

Effect of coconut husk extract on broiler chicken performance, pH and microbial composition of digesta, and small intestine histomorphology

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ABSTRACT

Plants that contain a large number of bioactive substances have been used as alternative phytobiotic additives. The objectives of the current study were to investigate the efficacy of coconut husk extract on broiler performance, ileal digesta characteristic and small intestine morphology. Two hundred and fifty day-old-chicks (DOC) were randomly placed in five treatment groups with five replicates each, and the chickens were reared until six weeks old. Basal ration was hand mixed to ensure the requirements in the starter period and finisher period. Treatments were basal ration only (TCT⁻), basal ration with 40 ppm tetracycline (TCT⁺), basal ration with 100 ppm coconut husk extract (T100), basal ration with 400 ppm coconut husk extract (T400) and basal ration with 700 ppm coconut husk extract (T700). The variables included feed intake, live weight gain (LWG), feed to gain ratio (FCR), breast meat quality, pH and microbial composition of digesta and small intestine morphology. Data were statistically analyzed using analysis of variances and comparison test using the Duncan's multiple range test. The results demonstrated that the feed additives significantly increased feed intake, LWG, breast meat protein content, villi height in the ileum, and the height: crypt depth ratio in the duodenum and ileum, and significantly decreased the abundance of *Escherichia coli* and *Staphylococcus aureus* and the pH of digesta ($P < 0.05$). Therefore, coconut husk extract could be applied as a phytobiotic feed additive for broiler chickens to stimulate the growth of broilers, suppress colonies of harmful bacteria in the small intestine and enhance the improvement of intestinal morphology.

Keywords: Additives, Extracts, Husk, Bacteria and Morphology

INTRODUCTION

Antibiotics as growth promoters in poultry diets are no longer permitted, because they cause resistance in the digestive tract micro-biota of hosts, presence of antibiotic residues in animal products and environmental pollution (Sugiharto and Ranjitkar, 2019, Sapsuha *et al.*, 2021). Accordingly, more attention should be given to searching for alternatives feed additives that do not induce the issues related to antibiotics. The alternatives are probiotics, prebiotics, phytobiotics, enzymes and organic acids, which are added to rations. Phytobiotics have been reported to be

safer, less poisonous, and less residual than antibiotic; therefore, they are ideal feed additives for animal production (Qureshi *et al.*, 2016). Phytobiotics also have advantages in the gut microbial ecosystem as they control pathogenic bacteria and improve digestive capacity (Hashemi and Davoodi, 2010), and improve feed efficiency by enhancing the secretion of enzymes from the gut and liver (Prakash and Srinivasan, 2010; Abou-Elkhair *et al.*, 2014). Phytobiotic feed additives (PFAs) are generated from plants or herbs and parts of such plants that have been used for generations as feed additives (Sugiharto, 2016), they generally consist of a

variety of bioactive compounds (Huyghebaert *et al.*, 2011; Hussein *et al.*, 2020). Previous studies have indicated that active compounds include a variety of secondary metabolites from plants, inducing antioxidant and antibacterial effects (Rusdi *et al.*, 2019), and potentially acting as growth stimulators for beneficial bacteria and growth inhibitors for harmful bacteria (Fasina *et al.*, 2015; Hussein *et al.*, 2020).

A wide range of studies have explored the effect of phytobiotics additive supplementation on animal performance. For instance, the inclusion of polyphenol mixtures as phytobiotics additives improved the performance of broiler chickens (An *et al.*, 2015; Starcevic *et al.*, 2015; Prihambodo *et al.*, 2021). The addition of phytobiotics reduced the pH in the digestive tract (Cherian *et al.*, 2013; Olukosi and Dono, 2014) and suppressed the development of several pathogenic bacteria in the digestive tract (Leusink *et al.*, 2010; Viveros *et al.*, 2011; Adriani *et al.*, 2015; Vlaicu *et al.*, 2017; Hussein *et al.*, 2020). Additionally, grape by-products or substrates containing tannins stimulated the development of intestinal villi in pigs (Bilic-Sobota *et al.*, 2016) and broilers (Viveros *et al.*, 2011; Prihambodo *et al.*, 2021). However, the efficacy of phytobiotic additives as growth promoters depends on plant species, plant parts, physical traits of the plant, plant genetic background, age of the plant, amount used, extraction method and time of harvesting (Sugiharto, 2016).

