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THE EFFECT OF COCONUT MEAT WASTE SUPPLEMENTED WITH THERMOPHILIC BACTERIA AND THERMOSTABLE MANNANASE ON PERFORMANCE, GUT HISTOMORPHOLOGY AND MICROBIOTA OF BROILER CHICKENS

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ABSTRACT: An experiment was conducted on Arbor Acress broiler chicks to evaluate the effects of coconut meat waste (CMW) supplemented with 10^{10} CFU/kg CMW of thermophilic bacteria (*Bacillus* sp. SM-1.4) and 800 U/kg diets of thermostable mannanase on performance and gut histomorphology of broilers. One hundred and fifty of day-old chicks (unsexed) were used in this study. The birds were fed five diets containing coconut meat waste supplemented with bacteria and mannanases (CBM); 0% CBM, 10% CBM, 20% CBM, 30% CBM, and 40% CBM. Feed and water were available at all times. A completely randomized design was applied in this experiment with five diets and five replicate cages. The parameters determined are performance (feed consumption, body weight gain, and feed conversion ratio), gut histomorphology, and microbiota of broilers. Data were analyzed by analysis of variance according to a completely randomized design and Duncan's Multiple Range Test. Data indicated that feed consumption, body weight gain, and gut histomorphology of birds fed the CMW supplemented with thermophilic bacteria and thermostable mannanase were up to 30% higher than those of birds fed the non-supplemented (0% CBM) diet. The birds fed a 20% coconut meat waste diet supplemented with 10^{10} CFU/kg diet thermophilic bacteria, and 800 U/kg CMW thermostable mannanase had higher body weight gain than others and significantly (P<0.05) affected on microbiota of broiler. Adding coconut meat waste supplemented with thermophilic bacteria and thermostable mannanase did not affect the feed conversion ratio. In conclusion, up to 20%, CBM level in the diet could improving performance, gut histomorphology, and composition microbiota of broiler.

Keywords: Broiler, Coconut meat waste, Histomorphology, Microbiota, Thermophilic bacteria, Thermostable mannanase.

INTRODUCTION

Indonesia is the second biggest producer of coconut in the world, with a total production of 15.5 billion grains per year, resulting in 573.7 tons of coconut meat waste (CMW). Coconut meat waste is a residue after extracting coconut milk from coconut meat. CMW is qualitatively poor due to low concentrations of several limiting amino acids (lysine 0,081%, methionine 0,024%, tryptophan 0,002%, phenylalanine 0,065%), and high dietary fiber. Nutritionally, CMW dry matter contains crude protein 5.81%, crude fiber 20.84%, ether extract 24.59% (Harnentis and Syahruddin, 2015), carbohydrate 56.7%., lignin 1.88% (Ng et al., 2010). On a dry matter basis, CMW contains 61% galactomannan and 26% mannan (Balasubramaniam, 1976; Purawisastra, 2001).

Most feed ingredients contain some anti-nutritional factors, and the non-digested part obstructs feed utilization. Coconut meat waste (CMW) is an important alternative feed source in broiler diets because of its abundant production. Coconut meat waste (CMW) contains anti-nutritive such as mannans and galactomannans, which are non-starch polysaccharides (NSP). It can form highly viscous solutions, thus increasing the intestinal digesta viscosity. Increased gut digesta viscosity usually limits contact between digesta enzymes and substrates and between nutrients and absorption sites in the intestinal mucosa. The anti-nutritive effect is manifested by lowering nutrient utilization followed by poor growth (Nguyen et al., 2021; Johnson et al., 2022). The low use of coconut meat waste in poultry is due to the low value of its nutrition and voluminous. The low nutritional value of coconut meat waste is associated with a high content of crude fiber, crude lipid, low crude protein content, and high content of mannan and galactomannan (Rethinam and Krishnakum, 2022). At the same time, poultry does not produce the enzymes to break down fibers mannan and galactomannan. However, scarce information exists to improve the quality and use of coconut meat waste in poultry diets. Numerous studies have suggested that the results of the study showed that inclusion of mannanase enzyme and probiotics in diets containing high Non-Starch Polysaccharides (NSP) such as mannan and galactomannan could improve growth performance (Khanongnuch et al., 2006; Sundu et al., 2006; Harnentis and Syahruddin, 2015; Hsiao et al., 2022), gut histomorphology (Awad et al., 2009; Rahimi et al., 2009; Aliakbarpour et al., 2012; Mikulski et al., 2012) of broilers.

