering, the animals were initially fed for 10 h so that the plasma glucose levels were below the normal

Table 5. Histomorphology of the duodenum, jejunum, and ileum including the villus height and width and crypt depth in male 35-day-old Indian River broilers fed with ration supplemented with palm kernel cake and enzymes

Variables (μm)	PKC (B)	Enzymes (A)			Average
		A1	A2	A3	
Duodenum					
Villus height	B1	1,630.84 ± 157.92^{bc}	2,054.16 ± 158.62^{a}	1,675.36 ± 109.71^{b}	1,786.79 ± 237.50^{a}
	B2	1,493.52 ± 138.64^{bc}	1,596.87 ± 121.55^{bc}	1,479.18 ± 89.87^{c}	1,523.19 ± 122.33^{b}
	Average	1,562.17 ± 157.68^{b}	1,825.52 ± 275.38^{a}	1,577.27 ± 140.11^{b}	
Villus width	B1	211.58 ± 19.68	223.69 ± 20.88	239.69 ± 26.05	224.99 ± 14.10
	B2	209.24 ± 13.12	228.31 ± 21.02	276.11 ± 50.02	237.89 ± 34.45
	Average	210.41 ± 1.65^{b}	226.00 ± 3.27^{b}	257.90 ± 25.75^{a}	
Crypt depth	B1	303.33 ± 31.42	294.78 ± 8.07	279.67 ± 28.06	301.59 ± 26.01^{b}
	B2	371.46 ± 73.65	286.97 ± 25.47	341.84 ± 21.43	333.42 ± 42.87^{a}
	Average	350.00 ± 29.08^{a}	290.88 ± 5.52^{b}	310.76 ± 43.96^{b}	
Jejunum					
Villus height	B1	1,322.73 ± 164.21	1,394.65 ± 32.82	1,324.83 ± 137.12	1,347.40 ± 40.93^{a}
	B2	1,199.63 ± 109.93	1,115.38 ± 194.97	1,255.15 ± 230.85	1,190.05 ± 70.38^{b}
	Average	1,261.18 ± 87.04	1,255.02 ± 197.47	1,289.99 ± 49.27	
Villus width	B1	217.75 ± 25.15	230.13 ± 52.73	243.78 ± 45.60	230.55 ± 13.02
	B2	203.86 ± 17.83	224.66 ± 13.70	193.32 ± 89.87	207.28 ± 15.95
	Average	210.81 ± 9.82	227.40 ± 3.87	218.55 ± 35.68	
Crypt depth	B1	272.73 ± 64.15	173.05 ± 29.31	187.26 ± 41.83	211.01 ± 13.02
	B2	203.51 ± 52.71	197.47 ± 94.16	213.55 ± 37.26	204.84 ± 8.12
	Average	238.12 ± 48.95	185.26 ± 17.27	200.41 ± 18.59	
Ileum					
Villus height	B1	1,041.28 ± 91.40^{b}	1,196.96 ± 109.22^{a}	893.70 ± 185.21^{cd}	1,041.98 ± 151.65^{a}
	B2	1,015.67 ± 138.64^{bc}	893.25 ± 41.98^{cd}	754.38 ± 11.61^{e}	887.75 ± 130.70^{b}
	Average	1,028.45 ± 89.24^{a}	1,045.11 ± 178.06^{a}	824.04 ± 143.87^{b}	
Villus width	B1	168.44 ± 14.32^{bc}	192.07 ± 15.12^{ab}	185.38 ± 15.16^{abc}	181.96 ± 12.18
	B2	209.34 ± 27.14^{a}	196.99 ± 7.66^{a}	161.85 ± 21.20^{c}	189.39 ± 24.64
	Average	188.89 ± 28.92	194.53 ± 3.48	173.62 ± 16.64	
Crypt depth	B1	162.67 ± 14.28	168.48 ± 16.88	161.86 ± 15.05	164.34 ± 3.61^{a}
	B2	175.73 ± 138.64	177.07 ± 9.31	173.46 ± 8.60	175.42 ± 1.82^{b}
	Average	169.20 ± 9.23	172.78 ± 6.07	167.66 ± 8.20	