A plant that has been commonly applied as a therapeutic agent is coconut. Coconut is very versatile and crucial plant for people in tropical regions. Coconut meat is rich in protein, calories, vitamins and minerals. It has high contents of K, Na, Mg and S (Waziri *et al.*, 2013). Coconut meat is also a source of phenols, phytosterol, flavonoids, proanthocyanidins, resveratrol and arginine (Bolling *et al.*, 2010). In addition to coconut being used for food, other parts such as shells, husks and roots can be applied for medicinal purposes. For example, coconut husk as a source of active compounds has been investigated by Silva *et al.* (2013) and Ramaswamy *et al.* (2015). The results of these studies demonstrated that coconut husk extract has numerous medicinal properties such as antioxidant, antibacterial, antifungal, antiparasitic and antidermatophytic activities. Furthermore, coconut husk extract impairs the existence of gastrointestinal parasites (Costa *et al.*, 2010; 2011). Recently, an *in vitro* study by Rusdi *et al.* (2019) found that co-

conut husk extract suppresses the growth of *Escherichia coli* and *Staphylococcus aureus* and to some extent stimulates the establishment of *Lactobacillus acidophilus*. However, there studies exploring the potency of coconut husk extract as phytobiotic in broiler chickens are lacking. Therefore, the goals of this study were to explore the efficacy of coconut husk extract on the performance, breast meat quality, ileal digesta pH and microbial composition, and also intestinal morphology in broiler chickens.

MATERIALS AND METHODS

Dry coconut husk was isolated from the outer shell and ground into a powder for further processing. The extraction process was conducted as described by Rusdi *et al.* (2019) using methanol solvent. Two hundred and fifty day-old-chicks (DOC) were purchased from the Malindo Mill Makassar hatchery. Chickens were reared for 6 weeks and given basal ration or experimental ration. The basal ration was mixed to contain 22% protein and 3000 kcal/kg ME, 20% protein and 3100 kcal / kg ME for starter and finisher, respectively (Table 1), and the ration composition met the recommendation of NRC (1994).

The research cages used comprised 25 plots with a size of 120 cm x 120 cm x 70 cm, and each plot contained 10 chickens. Cages were stage cages with wooden slats. Experimental chickens were randomly divided into five treatments with five replicates and 10 chickens per cage. Experimental rations comprised the basal ration and coconut husk extract (CHE). The five treatments were as follows: basal ration only (TCT⁻) as a negative control, basal ration supplemented with 40 ppm tetracycline (TCT⁺) as a positive control, basal ration supplemented with 100 ppm CHE (T100), basal ration supplemented with 400 ppm CHE (T400) and basal ration supplemented with 700 ppm CHE (T700). CHE was provided in a solid form. Rations and water were freely available during the study. The variables of interest were feed intake, live weight gain (LWG), feed conversion ratio (FCR), breast meat composition, pH and microbiology of the ileal digesta, and small intestine histomorphology.

The intake and live weight of the chickens were recorded weekly and were used to quantify LWG, feed intake and FCR. At 42 d of age, two chickens per cage were slaughtered and the small intestines were removed for digesta characteristics evaluation and histomorphology examina-

Table 1. Ingredients and Composition of Basal Ration

	Starter	Finisher
Feed ingredients (%)		
Maize	59.00	61.00
Soybean	17.80	14.50
Fish flour	16.50	14.00
Bran	6.00	9.50
Oil	0.00	0.60
Sodium chloride	0.02	0.05
Premix ¹	0.28	0.05
Lysine	0.20	0.10
DL-Methionine	0.20	0.20
Total	100	100
Composition (%)		
Metabolizable energy (kcal kg ⁻¹)	3219	3198
Protein	22.07	20.04
Fibre	3.06	3.45
Ca	1.08	0.90
P	0.45	0.35
Lys	1.10	1.00
Met	0.49	0.38