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These show that there is an increase in the rate of hydrolysis fiber greater with the addition of the enzyme in the diet than without the enzyme (Lin and Olukosi, 2021; Lannuzel et al., 2022). Inclusion of thermostable mannanase in CMW improved digestibility of crude fiber, hemicellulose (Harnentis. et al., 2015), nitrogen retention, metabolizable energy, and gut histomorphology of broilers (Harnentis and Syahruddin, 2016).

No information on using coconut meat waste supplemented with thermophilic bacteria and thermostable mannanase enzymes in poultry diets is available. This study was conducted to determine the effect of the use of coconut meat waste supplemented with thermophilic bacteria and thermostable mannanase enzyme in the diet on performance, gut histomorphology, and microbiota of the broilers.

MATERIALS AND METHODS

Bacteria preparation

Pure culture of *Bacillus* sp. SM-1.4 was isolated from hot springs in South Solok (Indonesian: Kabupaten Solok Selatan) is a landlocked regency (Kabupaten) of West Sumatra Province, Indonesia (Harnentis et al., 2013), which was then cultured in media nutrient broth (NB) and incubated at 60 ° C for 24 hours. Get the number of bacteria 10^{10} CFU/ml once the Standard Plate Count Agar has finished counting the bacteria. After that, the bacteria are kept in a bottle (Harnentis et al., 2013).

Production of enzymes

Mannanase thermostable enzyme produced by thermophilic bacteria (*Bacillus* sp. SM-1.4) according to the method (Harnentis et al., 2013). *Bacillus* sp. SM-1.4 were grown in a medium containing (g/L). 40.9 copra meal, NH_4NO_3 0.5, Na_2HPO_4 7.54, NaH_2PO_4 2.32, $MgSO_4.7H_2O$ 0.4, $FeSO_4.7H_2O$ 0.02, $CaCl_2.2H_2O$ 0.06, yeast extract (pH 7.0). *Bacillus* sp. SM-1.4 was utilized as the inoculum and was grown in NB medium at 60^{0}C on a shaker water bath (120 rpm) for 12 hours to produce the enzyme. The Minifors Benchtop Bioreactor, which has a 5 L capacity, is used to make enzymes. 10% inoculum (v/v) was added to the medium, which was then incubated at 60 ^{0}C for 18 hours before being centrifuged for 5 minutes at a speed of 5,000 rpm. After the initial enzyme activity test, the resulting supernatant is used as a crude enzyme in this study.

Composition diets

Before mixing the components of the diet with coconut meat waste, others added a mixture of crude enzyme mannanase with a dose of 800 U/kg of coconut meat waste and thermophilic bacteria (*Bacillus* sp. SM-1.4) 10^{10} CFU/kg diet (Harnentis and Syahruddin, 2015) is called the CBM (coconut meat waste supplemented with bacteria and mannanases). Diets were made using the ingredients and compositions listed in Table 1 and shaped into pellets using 3 mm die with a length of 0.5 cm.

Table 1 - Ration composition

Ingredients \ Treatments	D0	D1	D2	D3	D4
Corn	46.0	36.5	29.5	22.0	14.0
CBM[1]	0.0	10.0	20.0	30.0	40.0
Rice bran	12.0	10.0	6.5	3.0	0.0
Japfa BR I[2]	10.0	10.0	10.0	10.0	10.0
Soybean meal	11.5	13.0	13.5	14.5	15.5
Fish meal	20.0	20.0	20.0	20.0	20.0
Top mix[3]	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100
Calculated					
Crude protein (%)	22.28	22.40	22.16	22.0	22.0
ME (kcal/ kg)	3000	3000	3000	3000	3000
Crude fiber (%)	3.80	5.54	7.09	8.66	10.28
Lipid (%)	4.65	6.51	8.25	9.98	11.75
Calcium (%)	0.60	0.61	0.61	0.61	0.62
Phosporus - available (%)	0.35	0.35	0.34	0.34	0.33
Lysine (%)	1.29	1.28	1.28	1.27	1.26
Methionine (%)	0.49	0.48	0.48	0.47	0.46
Tryptophan (%)	0.24	0.23	0.23	0.22	0.21