Note: ^{abc} Different superscripts in the same raw/column indicate significant differences ($p<0.05$). PKC= palm kernel cake, A1= no supplementation of enzyme, A2= supplementation with NSPase at a dose of 200 g/ton, protease at a dose of 130 g/ton, and mannanase at a dose of 182 g/ton (Enzyme 1), A3= supplementation with NSPase at a dose of 400 g/ton, protease at a dose of 260 g/ton, mannanase at a dose of 182 g/ton (Enzyme 2), B1= 10% PKC, B2= 20% PKC.

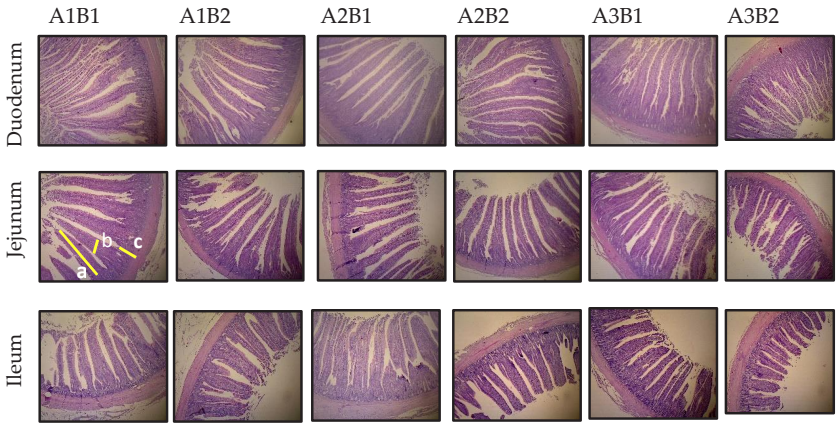

Figure 1. Histomorphology of the duodenum, jejunum, and ileum in male 35-day-old Indian River chicken fed with ration supplemented with palm kernel cake and enzymes. a= height of the villi, b= width of the villi, and c= crypt depth. A1B1, feed with 10% palm kernel cake (PKC) without enzyme supplementation; A1B2, feed with 20% PKC without enzyme supplementation; A2B1, feed with 10% PKC supplemented with NSPase at a dosage of 200 g/ton, protease at a dose of 130 g/ton, mannanase at a dose of 182 g/ton, A2B2, basal feed with 20% PKC supplemented with NSPase at a dose of 200 g/ton, protease at a dose of 130 g/ton, and mannanase at a dose of 182 g/ton; A3B1, feed with 10% PKC added with enzyme NSPase at a dose of 400 g/ton, protease at a dose of 260 g/ton, and mannanase at a dose of 182 g/ton; A3B2: feed with 20% PKC added with NSPase at a dose of 400 g/ton, protease at a dose of 260 g/ton, and mannanase at a dose of 182 g/ton.

standard for non-fasted chickens. Christensen *et al*. (2013) stated that chickens fed for >6 h before slaughtering had decreased blood glucose levels and 33-day-old chickens fed before slaughtering for 12 h had reduced plasma glucose levels by 22.6%. Proszkowiec-Weglarz *et al*. (2017) reported that during fasting, glucose levels decrease because of various physiological mechanisms and insulin regulates blood glucose levels. Fasting before slaughtering results in the depletion of energy reserves (Ali *et al*., 2008). Wu *et al*. (2024) reported that a long fasting period before slaughtering decreased blood glucose levels, liver glycogen levels, and leg muscle glycogen levels but not chest muscle glycogen levels. This was also seen in cattle that fasted for >8 h before slaughtering.