Provided the items per kg: Ca 32.5%; P 10%; Zn 6 g; Mn 4g; I 0.075g; Cu 0.3g; Zn 3.75g; vit. B12 0.5mg; vit. D3 50,000 IU; vit. A 1,200,000 IU; vit. E 800 IU; vit. K 0.2 g; vit. B1 0.2g; vit. B2 0.5 g; vit. B6 0.05 g; vit. C 2,5 g; Ca-D-panthotenate 0.6 g; Niacin 4 g; Met 3 g; Lys 3 g; Santoquin 1 g; Zinc bacitracin 2.1 g

tion. Digesta from the terminal ileum was gently removed and placed in a sterile container for pH and microbial count measurement. The pH of digesta was directly assessed using a digital pH metre (EZ9902), with a pH range of 0.00-14.00 and a resolution of 0.01. Each digesta sample was diluted to 10^{-3} to 10^{-6} and then spread on discs containing Eosin Methylene Blue Agar (EMBA, Merck KGaA, Darmstadt Germany), Baird Parker Agar (BPA, Merck KGaA, Darmstadt Germany) or Lactose Agar (Pronadisa, Laboratorios Conda S, A) media. The microbial colonies of *E. coli* and *S. aureus* that grew in each disc were counted using a colony counter, after incubation for 24 hours at 37°C. The number of microbial colonies analysed ranged from 30-300 cfu/ml.

Duodenum, jejunum and ileum samples of chickens from each cage were sectioned at approximately 3 cm for histological assay. Intestinal samples were placed in containers containing 10% formalin solution and shipped to the pathology laboratory for further histomorphology assessment. The tissue samples were dehydrated, cleared, and impregnated with paraffin. The tissues were coated with paraffin wax and sliced into 6µm sections on a microtome. The sliced tissues were smoothed by suspension in water at 55-60°C before positioning on 10% poly-L-

lysine coated slides. The slides were stained with haematoxylin and eosin. Villi height and crypt depth were determined by an electronic microscopic image analyser (Motic Images, 2000 1,2 Scion Image, Japan).

Breast meat was separated from each carcass and shipped to the laboratory for further analysis. The chemical composition of the breast meat was evaluated according to the procedure of AOAC (2005). Collected data were assessed by analysis of variance (ANOVA) in accordance with Steel and Torrie (1991) and Duncan's test was applied for means comparison, with $p < 0.05$ indicating significance.

RESULTS AND DISCUSSION

A previous study reported that coconut husk extract had antioxidant and antibacterial activities (Rusdi *et al.*, 2019). Under *in vivo* conditions, antioxidant effects may improve the quality of the ration by hampering fat oxidation and reducing the rancidity of the ration, improving palatability and therefore enhancing intake. Gheisar and Kim (2018) stated that the addition of phyto-biotic substances in the ration potentially increased intake by elevating the palatability of the ration due to the enhancement of taste and aroma. In fact, the presence of the additive in the

Table 2. Initial Liveweight, Feed Intake, Liveweight Gain and Feed Conversion Ratio of Broiler Chicken

Variables	Treatments					SEM	P-value
	TCT ⁻	TCT ⁺	T100	T400	T700		
Initial liveweight, g/head	44.80	44.88	43.20	42.60	45.60	0.36	0.057
Feed intake, g/head	3087.00 ^{ab}	2937.00 ^{ab}	2722.30 ^a	2899.00 ^{ab}	3153.80 ^b	40.43	0.035
Liveweight gain, g/head	1341.40 ^a	1440.60 ^{ab}	1408.10 ^{ab}	1426.40 ^{ab}	1610.30 ^b	24.88	0.044
FCR	2.32	2.05	1.94	2.04	1.96	0.05	0.153

FCR: feed conversion ratio. SEM: standard error mean. Values within the line different letters indicate significantly different

ration produced a substantial result on feed intake and feed intake in the 700 ppm group was significantly higher than that in the 100 ppm group (T100; $P < 0.05$) and was higher than those in the control groups (TCT⁻ and TCT⁺). This is in contrast with the results of Olukosi and Dono (2014) and Upadhaya *et al.* (2016) who demonstrated that the inclusion of phytobiotic additives in rations had no effect on feed intake, and Buyse *et al.* (2021) who reported a deleterious effect on broiler performance, when chestnut tannins were supplemented in a broiler diet at a rate of 0.2%.