[1] Coconut meat waste + Bacteria + Mannanase, [2] commercial feed from Japfa Comfeed Indonesia Tbk, [3] Top mix provided (in mg/kg): vit A 1200000 IU; vit D_3 200000 IU; vit E 800; vit K_3 200; vit B_1 200; vit B_2 500; vit B_6 50; vit B_{12} 1200µg; vit C 2500; Ca-D pantothenate 600; niacin 4000; choline chloride 1000; methionine 3000; lysine 3000; manganese 12000; iron 2000; iodine 20; zinc 10000; cobalt 20; copper 400; santoquin 1000; zinc bacitracin 2100

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Table 2 - Experimental diets

Diets*	Treatments
D0 = 0% CBM	No *Bacillus* sp. SM-1.4 and mannanase
D1 = 10% CBM	(10^{10} cfu/kg) and mannanase (800 U/kg)
D2 = 20% CBM	(10^{10} cfu/kg) and mannanase (800 U/kg)
D3 = 30% CBM	(10^{10} cfu/kg) and mannanase (800 U/kg)
D4 = 40% CBM	(10^{10} cfu/kg) and mannanase (800 U/kg)

*CBM = Coconut meat waste + Bacteria + Mannanase

Experimental design

One hundred fifty of day-old chick (DOC) broilers (unsex) were placed on a floor pen from 1 to 6 days and given a control diet. After six days (the initial 6-day period), 125 chickens were weighed between 98 and 116 g (107.0 ± 0.65; mean ± SE) selected to minimize animal variations and transferred into 20 units box and lighted. Each box contains five chickens and is maintained until six weeks. The design was completely randomized with five levels of coconut meat waste supplemented with thermophilic bacteria and thermostable mannanase (0, 10, 20, 30 and 40%) with five replications. Diets were formulated in iso-nitrogenous (22% crude protein) and isocaloric (3000 kcal/kg), as presented in Table 1. Diets and water were given ad libitum. The five diets imposed are described in Table 2.

Histological preparations

Making prep diets for histological performed in chickens aged 42 days. Duodenal each chicken cut along the 3 cm and then inserted into 10% buffered formalin for 24 hours. After 24 hours, the sample was made histological preparations with Hematoxylin-eosin staining. Villi length and width measurements are done by shooting prepadiets with magnification four times and then measuring with a predetermined scale (Incharoen et al., 2010).

Viscosity

Digesta was collected from each chicken's duodenum, jejunum, and ileum for viscosity measurements. Digesta diluted (1.1) with distilled water and homogenized for 20 minutes at room temperature, then centrifuged at 3500 rpm for 15 minutes. The viscosity of the supernatant was measured at a temperature of 29°C and 60 rpm using a viscometer with coaxial cylinders (model NDJ-8S; Piel et al., 2005).

Microbial count

Digesta was collected from each bird's duodenum, ileum, caecum, and colon for the microbial count, stored in a sterile tube, and cooled at 4°C (Cowan, 2004). Digesta was mixed in a 10 ml pre-reduced salt medium and serially diluted according to the procedure described (Engberg et al., 2004) to examine the count of Lactobacilli (Rogosa, CM 0627, incubated anaerobically 48h) and coliforms (Mackonkey, CM 0115, incubated aerobically 24h). Gut tissue samples were serially diluted from 10-3 to 10-7, and 0.1 ml of each dilution was spread evenly on a medium for enumediet bacteria.

Organ weights

On day 42, one bird per replicate was sacrificed through cervical dislocation. They were subsequently opened, and the liver, heart, gizzard, and pancreas were Harvested and weighed.

Statistical analysis

All data were analyzed using analysis of variance (ANOVA) on a completely randomized design according to (Steel and Torrie, 1991). Duncans Multiple Range Test (DMRT) was used to determine differences between diets (Steel and Torrie, 1991).

Ethical regulations and considerations

The experimental procedures were approved by the Universitas Andalas Animal Care and Use Committee (Padang, Indonesia; NO. 1020/UN.16.2/KEP-FK/2022). This research was conducted in the poultry research enclosure of the Teaching Farm of the Faculty of Animal Science, Universitas Andalas.

