Review Article

Effects of the Encapsulation of Liquid Smoke on Growth Performance, Intestinal Profile, and Microbial Profile of Broiler Chickens

Andy[1,4*], Ratmawati Malaka[2], Sri Purwanti[3], Hikmah M Ali[2], Tutik Lusyta Aulyani[4], Danung Nur Adli[5]

[1,4]Doctoral Student of Animal Science Study Program, Faculty of Animal Science, Universitas Hasanuddin. Jl. Perintis Kemerdekaan Km.10, Tamalanrea Indah, Makassar 90245, South Sulawesi, Indonesia; [2]Departement of Animal Product, Faculty of Animal Science, Universitas Hasanuddin. Jl. Perintis Kemerdekaan Km.10, Tamalanrea Indah, Makassar 90245, South Sulawesi, Indonesia; [3]Department of Animal of Feed and Nutrition, Faculty of Animal Science, Universitas Hasanuddin, Jl. Perintis Kemerdekaan Km.10, Tamalanrea Indah, Makassar 90245, South Sulawesi, Indonesia; [4]Gowa Polytechnic Agriculture Development, Jl. Malino Km.7 Bontomarannu, Kabupaten Gowa 92171, south Sulawesi, Indonesia; [5]Faculty of Animal Science, Universitas Brawijaya, Malang, East Java, Indonesia.

Abstract | This study aims to determine the effect of supplementation of the encapsulated liquid smoke on growth performance, intestinal profile, and microbial profile of broiler chickens. A total 100 broiler chickens were reared for five weeks on the experimental diets. A general linear models with five treatments and four replications was employed. The treatments consisted of T_0 = control, T_1 = basal diet + acidal, T_2 = basal diet + 0.5% encapsulation of liquid smoke, T_3 = basal diet + 1.0% encapsulation of liquid smoke, T_4 = basal diet + 1.5% encapsulation of liquid smoke. Results were analysed using general linear model using SAS Demand on Academics, least significant differences were applied if there are significance. The result showed that using encapsulation of liquid smoke was significantly difference (p < 0.05) on the body weight gain, feed conversion ratio (FCR), crypt depth, and reduce total population of *Escherichia* coli in the intestinal. To sum up, using liquid smoke encapsulation successfully increasing villus height and reduce feed conversion ratio without any adverse effect.

Keywords | Broiler, Encapsulation, Growth Performance, Intestinal Profile, Liquid Smoke

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***Correspondence** | Andy, Doctoral Student of Animal Science Study Program, Faculty of Animal Science, Universitas Hasanuddin. Jl. Perintis Kemerdekaan Km.10, Tamalanrea Indah, Makassar 90245, South Sulawesi, Indonesia; **Email:** andyhasan13@gmail.com
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INTRODUCTION

Indonesia production of poultry reached 5 million tonnes / year in Indonesia (Adli, 2021). Indonesia has imposed a ban on the use of antibiotics growth promoters (AGPs) on early January 2018 (Ardiansyah et al., 2022). Several products were listed as an antibiotics growth promoters such as of using zinc bacitracin, penicillin, tylosin, and kanamycin. A lot of researcher were undertaken to seeking alternative of this regulation. One of potential that can be used were liquid smoke. Nowadays, liquid smoke act as flavoring for food. Liquid smoke has a phenolic compound, taste, and fluid for color changes (Smaoui et al., 2021; Drosou et al., 2017). Liquid smoke are recommended as an antibiotics growth promoters replacement since extracted from coconut shells. The phenolic compounds that is consisted in the liquid smoke has potential to reduce several harmful microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* (Jiang, 2005; Yamauchi et al., 2010). As alternatives to synthetic antibiotics, bioactive substances in plants that are sources of natural antibiotics have received attention from both the public and researchers. Plant extracts, phytogenic additives, essential oils, prebiotics and probiotics have been extensively studied as replacements for synthetic antibiotics and have been tested to identify the best options (Ardiansyah et al., 2022). To enhance the liquid smoke there were method called encapsulation. Encapsulation is method for covering raw material before subjected into object (Smaoui et al., 2021). Encapsulation is an alternative to giving liquid smoke to livestock which is expected to protect and streamline the use of active ingredients contained in liquid smoke (Smaoui et al., 2021; Drosou et al., 2017). In addition, balancing the microorganism is important to enhance general health, productivity, and physiological status. Therefore, this study aims to determine the effect of supplementation of the encapsulated liquid smoke on growth performance, intestinal profile, and microbial profile of broiler chickens.

MATERIALS AND METHODS

Ethical approval

Ethical approval for the study was given by the Universitas Hasanuddin Animal Science with number 355/UN4 .6.4.5.31.1 PP36/ 2022 and number protocol UH22460290 with date 19 July 2022.