The addition of synthetic antibiotics and coconut husk extract enhanced the LWG, and the LWG of the chickens in the 700 ppm group was higher than that in untreated group ($P < 0.05$). This supports the finding of Starcevic *et al.* (2015), who observed an enhancement in the performance of chickens consuming rations containing polyphenol mixtures, such as gallic acid and tannin acid. The beneficial effect of 700 ppm coconut husk extract on LWG can be attributed to the tendency of higher feed intake than that associated with other treatments and possibly the enhancement of absorption of feed substances as a result of the improvement in the environment and histomorphology of the small intestine, as presented in Tables 3 and 4. However, there is no guarantee for further improvement in LWG with additional increases in the level of coconut husk extract in the ration; this topic needs further study.

A previous study by Rusdi *et al.* (2016) reported that intake and LWG were low with higher levels of onion extract in the ration. The high concentration of onion extract in the ration had a beneficial effect on livestock due to its high antibacterial activity against *E. coli* (Rusdi *et al.*, 2019). An increase in bioactive mixtures, especially tannins, will however increase the quantity of undigested feed components in the digestive tract due to the interaction between tannins and feed ingredients, especially protein, forming a

protein-tannin complex; that cannot be absorbed in the digestive tract. There is also an interaction between tannins and enzymes, reducing the activity of digestive enzymes. These interactions reduced feed ingredient digestibility as reported by Woyengo and Nyachoti (2012), who found that condensed tannins from faba bean (*Vicia faba*) reduced the digestibility of protein and amino acids in broiler chickens, moreover, Hejdysz *et al.* (2016) found a negative correlation between nutrient digestibility and the tannin content in faba bean. The inclusion of shallot extract had no substantial effect on intake and slightly increased the LWG of chickens (An *et al.*, 2015). Additionally, application of coconut husk extract in the present study tended to improve the FCR, which was lowest in the 100 ppm group (T100).

Administration of coconut husk extract in the ration did not influence the water and fat content of the breast meat ($P > 0.05$). The current results showed that the protein content of breast meat in the 700 ppm of coconut husk extract group was higher than that in the negative control group ($P < 0.05$), which is similar to the result of Hossain *et al.* (2012) who reported an increase in protein meat of pigs that consumed polyphenols from green tea by-product. In contrast, Glamoclija *et al.* (2016) found a nonsignificant decline in the meat protein of broiler chickens who were fed phytobiotic mixtures of cumin, mint, clove and anise. Starcevic *et al.* (2015) found that the inclusion of gallic acid in the ration had no effect on the protein content, but tannin acid reduced the protein content of the meat. The by-product resulting from the production process of carrageenan, which contains bioactive compounds (Makkar *et al.*, 2016), reduced the fat content and cholesterol level in breast meat, but the protein content in breast meat remained unchanged (Hasanuddin *et al.*, 2017). The anomalies in the response of polyphenols to breast meat quality are partly determined by the

type of polyphenol mixture in the feed additive and diet composition.

Furthermore, feed additives tended to induce a favourable environment for the growth of nonpathogenic bacteria as demonstrated by the pH value and the colony counts of bacteria shown in Table 3. There was a reduction in the pH of digesta with increasing levels of coconut husk extract in the ration ($P < 0.05$). This result indicated that the fermentation process in the intestine was more intensive and created a more acidic environment, thereby hampering the population of harmful bacteria and improving the health of livestock. The current results are in line with those of Cherian *et al.* (2013) and Olukosi and Dono (2014) who found that adding phytobiotics to the ration decreased the pH of the gut environment in broiler chickens. The decrease in pH is caused by not only additives that are acidic, but also additives that do not have an acidic nature (Olukosi and Dono, 2014). According to Pearlin *et al.* (2019), maintaining a low pH in the hindgut is very important to improve the health of the digestive tract because an acidic environment can suppress harmful bacteria in the alimentary tract.