Glycogen is a form of energy stored in animal cells that helps keep blood glucose levels stable. The majority of animal glycogen is stored in the muscles and liver, known as liver glycogen and muscle glycogen, respectively. Excess monosaccharides taken by the intestines are converted into glycogen and stored in the liver and muscles to prevent high glucose levels (Do Nascimento, 2018). During fasting, blood glucose is derived from glycogen stores in the liver and muscles. Glycogen is made up of polymerized glucose monosaccharide chains that are converted into energy during glycogenolysis. After approximately 24 h of fasting, glycogen stores are depleted, forcing the body to rely on energy stores from the adipose tissue and protein storage (Sanvictores *et al*., 2023). After fasting, glucose absorption in the intestine decreases, glycogen stored in the liver decomposes quickly, fat and protein conversion is relatively slow, and blood sugar oxidation and energy supply increase, resulting in a drop in blood sugar levels (Delezi *et al*., 2007). When the blood glucose levels drop after fasting, the diminished liver glycogen degrades into glucose and enters the bloodstream to keep the blood glucose levels stable. Muscle glycogen is partially degraded into lactic acid by oxidation for energy supply, and the remaining stored glycogen is transported to the liver via blood and transformed into liver glycogen by gluconeogenesis (Jensen *et al*., 2011).

The plasma albumin levels in the present study were consistent with the findings of Saleh *et al.* (2020), who found that increasing protease supplementation from 200 to 300 mg/kg raised the plasma albumin levels. Protease supplementation could increase dry matter digestibility and protein digestibility. According to Moati *et al.* (2022), the addition of probiotics and mixed enzymes (xylanase, amylase, and protease) could raise plasma albumin levels compared with the control treatment. Probiotic supplements, mixed enzymes, and their combination affect growth performance, immunological condition, and microbiota in the small intestines. According to Lee *et al*. (2018), the addition of protease and NSPase could increase amino acid digestibility, promote nutrient utilization, and help in protein synthesis, which causes an increase in blood plasma levels in experimental chickens fed ration supplemented with the given enzyme. Supplementation of β-mananase in poultry feed has positively improved blood glucose and anabolic hormone homeostatis, FCR, energy digestibility, and amino acid digestibility (Saeed *et al*., 2019; Caldas *et al*., 2018).

Gastrointestinal Health

Okukpe *et al*. (2019) found that the weight of the proventriculus of broilers fed 30% PKC was higher than those fed 10% or 20% PKC. Feeds containing 10% and 20% PKC increased the relative weight percent of gizzard by 9.42%–13.79% compared with feed without PKC. Moreover, Okeudo *et al*. (2006) found that 0%, 15%, 30%, and 45% PKC increased the relative weight percent of broiler gizzard from 2.43% to 3.49%. Increasing PKC concentration increased the relative weight percent of the gizzard but not the liver (Alshelmani *et al*., 2017).

Animals fed 20% PKC had heavier gizzards because PKC has high crude fiber, which increased the frequency of contractions to digest fibrous feed smoothly and stimulative effect of the grinding activity on the gizzard muscles; thus the development of gizzard muscles (Koranteng *et al*., 2022; Alshelmani *et al*., 2017). The ventriculus of the group fed 20% PKC had the highest relative weight percent because the addition of NSPase and mannanase helps in the hydrolysis of NSP into mano-oligosaccharides and protease promote protein utilization by hydrolyzing proteins into amino acids, reducing the workload of the gizzard (Singh *et al*., 2015). The relative weight percent of the liver, pancreas, duodenum, and ileum were not significantly different. This finding was consistent with a previous report that feed supplemented with PKC and enzyme altered the height of the duodenal villi. Still, it did not affect the relative weight percent of the heart, liver, and lymph (Koranteng *et al*., 2022). Pushpakumara *et al*. (2017) reported that the addition of 0%–20% PKC did not affect the weight of the liver.