RESULTS AND DISCUSSION

The effect of treatment on performance broiler

The effect of diets on feed consumption is presented in Table 3. Diets significantly affect feed consumption. Feed consumption increased with increasing levels of CBM up to 20%, and then decreased with increasing up to levels of 40% CBM. The highest feed consumption was obtained at the level of CBM 20%, while feed consumption to the level of CBM 40% is not different from control diet (0% CBM), as well as feed consumption in the D2 diet (10% CBM) had no significant (P>0.05) with D3 diet (30% CBM). The diet significantly affected the body weight gain of broilers (P<0.05). The body weight gain broiler increased with increasing levels of CBM to the level of 30% in the diet and significantly higher than the control and D4 diet (CBM 40%). Usage levels of CBM 10% have a significantly different effect (P<0.05) with the use of CBM 20%, but had no significant with the use of CBM 30%, while the use of CBM 40% in the diet had no significant (P>0.05) with a control diet 0% CBM. The feed conversion ratio is presented in Table 3. Feed conversion of broiler fed the different levels of CBM had no significant (P>0.05) on feed conversion of broiler chickens.

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Table 3 - Feed consumption, body weight gain, and feed conversion					
Performance \ Treatments	D0	D1	D2	D3	D4
Feed consumption (g/week)	548.34[c]	589.0[b]	598.08[a]	589.68[b]	533.88[c]
Body weight gain (g/week)	296.4[c]	322.1[b]	337.9[a]	324.0[b]	296.6[c]
Feed conversion	1.85	1.83	1.77	1.82	1.80

[a,b,c] Means within a lines with different superscripts differ significantly (P<0.05)

Several studies have shown that including mannanase enzymes in the diets containing high NSP, such as mannan and galactomannan, could improve feed consumption and body weight gain of broilers (Pluske et al., 1997; Sundu et al., 2006). This shows that there is an increase in the rate of hydrolysis fiber more significant with the addition of the enzyme in the diet. In this study, feed consumption increased with increased levels of coconut meat waste supplemented with thermophilic bacteria as probiotic and thermostable mannanase up to 20% (20% CBM) and a decrease in the level of 30% CBM. With increasing levels of CBM in the diet, the amount of enzyme so that more coconut meat waste is hydrolyzed by mannanase, which ultimately accelerates the rate of digestion. This result is different from that reported Agboola et al. (2015) there is no effect of probiotic supplementation and carbohydrase enzyme in the diet based on wheat-soybean on feed consumption of broilers.

No information is available about the use of coconut meat waste supplemented with thermophilic bacteria and mannanase thermostable enzymes in the pellet form diet on the performance of broiler chickens. However, compared with studies on the diets containing feedstuff in high NSP such as coconut meal and palm kernel cake, coconut meat waste supplemented with thermophilic bacteria and thermostable mannanase improved feed consumption and was higher than coconut meal and palm kernel cake. The results of this study, similarly to those reported Sundu et al (2006), showed an increase in the feed consumption of broiler 4-14 days old fed diets containing coconut meal supplemented with mannanase. Adrizal et al. (2011) reported an increase in feed consumption with increasing levels of palm kernel meal from 0% - 15% and 30% in laying hens. Higher feed consumption in foods fed chicken containing palm kernel flour also (Sundu et al., 2005; Ezieshi and Olomu, 2008). This is caused by its faster passage rate in the digestive tract (Onifade and Babatunde, 1998), high bulk density, and low water holding capacity (Sundu et al., 2006). The lower feed consumption in the D0 diet (0% CBM) is caused by pellet durability because of the composition of corn and soybean meal, which causes high amylose content, resulting in a high gelatinization degree in pelleting. This pellet's durability will affect the work gizzard, so the diet is longer in the gizzard. Consequently, the passage rate of feed will be lower. The lower feed consumption D0 (0% CBM) is due to the high viscosity of the corn and soybean meal-based diet, so the rate of feed passage is low. At the same time, the lower feed consumption in the D4 diet (40% CBM) results from the voluminous nature of coconut meat waste. Most of the probiotics in the diets had no significant effect on broiler feed consumption if compared without probiotics (Applegate et al., 2010; Mikulski et al., 2012; Agboola et al., 2015).