Liquid Smoke Encapsulation

The preparation of encapsulation of liquid smoke were following Ali et al. (2014) with adjustment of protocol. First, the liquid smoke was put down into formulated liquid with malt dextrin 8.5% w/v: Chitosan is 1.5% w/v. Second, the formulated liquid was homogenized using a magnetic stirrer at 200 rpm for 30 minutes. The solution was then centrifuged at 400 rpm for 5 minutes. Drying using a spray dryer (Armfield SD-Basic, USA), then dried at an inlet temperature of 148^{O}C with a flow rate of 4-6 mL/min, with pressure on the nozzle atomizer 1 bar. The liquid smoke powder is taken from the container and then stored in a dark bottle lined with aluminum foil.

Experimental design

A total 100 broiler chickens were reared for five weeks on the experimental diets. A general linear models with five treatments and four replications was employed. The treatments consisted of T_0 = control, T_1 = basal diet + acidal, T_2 = basal diet + 0.5% encapsulation of liquid smoke, T_3 = basal diet + 1.0% encapsulation of liquid smoke, T_4 = basal diet + 1.5% encapsulation of liquid smoke. All broiler chickens were housed in environmentally controlled rooms with rice-hull-littered pens. All broiler chickens were allowed *ad libitum* access water. The formulated feed was provided by Charoen Pokphand Tbk. consisted yellow maize, soybean meal, bone meal, meat meal, soy oil, leaf meal, and custom mineral mix. Representative of the formulated feed were analyzed for metabolizable energy (Kcal/kg), crude protein (CP), crude fibre (CF), calcium (Ca), and phosphorus (P) according to established procedures described by (AOAC, 2000). The composition of formulated feed showed in the Table 1 and 2.

Growth performance

Each end of week the broiler chickens were selected individually by body weight group selection and weighed at the beginning and end of the experiment (Adli et al., 2020). Feed intake recorded as the differences between amount of feed given at the early week and end of the week. As long as, water intake was recorded with amount of feed intake. The gain feed ratio was recorded amount of the body weight with feed intake.

Intestinal profile

Specimens preparation:The intestinal profile was recorded by following protocol from Ardiansyah et al. (2022) first specimens were cut approximately 2 mm at duodenum section. After carefully evaluation, the specimens were immediately stored at formulated liquid consisted 70,80,90% of alcohol. The specimens stored for remained two hours and one hour for xylolite liquid. Third, the selected tissue put down into paraffin and drying at 60^0 for one hours.

Hematoxylin eosin preparation: The hematoxylin eosin preparation was expressed by following Sjofjan and Adli (2020) protocol. First, the selection tissue from intestinal were cut with range 3-4 mm. The selection tissue was immediately put down into object glass with covered. First, the staining began with deparaffinization and rehydration. After carefully preparation, the step continued with preparation of formulated liquid consisted xylolite, 70,80,90% of alcohol, and reverse osmosis water. Third, hematoxylin liquid was used in this step and soak for 10 minutes at least. Fourth, the specimen was put down into eosin staining liquid for 20 minutes and put down into reverse osmosis water. Fifth, the specific sample were covered with cover slip and put down into microscope with 400x zoom scale.

Microbial profile

The microbial profile was determined by following Sjofjan and Adli (2020) protocol. First, selected chime was taken from duodenum. Each broiler chicken was collected and selected chime was diluted with 9 ml of 10 g/kg peptone broth and homogenized. Second, 10-fold dilution of chime were ranging with 10^{-4} MacConkey Agar broth of Escherichia coli and Mueller Hinton Agar (MHA) for *Streptococcus sp*. The MacConkey Agar Broth were incubated for 24 hours last at 37^{0}C. The Mueller Hinton Agar were incubating for 72 hours at 37^0 C under anaerobic conditions.

Data analysis

Prior to statistical analysis, analysis of variance (ANOVA) using general linear model (GLM) was carried out using SAS OnDemand for Academics (ODA, Cary, NC,USA). The results were presented as standard error mean (SEM). Moreover, probability values were calculated using least significant different testing. The following model was used:

$Y_{ij} = \mu + Ti + e_{ij}$

Where Yij was parameters observed, μ was the overall mean, Tithe effect level of liquid smoke encapsulation, and eij the amount of error number.