Intestinal pH

The feed supplemented with PKC increases the pH of the duodenum but not that of the jejunum and ileum. Feed supplemented with PKC and enzymes could boost the nutritional value of the feed by reducing the crude fiber content, which will influence the digestive tract environment (Stein *et al*., 2015). The enzymes added in PKC feed can trigger the release of luminal ATP that activates submucosal H^+ chemosensors, which have the potential for bicarbonate secretion in the duodenum and can increase duodenal pH (Wang *et al*., 2011). Feed supplemented with PKC and enzymes could improve the health of the digestive tract and microbial gut composition and, thus gut pH (Saenphoom *et al*., 2013). The high fiber content of PKC may stimulate the growth of mucosal epithelial cells, which will affect the morphology of the villi, potentially affecting pH regulation in the duodenum (Alyileili *et al*., 2020). The ration supplemented with PKC and β-mannanase can increase fiber digestibility, which may modify nutrient absorption and fermentation in the gut, thus affecting pH (Wilkinson & Young, 2020). Enzymatic treatment of PKC could increase digestibility and improve gastrointestinal health,

which will indirectly affect pH (Habte-Tsion & Kumar, 2018).

Intestinal Histomorphology

Saenphoom *et al*. (2013) found no significant difference in the villus height in the duodenum, jejunum, and ileum but decreased the crypt depth in animals fed ration supplemented with PKC and enzyme. Yaophakdee *et al*. (2018) reported no significant difference in the villus height, villus width, ileal crypta depth, and ileal villus surface area in broiler chickens. Hakim *et al*. (2022) reported that fermented PKC could increase duodenal villus height, jejunal, and ileal crypt depth compared to PKC without fermentation. Rahim *et al*. (2007) observed that duodenal villus height, jejunum, and ileum decreased with the addition of fermented PKC at the levels of 0%, 9%, 18%, 27%, and 36%, respectively. Alshelmani *et al*. (2016) reported that feeding PKC without fermentation or with fermentation using 0%, 5%, 10%, and 15% showed no significant difference in villus height and crypt depth of the duodenum, jejunum, and ileum.

This study showed that the addition of enzymes could increase the villus height. As the villus height in the small intestine increases, the surface becomes wider, resulting in the increased nutrient absorption, which leads to faster chicken growth (Gopinger *et al*., 2014). Mannan breakdown by enzymes may increase the levels of mannan oligosaccharides (MOS), which have a prebiotic effect (Chen *et al*., 2014). The addition of prebiotic MOS to feed may promote the development of the intestinal shape, particularly by increasing the villus height, producing more goblet cells to generate mucin, increasing the abundance of beneficial bacteria, and decreasing the number of pathogenic bacteria. The increase in villus height is related to the surface area for nutrient absorption, which affects gut health, immunity, and body weight gain (Ravindran & Abdollahi, 2021).

The crypt cells of the small intestine provide stem cells for the renewal of the intestinal epithelium. In the crypt base, stem cells are continuously divided to produce epithelial cells in the crypt and villus, and some epithelial cells are coated with younger epithelial cells involved in secretion. The addition of *Enterococcus faecium* could increase the villi height and the ratio of villi height to crypta depth, while *Escherichia coli* infection could cause swelling of the villi tips and increase the crypta depth (Huang *et al*., 2018). The addition of prebiotics regenerates the crypts in intestinal epithelial cells and increases goblet cells in the intestines (Bogucka *et al*., 2017). PKC feed fermented with lactic acid bacteria improved the intestinal growth and expression of glucose transporters and amino acids but not the nutrient digestibility or digestive enzyme activity of broiler hens (Hakim *et al*., 2022).

CONCLUSION

The feed supplemented with PKC and enzymes improved the albumin levels, gastrointestinal health, and histomorphology of the small intestine. The ration supplemented with PKC at a dose of 10% with enzyme supplementations of mannanase at a dose of 182 g/ton, NSPases at a dose of 200 g/ton, and protease at a dose of 130 g/ton (A2B1) had the best health status.

CONFLICT OF INTEREST

The authors declare no conflict of interest with any organization regarding the content discussed in this study.