Generally, antibiotics suppress bacterial growth, but the results of studies are not always consistent and rely on the classification and amount of antibiotics used, environmental hygiene and health status of the livestock (Viveros *et al.*, 2011). Adding coconut husk extract to the ration suppressed pathogenic bacterial growth, which was indicated by a reduction in the number of bacteria in the ileum (Table 3), and an increase in the quantity of coconut husk extract in the ration ($P < 0.05$). This trend corroborates the findings of Rusdi *et al.* (2016; 2019), who reported that the antibacterial activity of onions and coconut husk extracts was due to bioactive contents in these ingredients such as flavonoids, gal-

lic acid and tannins. Moreover, an *in vivo* study on jengkol (*Pithecellobium jiringa*) extract, also reported a significant reduction in *E. coli* colonies in the digestive tract with an increased amount of jengkol extract in the ration (Adriani *et al.*, 2015). This is in line with the results of Ifesan *et al.* (2010), who found that onions (*Eleutherine americana*) can be used as herbal medicine (medical plants), because onions contain active compounds including alkaloids, saponins, terpenoids, steroids, glycosides, tannins, phenolics and flavonoids. Furthermore, it was reported that onion (*E. americana*) extracted in ethanol has antimicrobial effects against gram-positive bacteria and a small number of gram-negative bacteria, and even has antifungal activity, especially against *Aspergillus niger*, *Penicillium spp* and *Rhizopus spp.* (Ifesan *et al.*, 2010). The results of other studies proved that feed additives containing active compounds suppressed the growth of several pathogenic bacteria in the alimentary tract (Leusink *et al.*, 2010; Viveros *et al.*, 2011; Vlaicu *et al.*, 2017).

A reduction in the abundance of pathogenic bacteria as a result of the supplementation of coconut husk extract in the current study is in accordance with the findings by Rusdi *et al.* (2019). Thus, lowering the abundance of *E. coli* and *S. aureus* will provide benefits to nonpathogenic bacteria such as *Lactobacillus*. For instance, coconut husk extract has the ability to stimulate the growth of *Lactobacillus* (Rusdi *et al.*, 2019). Similarly, Viveros *et al.* (2011); Vlaicu *et al.* (2017) and Saracila *et al.* (2018) found that phytobiotic elements suppress the development of pathogenic bacteria and are able to stimulate the development of nonpathogens bacteria such as *Lactobacillus*. The current study also proved that coconut husk extract at a dose 2-17 times higher than that of tetracycline was able to exceed the antibacterial capacity of tetracy-

Table 3 .Chemical Composition of Breast Meat and Ileal Digesta Characteristics

Variables	Treatments					SEM	P-value
	TCT ⁻	TCT ⁺	T100	T400	T700		
Breast meat composition							
Water,%	72.82	71.27	73.02	71.80	71.15	0.32	0.256
Protein,%	17.68 ^a	19.90 ^{ab}	19.82 ^{ab}	18.88 ^{ab}	20.60 ^b	0.19	0.002
Fat,%	3.40	3.81	3.33	3.65	3.68	0.09	0.523
Ileal digesta characteristics							
pH	5.92 ^a	5.78 ^{ab}	4.86 ^{bc}	4.92 ^{bc}	4.65 ^c	0.09	0.004
<i>E.coli</i> ,(x10 ⁶) cfu/g	1.72 ^a	1.52 ^b	1.35 ^c	0.84 ^d	0.64 ^e	0.01	0.000
<i>S.aureus</i> , (x10 ⁶) cfu/g	2.78 ^a	1.70 ^b	1.39 ^c	0.96 ^d	0.76 ^e	0.01	0.000

SEM: standard error mean. Values within the line different letters indicate significantly different

Table 4. The Values of Villi Height, Crypt Depth and Ratio of Villi Height:Crypt Depth