RESULTS AND DISCUSSION

Effects of adding encapsulation of liquid smoke on the growth Performance of broiler chickens

The results showed that the average feed consumption data ranged from 1515.70 to 1731.40 g/head. The results of statistical analysis showed that the effect of liquid smoke encapsulation in drinking water was not significantly different (p >0.05) on feed consumption. (Table 3) The effect that was not significantly different was thought to be because the encapsulation of coconut shell liquid smoke in drinking water up to a level of 1.5% did not affect the palatability of the feed. Several factors might be influenced this condition such as the liquid smoke are not mixed well with water, temperature, and humidity during experiment. The nutritional content of feed on metabolic energy and crude protein in both the starter and finisher phases has met the nutritional requirements of broilers. The nutrition and quantity of feed used in the study were given the same in all treatments, so it did not show results that had a significant effect.

Effect of Treatment on Weight Gain The results of the study on the appearance of production in table 3 show that the average data on body weight gain ranged from 988.75 to 1190.25 g/head. Based on statistical analysis, it was known that the addition of coconut shell liquid smoke encapsulation in drinking water had a significant effect (p < 0.05) on T2 and T4 treatments. Treatment with the addition of encapsulation with a level of 0.5% showed a lower body weight gain than T1 (as a positive control), this was because phytochemical compounds (phenol, carbonyl, and organic acids) which were given up to a level of 0.5% had not been able to effect on body weight gain. The encapsulation treatment causes a decrease in the content of coconut shell liquid smoke compounds which is the cause of these compounds not being able to show the effect of their functional properties as antimicrobials to help the digestive process to increase body weight.

Based on the increase in the level of treatment (T2, T3, and T4) showed an increase in body weight gain along with the increase in the level of liquid smoke encapsulation. The increasing level means increasing the content of phytochemical compounds such as phenol, carbonyl, and organic acids consumed. These phytochemical compounds have functional properties as an antibacterial which can help control the growth of pathogenic bacteria in the digestive tract. Achievement of body weight can be obtained by optimizing feed nutrients that can be absorbed, one of which is influenced by the balance of microflora in helping the process of feed metabolism. Research conducted by Natsir et al. (2010) stated that encapsulated lactic acid showed better results on the appearance of production, intestinal microflora, and intestinal characteristics compared to liquid lactic acid. This effect is related to the encapsulation technology in lactic acid which can protect from degradation in the digestive tract before it reaches the small intestine so that lactic acid can work optimally in the small intestine in the process of feed metabolism and absorption of feed nutrients. There is method called meta-analysis to prove each experimental were work properly (Adli et al., 2022).

The results showed that the average FCR data ranged from 1.4 to 1.7. The results of statistical analysis showed that the effect of liquid smoke encapsulation in drinking water was significantly different (p<0.05) in the T2 and T4 treatments on the FCR value. Feed conversion becomes a benchmark in assessing the efficiency of feed use by comparing the consumption of feed used during maintenance with the body weight gain obtained. Feed conversion indicates the efficiency of the absorption of feed nutrients that can be converted for meat formation.

Effects of adding encapsulation of liquid smoke on the intestinal profile of broiler chickens

The results showed that the mean height of the villi ranged from 391.775 µm - 475.925 µm. The results of statistical analysis showed that the treatment had no significant effect (p>0.05) on the height of the villi ileum of the broilers in the study (Table 4) (Figure 3-4). A few reports and study reported correlation between related phenolic compound and contribution changes in the intestinal profile. It is assumed that an increased villus height is paralleled by an increased digestive and absorptive function of the intestine as a result of increased absorptive surface area, expression of brush border enzymes, nutrient transport systems (Viveros et al., 2011). The increasing of villus area was in line with increasing of positive microorganism in the gut (Viveros et al., 2011).

The results showed that the average depth of the crypt data ranged from 122.025 µm - 196.250 µm. The results of sta tistical analysis showed that the treatment had a significant

Table 1: Ingredient of Broiler chicken diets (0-21 days)

Ingredients (% as is basis)	T_0	T_1	T_2	T_3	T_4
Yellow Maize	50.00	50.00	50.00	50.00	50.00
Broken wheat	20.00	20.00	20.00	20.00	20.00
Soybean	20.00	20.00	20.00	20.00	20.00
Bone meal	7.00	7.00	7.00	7.00	7.00
Meat meal	4.00	4.00	4.00	4.00	4.00
Canola oil	2.00	2.00	2.00	2.00	0.90
Custom Mineral premix*	1.00	1.00	1.00	1.00	1.00
Probiotics	0.00	0.00	0.00	0.10	0.60
Encapsulation liquid smoke	0.00	0.00	0.50	1.00	1.5
Acidal	0.00	0.00	0.00	0.00	0.00
Total	100.00	100.00	100.00	100.00	100.00
Calculated Composition					
ME (Kcal/kg)	2833.21	2821.00	2834.32	2856.34	2764.44
Crude Protein (CP)	21.30	20.30	21.00	21.00	22.00
Crude fibre (CF)	4.00	4.00	4.00	4.00	4.00
Calcium (Ca)	0.60	0.60	0.60	0.60	0.60
Phosphorus (P)	0.40	0.40	0.40	0.40	0.40
Proximate composition (Wet chemical analysed)					
ME (Kcal/kg)	2721.03	2734.02	2678.01	2710.00	2710.00
Crude Protein (CP)	18.20	18.20	18.10	18.00	18.00
Crude fibre (CF)	4.05	4.05	4.01	4.03	4.11