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REFERENCES

Abdollahi, M. R., B. J. Hosking, D. Ning, & V. Ravindran. 2016. Influence of palm kernel meal inclusion and exogenous enzyme supplementation on growth performance, energy utilization, and nutrient digestibility in young broilers. Asian-Australas. J. Anim. Sci. 29:539–548. https://doi.org/10.5713/ajas.15.0224

Ali, M. S., G. H. Kang, & S. T. Joo. 2008. A Review: Influences of pre-slaughter stress on poultry meat quality. Asian-Australas. J. Anim. Sci. 21:912-916. https://doi.org/10.5713/ajas.2008.r.06

Alshelmani, M. L., T. C. Loh, & H. L. Foo. 2017. Effect of solid states fermentation on nutrient content and ileal amino acids digestibility of palm kernel cake in broiler chickens. Indian J. Anim. Sci. 87:1135-1140. https://doi.org/10.56093/ijans.v87i9.74331

Alshelmani, M. L., T. C. Loh, H. L. Foo, A. Q. Sazili, & W. H. Lau. 2016. Effect of feeding different levels of palm kernel cake fermented by *Paenibacillus polymyxa* ATCC 842 on broiler growth performance, blood biochemistry, carcass characteristics, and meat quality. Anim. Prod. Sci. 57:216-224. https://doi.org/10.1016/j.anifeedsci.2016.03.019

Alshelmani, M. I., T. C. Loh, H. L. Foo, W. H. Lau, & A. Q. Sazili. 2014. Biodegradation of palm kernel cake by cellulolytic and hemicellulolytic bacterial cultures through solid state fermentation. Scientific World Journal 14:1-8. https://doi.org/10.1155/2014/729852

Alyileili, S. R., K. A. El-Trabily, I. E. H. Belai, W. H. Ibrahim, M. Sulaiman, & A. S. Hussein. 2020. Intestinal development and histomorphometry of broiler chickens fed *Trichoderma reesei* degraded date seed diets. Front. Vet. Sci. 7:1–9. https://doi.org/10.3389/fvets.2020.00349

Azizi, M. N., T. C. Loh, H. L. Foo, & E. L. T. Chung. 2021. Review: Is palm kernel cake a suitable alternative feed ingredient for poultry? Animals 11:4-15. https://doi.org/10.3390/ani11020338

Bogucka, J., A. Dankowiakowska, G. Elminowska-Wenda, A. Sobolwska, J. Jankowski, M. Szpinda, & M. Bednarczyk. 2017. Performance and small intestine morphology and ultrastructure of male broilers injected *in ovo* with bioactive substances. Annals Animal Science 17:179-195. https://doi.org/10.1515/aoas-2016-0048

Caldas, J. V., K. Vignale, N. Boonsinchai, J. Wang, M. Putsakum, J. A. England, & C. N. Coon. 2018. The effect of β-mannanase on nutrient utilization and blood parameters in chicks fed diets containing soybean meal and guar gum. Poult. Sci. 97:2807–2817. https://doi.org/10.3382/ps/pey099

Chacher, M. F. A., Z. Kamran, U. Ahsan, S. Ahmad, K. C. Koutoulis, H. G. Qutab Ud Din, & Ö. Cengiz. 2017. Use

of mannan oligosaccharide in broiler diets: an overview of underlying mechanisms. Worlds Poult. Sci. J. 73:831-844. https://doi.org/10.1017/S0043933917000757

Chen, W. L., J. B. Liang, M. F. Jahromi, N. Abdullah, Y. W. Ho, & V. Tufarelli. 2014. Enzyme treatment enhances release of prebiotic oligosaccharides from palm kernel expeller. Bioresource 10:196-209. https://doi.org/10.15376/biores.10.1.196-209

Christensen, K., J. P. McMurtry, Y. V. Thaxton, A. Corzo, C. McDaniel, & C. G. Scanes. 2013. Metabolic and hormonal responses of growing modern meat-type chickens to fasting. Br. Poult. Sci. 54:199–205. https://doi.org/10.1080/00071668.2013.772953

Delezi, E., Q. Swennen, J. Buyse, & E. Decuypere. 2007. The effect of feed withdrawal and crating density in transit on metabolism and meat quality of broilers at slaughter weight. Poult. Sci. 86:1414–1423. https://doi.org/10.1093/ps/86.7.1414