Intestine section	Treatments					SEM	P-value
	TCT ⁻	TCT ⁺	T100	T400	T700		
Duodenum							
Villi height, μm	965.00	1463.00	1012.00	1103.00	1254.00	65.01	0.181
Crypt depth, μm	275.60 ^{ab}	203.00 ^a	243.70 ^a	240.04 ^a	359.20 ^b	14.54	0.040
Ratio Vh:Cd	3.48 ^a	7.18 ^b	4.49 ^{ab}	4.61 ^{ab}	3.78 ^{ab}	0.33	0.039
Jejunum							
Villi height, μm	1695.00	1601.30	1418.66	1720.10	1571.40	45.43	0.308
Crypt depth, μm	264.40	278.10	220.90	301.80	311.60	20.19	0.655
Ratio Vh:Cd	6.39	5.87	6.47	6.65	5.34	0.46	0.889
Ileum							
Villi height, μm	992.70 ^{ab}	849.00 ^b	1245.30 ^{ab}	1333.00 ^a	1592.40 ^a	46.86	0.007
Crypt depth, μm	145.20 ^a	197.70 ^b	239.00 ^b	244.90 ^b	226.30 ^b	8.85	0.005
Ratio Vh:Cd	6.84 ^{ab}	4.34 ^b	5.25 ^{ab}	5.39 ^{ab}	7.26 ^a	0.28	0.044

Vh: villi height and Cd: crypt depth. SEM: standard error mean. Values within the line different letters indicate significantly different

cline ($P < 0.05$).

The application of polyphenols such as tannins in livestock diets has an effect on the morphology of the intestine. Grape by-products and substrates containing tannins influenced the morphology of the intestine in pigs (Bilic-Sobota *et al.*, 2016) and broilers (Viveros *et al.*, 2011; Prihambodo *et al.*, 2021), by enhancing the growth of villi. The height of villi is associated with the health status of the alimentary tract (Ologhobo *et al.*, 2015), and increase in height and width of the villi enhance digestive function and nutrient uptake (Viveros *et al.*, 2011), because the surface area for absorption is increased, in turn increasing the LWG of livestock. In this study, the feed additive had no significant effect ($P > 0.05$) on the height of villi in the duodenum and jejunum, but there was an effect on the ileum ($P < 0.05$). This result is supported by that of Olukosi and Dono (2014), who found that the antioxidant properties of additives may help to support growth and improvement of the digestive tract, and overall gut function. Bilic-Sobota *et al.* (2016) found that villus development was enhanced by the addition of a hydrosable tannin rich extract in pig rations. In contrast, Dialoke *et al.* (2020) found that the inclusion of a material containing chestnut tannins at concentration of 15% suppressed villus development in the ileum of broiler chickens.

Interesting results regarding the morphology of the ileum were observed (Table 4). Tetracycline and coconut husk extract had a substantial

effect on villi height, crypt depth and the villi height: crypt depth ratio ($P < 0.05$). Additionally, the feed additive also influenced the crypt depth and the villi height: crypt depth ratio in the duodenum. The current findings are in line with the results of Bilic-Sobota *et al.* (2016); Qureshi *et al.* (2016) and Prihambodo *et al.* (2021). Increased villi height increases the surface area for absorption and the activity of enzymes secreted from the villi. Improvements in villi characteristics reflects the health status of the digestive tract of livestock (Ologhobo *et al.*, 2015). According to Viveros *et al.* (2011), long villi and a short crypt depth or a high villi height: crypt depth ratio allow better absorption of feed ingredients, increased disease resistance and improve livestock performance. Furthermore, Incharoen *et al.* (2010) reported that heavier broilers generally have longer villi and larger villi surface areas. The constructive effect of coconut husk extract on intestinal morphology might be related to its antimicrobial activity, reducing inflammatory reactions in the mucosa (Qureshi *et al.*, 2016). The present study also found that the best response was achieved by the inclusion of 700 ppm coconut husk extract in the ration (T700). This can be interpreted as an increase in nutrient uptake and reduced competition for the use of feed ingredients by pathogenic bacteria such as *E. coli* and *S. aureus* in the digestive tract (Table 3). These results are also in line with the pattern of LWG, in which the inclusion rate of 700 ppm

coconut husk extract produced the highest value of LWG (Table 2).

CONCLUSION

The addition of coconut husk extract to the ration improved broiler performance, improved breast meat quality, reduced the colony count of harmful bacteria in the ileum and improved the morphology of the villi and crypt depth in the intestine. Therefore, husk extract as a phytobiotic can improve the growth of broiler chickens by improving feed intake, suppressing the colony count of pathogenic bacteria in the ileum and improving the development of small intestinal morphology.

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