Feeding the CBM 10% and 20% in the diet increases the body weight gain of broilers. This is closely related to the supplementation of thermophilic bacteria (*Bacillus* sp. SM-1.4) as a probiotic and thermostable mannanase enzyme. Probiotic supplementation's beneficial effects on the broiler diet lead to an increase in body weight gain, and the feed conversion ratio is documented in a study of several research groups (Timmerman et al., 2006; Onderci et al., 2008; Bansal et al., 2011). According to Applegate et al. (2010), giving probiotics to birds fed at six weeks had a significantly higher body weight gain than the chickens fed a diet without probiotics. The body weight improvement at the end of the growth phase caused by probiotic supplementation will reduce the phatogen bacteria colonies, thereby reducing nutrient competition and lowering toxic compounds or immune system modulation. El-Nagmy et al. (2007) has also stated that the increase in body weight gain of broilers is associated with the effect of probiotics in improving nutrient absorption and reducing the harmful bacteria that cause suppression of growth. Several research results support the results of this study (Kabir, 2009; Rahman et al., 2013).

The effect of supplementation of poultry fed with mannanase enzyme has been shown by several studies (Daskiran et al., 2004; Sundu et al., 2005; Zou et al., 2006; Williams et al., 2014). Abdollahi et al. (2016), reported that supplementation of the exogenous enzyme (mannanase, xylanase, protease, amylase, cellulase, and β-glucanase) in a diet containing 24% Palm Kernel Meal (PKM) impaired weight gain and feed efficiency of broilers. In this study, broilers increased body weight gain with increasing levels of CBM in the diet up to the level of 30%. Increased levels of CBM cause it to produce greater mannose and mannooligosaccharide (MOS). This MOS is a result of mannan hydrolysis by mannanases, so that more energy contribution, and MOS as a prebiotic that can stimulate the growth of bacteria on the ceca, as reported Baurhoo et al. (2009), supplementation of MOS in broilers diet produce in higher Bifidobacteria concentration in their ceca in conjunction with in an increase in villus length and the goblet cells count in the segment of intestine. There was also an increase in the other nutrients, such as protein and intracellular lipids. Adding enzymes degrading mannan causes the digestive enzymes to digest the cell contents easily. The endosperm cell walls close the proteins and lipids that inhibit intra-cellular digestion and absorption (Knudsen, 1997). The digestibility of crude fiber, hemicellulose digestibility, nitrogen retention, and energy metabolism corrected by nitrogen (MEn) increases (Harnentis and Syahruddin, 2015). In turn, it affects the increase in broilers' weight gain. It is also supported by the supplementation

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of thermophilic bacteria (*Bacillus* sp. SM-1.4), as probiotics improve intestinal morphology and animal health to increase nutrient absorption. Improvement in weight gain of broilers by probiotics and these mannanases for improvement of growth caused by the two feed additives. Probiotics ultimately improve nutrient efficiency by reducing competition between host and intestinal microbial populations, thus providing a favorable effect on livestock health by improving intestinal microbial balance. Jackson et al. (2004), reported improved body weight and feed efficiency in broiler chickens fed corn-soybean-based diets supplemented with β-mannanase.

Effect of treatment on intestinal villi length and width of broilers (gut histomorphology)

The effect of diet on intestinal villi length and width of broilers are presented in Table 4. The diet affected the length and width of the villi duodenum significantly (P<0.05). Duodenal villi length and width increased with increasing levels of use of CBM in the diet. The length of the diet of duodenal villi D2 diet (20% CBM) had no significant (P>0.05) with the D3 diet (30% CBM) and was significantly higher (P<0.05) compared with other diets, while the lowest duodenal villi length obtained in the diet of D0 (0% CBM). Duodenal villi width at D2 diet (20% CBM) had no significant (P>0.05) with D3 diet (30% CBM) and was significantly higher (P<0.05) compared with other diets, while the width of the duodenal villi D1 diet (10% CBM) and D4 (40% CBM) had no significant (P>0.05) compared with controls.