Do Nascimento, G. S., R. P. Constantin, E. H. Giglioni, C. V. d. C. Ghizoni, A. Bracht, K. S. Utsunomiya, N. S. Yamamoto, E. L. I. Iwamoto, J. Constantin, & R. P. Constantin. 2018. The acute of citrus flavanones on the metabolism of glycogen and monosaccharides in the isolated perfused rat liver. Toxicol. Lett. 291:158-172. https://doi.org/10.1016/j.toxlet.2018.04.001

Fan, S. P., L. Q. Jiang, C. H. Chia, Z. Fang, S. Zakaria, & K. L. Chee. 2014. High yield production of sugars from deproteinated palm kernel cake under microwave irradiation via dilute sulfuric acid hydrolysis. Bioresour. Technol. 153:69–78. https://doi.org/10.1016/j.biortech.2013.11.055

Farouk, M. M., H. M. Al-Mazeedi, A. B. Sabow, A. E. D. Bekhit, K. D. Adeyemi, A. Q. Sazili, & A. Ghani. 2014. Halal and kosher slaughter methods and meat quality: A review. Meat Sci. 98:505-519. https://doi.org/10.1016/j.meatsci.2014.05.021

Gomes-Osorio, L. M., J. U. Nielson, H. J. Martens, & R. Wimmer. 2022. Upgrading the nutritional value of PKC using a *Bacillus subtilis* derived monocomponen β-mannanase. Molucules 27:1–4. https://doi.org/10.3390/molecules27020563

Gopinger, E., E. G. Xavier, M. C. Elias, A. A. S. Catalan, M. L. S. Castro, A. P. Nunes, & V. F. B. Roll. 2014. The effect of different dietary levels of canola meal on growth performance, nutrient digestibility, and gut morphology of broiler chickens. Poult. Sci. 93:1130-1136. https://doi.org/10.3382/ps.2013-03426

Greenacre, C. B. & T. Y. Moroshita. 2021. Backyard Poultry Medicine and Surgery: A Guide for Veterinary Practitioner. Second Edition. John Wiley and Son, Inc. Hoboken, USA. p. 522.

Habte-Tsion, H. M. & V. Kumar. 2018. Nonstarch Polysaccharide Enzymes-General Aspects. Enzymes in Human and Animal Nutrition. Chapter 9. p. 183–209. Academic Press. https://doi.org/10.1016/B978-0-12-805419-2.00009-5

Hakim, A. H., I. Zulkifli, A. S. Farjam, E. A. Awad, & S. K. Ramiah. 2022. Impact of feeding fermented palm kernel cake and high dietary fat on nutrient digestibility, enzyme activity, intestinal morphology and intestinal nutrient transporters mRNA expression in broiler chickens under hot and humid conditions. Animals 12:1–14. https://doi.org/10.3390/ani12070882

Huang, L., L. Luo, Y. Zhuang, Z. Wang, & Z. Xia. 2018. Effect of the dietary probiotic, *Enterococcus faecium* NCIMB11181, on the intestinal barrier and system immune status in *Escherichia coli* O78-challenged broiler chickens. Probiotics Antimicrob. Proteins 11:946–956. https://doi.org/10.1007/s12602-018-9434-7

Jensen, J., P. I. Rustad, A. J. Kolnes, & Y. C. Lai. 2011. A Review: The role of skeletal muscle glycogen breakdown for regulation of insulin sensitivity by exercise. Front. Physiol. 2:1–11. https://doi.org/10.3389/fphys.2011.00112

Koranteng, A. A. A., K. A. Gbogbo, B. A. Mensah, T. Boussai, C. T. F. Aina, & J. Glago. 2022. Impact of palm kernel cake with or without multi-blend enzyme on the growth performance and carcass traits of Sasso broilers. Int. J. Vet. Sci. Med. 10:80-89. https://doi.org/10.1080/23144599.2022.2125735

Lee, S. A., M. R. Bedford, & C. L. Walk. 2018. Meta-analysis: Explicit value of mono-componen proteases in monogastric diets. Poult. Sci. 97:2078–2085. https://doi.org/10.3382/ps/pey042

National Research Council (NRC). 1994. Nutrient Requirement for Poultry. Ninth Revised Edition. National Academy Press, Washington D. C. p. 27.

Moati, A. Y. A., N. M. Eissa, K. F. M. Aboulezz, & M. Younis. 2022. Effect of dietary supplementation of probiotics, enzymes and their combination on growth performance, meat yield, intestinal microbiota and plasma analysis of broiler chicks. Archives Agricultural Sciences Journal 5:136–152. https://doi.org/10.21608/aasj.2022.144587.1121

Okukpe, M. K., T. A. Aderibigde, & J. O. Atleh. 2019. Effect of supplementation of palm kernel cake (PKC) with enzyme xylanase on performance and gut microbiota of broiler chickens. Vidyodaya Journal Science 22:12–28. https://doi.org/10.4038/vjs.v22i1.6061

Okeudo, N. J., I. L. Onyike, C. V. Okoli, & I. L. Chielo. 2006. Production performance, meat quality and feed cost implications of utilizing high levels of palm kernel cake in broiler finisher diets. Int. J. Poult. 12:1160-1163. https://doi.org/10.3923/ijps.2006.1160.1163

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

Pasaribu, T., E. B. Laconi, & I. P. Kompiang. 2019. Evaluation of the nutrient contents of palm kernel cake fermented by microbial cocktails as a potential feedstuff for poultry. J. Indones. Trop. Anim. Agric. 44:295–302. https://doi.org/10.14710/jitaa.44.3.295-302

Prakatur, I., M. Miskulin, M. Pavic, K. Marjanovic, V. Blazicevic, I. Miskulin, & M. Domacinovic. 2019. Intestinal morphology in broiler chickens supplemented with propolis and bee pollen. Animals 9:1-12. https://doi.org/10.3390/ani9060301

Proszkowiec-Wegla, M., J. Dupont, N. Rideau, C. Gespach, J. Simon, & T. E. Porter. 2017. Insulin immuno-neutralization decreases food intake in chickens without altering hypothalamic mRNA levels for genes involved in regulation of food intake and metabolism. Poult. Sci. 96:4409–4418. https://doi.org/10.3382/ps/pex247

Pushpakumara, D. M. S., N. Priyankarage, W. A. D. Nayananjalie, D. L. Ranathunge, & D. Dissanayake. 2017. Effect of inclusion of palm (*Elaeis guineensis*) kernel cake in broiler chicken rations. Int. J. Livest. 7:103–109. https://doi.org/10.5455/ijlr.20170201053413

Rahim, F., Sabrina, Rusmawati, & M. Syibli. 2007. Broiler small intestine villi response to feed containing palm kernel cake which fermented with *Rhizopus* sp. J. Indones. Trop. Anim. Agric. 32:251-256.

Ravindran, V. & R. Abdollahi. 2021. Review: Nutrition and digestive physiology of broiler chick: State the art and outlook. Animals 11:1–23. https://doi.org/10.3390/ani11102795

Saeed, M., T. Ayasan, M. Alagawany, M. E. El-Hack, M. A. Abdel-Latif, & A. K. V. Patra. 2019. The role of β-mannanase (Hemicell) in improving poultry productivity, health and environment. Rev. Bras. Cienc. Avic. 21:1–8. https://doi.org/10.1590/1806-9061-2019-1001

Saenphoom, P., J. H. Liang, Y. W. Ho, T. C. Loh, & M. Rosfarizan. 2013. Effects of enzymes treated palm kernel expeller on metabolizable energy, growth perfoamce, villus height and digesta viscosity in broiler chickens. Asian-Australas. J. Anim. Sci. 26:537-544. https://doi.org/10.5713/ajas.2012.12463

Saleh, A. A., M. Mustafa, Dawood, N. A. Badawi, T. A. Ebeid, K. A. Amber, & M. M. Azzam. 2020. Effect of supplemental serine-protease from *Bacillus licheniformis* on growth performance and physiological change of broiler chickens. J. Appl. Anim. Res. 48:86-92. https://doi.org/10.1080/09712119.2020.1732986

Sanvictores, T., J. J. Casale, & M. R. Huecker. 2023. Physiology, Fasting. StatPealrs Publishing. National Library of Medicine. Physiology, Fasting - StatPearls - NCBI Bookshelf (nih.gov). Treasure Island, Florida USA.