Table 4 - Effect of treatment on intestinal villi length and width of broilers

Intestinal \ Treatments	D0	D1	D2	D3	D4
Villous length (µm)	608.5[c]	1016.3[b]	1044.8[a]	1038.2[a]	1019.6[b]
Villous width (µm)	78.9[b]	80.9[b]	89.2[a]	86.9[a]	80.6[b]
Digesta viscosity (dPas)	0.39	0.32	0.30	0.27	0.30

[a,b,c] Means within a lines with different superscripts differ significantly (P<0.05)

Current studies suggest that adding thermophilic probiotics has improved intestinal villus length (Chichlowski et al., 2007; Awad et al., 2009; Rahimi et al., 2020). In this study, the intestinal villus in coconut meat waste treated with thermophilic bacteria and mannanase thermostable enzyme supplementation improved villus length and width more than without supplementation. It cannot be apart from two additives, namely thermophilic bacteria Bacillus sp. SM-1.4 as probiotic and mannanase thermostable. Improving intestinal villus length and width increases the digestive and absorption function of the intestine (Caspary, 1992) and can improve digestion by increasing the surface area for absorption and enhancing the nutrient transport system because it increases the production of digestive enzymes (Awad et al., 2009; Rahimi et al., 2009; Rahimi et al., 2020). In this study, coconut meat waste hydrolyzed by mannanase enzyme results from mannose and MOS. According to Sims et al. (2004), feeding MOS could improve intestinal villus length and width in Turkey poults. An increase in the length and width of the villus results because the fermentation of MOS by microflora in the cecum and colon produces short-chain fatty acids, especially butyric acid, which could increase intestinal proliferation villus (Ferket et al., 2002). An enhancement of intestinal villus length in the chicken by administering probiotics *Bacillus* subtilis is also reported by Samanya and Yamauchi (2002).

Broiler digesta viscosity was not affected by the level of use of CBM in the diet. Although statistically not significant, numerically digesta viscosity decreased with increasing levels of CBM in broiler diets. High digesta viscosity reduces the rate of substrate diffusion and digestive enzymes and inhibits their effective interaction with intestinal mucosa (Ikegami et al., 1990). The Inclusion of cereals rich in NSP increases the digesta viscosity, bacteria profile, and gut physiology (Aliakbarpour et al., 2012; Zduńczyk et al., 2020). In this research, coconut meat waste content is higher in insoluble NSP compared with soluble NSP, so not much impaired the viscosity, coupled with supplementation of mannanase, resulting in lower digesta viscosity.

Effect of treatment on microbiota digesta

The effect of diet on microbiota digesta showed that in Table 5. The diet significantly affected the broiler's total bacteria count in intestinal on the duodenum, ileum, caecum, and colon (P<0.05). The intestinal Total Plate Count (TPC) of the broiler decreased with increasing levels of CBM to 40% in the diet and significantly higher than in the control diet. The effect of diet on *E. coli* is presented in Table 5. *E. coli* of broilers significantly (P<0.05) affected by the diet. *E. coli* of broilers increased with increasing levels of use of CBM in the diet to the level of 40%. *E. coli* of broilers in the diet of D2 (20% CBM) had no significant (P>0.05) with D3 of diet (30% CBM) and significantly lower (P<0.05) compared with other diets. *E. coli* in the D1 diet (10% CBM) had no significance (P>0.05) with the D4 diet (40% CBM) and was significantly higher (P<0.05) than in the D2 and D3 diets.

The effect of diet on Lactic acid bacteria (LAB) is presented in Table 5. The diet significantly affected intestinal LAB intestines of broilers (P<0.05). Intestinal LAB of broilers increased with increasing levels of use of CBM in the diet to the level of 40%. Intestinal LAB of broilers in the D2 diet (20% CBM) had no significance (P>0.05) with the D3 diet (30% CBM) and was significantly higher (P<0.05) than in other diets. LAB on D1 diet (10% CBM) had no significant (P>0.05) with D4 diet (40% CBM) and significantly lower (P<0.05) than D2 and D3 diet.