Shastak, Y., P. Ader, D. Feuerstein, R. Ruehle, & M. Matuschek. 2019. ß–Mannan and mannanase in poultry nutrition. Worlds Poult. Sci J. 71:161–174. https://doi.org/10.1017/S0043933915000136

Shehata, A. A., S. Yalcin, J. D. latorre, S. Basiouni, Y. A. Attia, A. A. El-Wahab, C. Visscher, H. R. El-Seedi, C. Huber, H. M. Hafez, W. Eisenreich, & G. Tellez-Isaias. 2022. A Review: Probiotics, rebiotics, and phytogenic substances for optimizing gut health in poultry. Microorganism 10:1–15. https://doi.org/10.3390/microorganisms10020395

Singh, P. K., Chandramoni, A. Kumar, & S. Kumar. 2015. Animal Feed Additives. New India Publishing Agency, New Delhi. p. 18.

Steel, R. G. & J. H. Torrie. 1981. Principle and Procedures of Statistics: A Biometrical Approach. 2nd edition. McGraw Hill Book Co., Singapore. p. 355.

Stein, H. H., G. A. Casas, J. J. Abelilla, Y. Loiu, & R. S. Sulabo. 2015. Nutritional value of high fiber co-products from the copra, palm kernel, and rice industries in diets fed to pigs. J. Anim. Sci. Biotechnol. 6:1-9. https://doi.org/10.1186/s40104-015-0056-6

Sundu, B., A. Adjis, H. Hafsah, & M. Pamulu. 2024. The use of enzymatic pre-digestion of fermented palm kernel cake in the laying hens diet on production performance, nutrient digestibility, egg quality, and egg chemical content. Trop. Anim. Sci. J. 47:61–67. https://doi.org/10.5398/tasj.2024.47.1.61

Sureshkumar, S., J. Song. V. Sampath, & I. Kim. 2023. Exogenous enzymes as zootechnical additives in monogastric animal feed: A Review. Agriculture 13:1–14. https://doi.org/10.3390/agriculture13122195

Wang, J. H., T. Inoue, M. Higashiyama, P. H. Guth, E. Engel, J. D. Kaunits, & Y. Akiba. 2011. *Umami* receptor activation increases duodenal bicarbonate secretion via glucagon-like peptide-2 release in rats. J. Pharmacol. Exp. Ther. 339:464-473. https://doi.org/10.1124/jpet.111.184788

Wilkinson, J. M. & R. H. Young. 2020. A Review: Strategies to reduce on soya meal and palm kernel meal in livestock nutrition. J. Appl. Anim. Nutr. 8:75-82. https://doi.org/10.3920/JAAN2020.0007

Wu, X., Y. Zhou, Z. Lu, Y. Zhang, & T. Zhang. 2024. Effect of pre-slaughter fasting time on carcass yield, blood parameters and meat quality in broilers. Anim. Biosci. 37:315-322. https://doi.org/10.5713/ab.23.0262

Yaophakdee, N., Y. Ruangpanit, & S. Attamangkune. 2018. Effects of palm kernel meal level on live performance and gut morphology of broilers. Agric. Nat. Resour. 52:72–78. https://doi.org/10.1016/j.anres.2018.05.007

Zubaidah, S., C. Hanim, B. Ariyadi, A. P. Baskara, & Zuprizal. 2024. Nutrient composition and cell-wall structure of palm kernel cake supplemented with enzymes. Adv. Anim. Vet. Sci. 12:1191–1198. https://doi.org/10.17582/journal.aavs/2024/12.6.1191.1198