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Table 5 - Microbiota digesta of broilers					
Microbiota \ Treatments	D0	D1	D2	D3	D4
TPC (10^6 cfu/g)[1]	4.1[a]	3.3[b]	3.0[b]	2.9[b]	3.0[b]
E. coli (10^5 cfu/ g)	3.6[a]	3.1[b]	0.5[c]	1.3[c]	2.9[b]
LAB (10^8 cfu/g)[2]	0.4[c]	1.3[b]	2.8[a]	2.2[a]	1.4[b]

[a,b,c] Means within a lines with different superscripts differ significantly (P<0.05), [1] TPC = Total Plate Count, [2] LAB = Lactic Acid Bacteria

The TPC and *E. coli* count decreased with increasing levels of use of CBM in the diet, otherwise with LAB count. Several researchers reported the influence of probiotics (Higgins et al., 2008; Vilà et al., 2009) and enzymes (Bedford, 2000) on microbial populations in poultry. In this study, supplementation of thermophilic bacterium *Bacillus* sp. SM-1.4 and mannanase thermostable improved the health of the broiler, where lowering *E. coli* count. The *E. coli* count determines the presence and population of the pathogen in the intestine. The *E. coli* count was reduced in the ileum of broiler chicken supplemented with probiotics and mannanase enzymes, as reported by Agboola et al. (2014), which lowered the number of *E. coli* in the ileum of turkey given the probiotics and symbiotic diets. The result of this study, the decline *E. coli* count was followed by an increase in the LAB count in digesta of the broiler. According to Kizerwetter-Swida and Binek (2009), the Lactobacilli reduce coliform with competitive exclusion. The results of the number of E. coli in this study showed an effective modulation of intestinal microflora and significant obstruction of microflora pathogenic with the competition of nutrients, creating conditions and producing antimicrobial (free fatty acids, low pH, and bacteriocin), competition of site locations at ephitel intestines and immune system stimulation (Jin et al., 1996). The enzyme reduces the number of bacteria by increasing the rate of digestion and restricting the number of available substrates to the microflora in the ileum (Bedford, 2000). The mannanase supplemented with coconut meat waste hydrolyzes mannan and results from mannose and mannooligosaccharide (MOS). MOS can increase the use of nutrients by stimulating the population of certain bacteria in the digestive tract, such as Lactobacillus and Bifidobacterium (Jana et al., 2021). Addition of *Bacillus* sp. SM-1.4 can reduce *E. coli* colonization and TPC and improve the LAB. As well as, the addition of *Bacillus* subtilis in drinking water can reduce the colonization of *E. coli*, Salmonella enteritidis, and Clostridium perfringens in the chickens (Griggs and Jacob, 2005). Besides that, *Bacillus* subtilis can also increase the proliferation of Lactobacillus, which produces lactic acid so that it can control the bacterial pathogen.

Effect of treatment on physiological organs

The effect of diet on the percentage of broiler chicken's liver, heart, gizzard, and pancreas is shown in Table 6. The use of the different levels of CBM in the broiler diet had no significant effect (P>0.05) on the liver, heart, gizzard and chicken pancreas broiler percentage. Some of the study results found that the relative weight of the physiological organs of poultry is not affected by the feeding of probiotics (Konca et al., 2009; Agboola et al., 2014). In this study, liver, heart, gizzard, and pancreas weight were not affected by the CBM level in the diet, but numerically the relative weight of liver and heart tended to decline, and the relative weight of gizzard and pancreas tended to increase with increasing CBM levels in the diet. According to Iyayi and Yahaya (1999) relative weight of the liver, spleen, and heart are not affected by enzyme supplementation in broiler diets.

Table 6 - Percentage of physiological organs of broiler					
Physiological organs \ Treatments [NS]	D0	D1	D2	D3	D4
Liver (%)	2.37	1.77	1.81	1.81	1.76
Heart (%)	0.52	0.48	0.45	0.42	0.43
Gizzard (%)	2.81	2.79	3.25	3.27	3.56
Pancreas (%)	0.20	0.16	0.18	0.20	0.23

NS = not significant

CONCLUSION

It was concluded that coconut meat waste supplemented with thermophilic and thermostable mannanase shows potential for improving the performance, gut histomorphology, and composition microbiota of broiler in the pellet form diet containing up to 20% CBM level.

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Authors' contribution

HARNENTIS, Robi AMIZAR, Yuliaty Shafan NUR, and Nurul HUDA contribute on experiment, data analysis and the write up of the manuscript.

Conflict of interests

The authors have not declared any conflict of interests.

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