**: Vitamin A, 6000IU, Vitamin D3, 1000IU, Vitamin E, 10mg, Vitamin K3, 1.5mg, Vitamin B1, 5mg, Vitamin B2, 2.5mg, Vitamin B6 0.5mg,
Vitamin B12, 2.0mg, niacin, 5.5mg, pantothenic acid, 0.2mg, betaine, 30mg.
*: Iron, 12.50mg, copper, 3mg, manganese, 37.5mg, zinc, 31.32mg, iodine, 5mg and selenium 0.0625mg***Carrier was $CaCo_3$

Table 2: Ingredient of Broiler chicken diets (21-35 days)

Ingredients (% as is basis)	T_0	T_1	T_2	T_3	T_4
Yellow Maize	45.00	45.00	45.00	45.00	45.00
Broken wheat	35.00	35.00	35.00	35.00	35.00
Soybean	5.00	5.00	5.00	5.00	5.00
Bone meal	2.50	2.50	2.50	2.50	2.50
Meat meal	2.50	2.50	2.50	2.50	2.50
Canola oil	2.00	2.00	2.00	2.00	0.90
Custom Mineral premix*	1.00	1.00	1.00	1.00	1.00
Probiotics	0.00	0.00	0.00	0.10	0.60
Encapsulation liquid smoke	0.00	0.00	0.50	1.00	1.5
Acidal	0.00	0.00	0.00	0.00	0.00
Total	100.00	100.00	100.00	100.00	100.00
Calculated Composition					
ME (Kcal/kg)	3112.21	3124.00	3123.23	3118.46	3119.34
Crude Protein (CP)	19.00	19.25	18.75	19.25	19.00
Crude fibre (CF)	4.00	4.00	4.00	4.00	4.00
Calcium (Ca)	0.60	0.60	0.60	0.60	0.60

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Phosphorus (P)	0.40	0.40	0.40	0.40	0.40
Proximate composition (Wet chemical analysed)					
ME (Kcal/kg)	2721.03	2734.02	2678.01	2712.01	2713.22
Crude Protein (CP)	18.20	18.25	18.20	18.00	18.00
Crude fibre (CF)	4.05	4.05	4.05	4.05	4.05

Table 3: Growth performance of broiler chicken after adding liquid smoke encapsulation

Parameters	T0	T1	T2	T3	T4	SEM
Feed intake (g/head)	1731.40	1515.70	1693.70	1567.00	1628.00	78.00
Water intake (ml/head)	3773.80	3283.30	3448.30	3752.30	3904.50	123.24
BWG (g/head)	1066.25^{ab}	1008.00^{ab}	988.75^{a}	1089.25^{ab}	1190.25^{b}	12.71
FCR	1.69^{ab}	1.5^{ab}	1.7^{b}	1.4^{a}	1.4^{a}	0.04

^{a,b,c,d} Means with different superscripts in the row differ significantly ($p \leq 0.05$). T_0 = control, T_1 = basal diet + acidal, T_2 = basal diet + 0.5% encapsulation of liquid smoke, T_3 = basal diet + 1.0% encapsulation of liquid smoke, T_4 = basal diet + 1.5% encapsulation of liquid smoke. BWG – Body weight gain; FCR – Feed conversion ratio.

Table 4: Intestinal profile of broiler chicken after adding liquid smoke encapsulation

Parameters	T0	T1	T2	T3	T4	SEM
Villus height (μm)	475.58	391.36	454.96	441.80	410.05	1.93
Crypt depth (μm)	122.28^{a}	152.69^{ab}	155.46^{ab}	194.75^{b}	152.86^{ab}	1.48
Surface area of villi (μm)	1349.15	1330.17	1828.60	1612.17	1379.67	2.34

^{a,b,c,d} Means with different superscripts in the row differ significantly ($p \leq 0.05$). T_0 = control, T_1 = basal diet + acidal, T_2 = basal diet + 0.5% encapsulation of liquid smoke, T_3 = basal diet + 1.0% encapsulation of liquid smoke, T_4 = basal diet + 1.5% encapsulation of liquid smoke. BWG – Body weight gain; FCR – Feed conversion ratio.

effect (p <0.05) on the T0 and T3 treatments, while the T1, T2 and T3 treatments had no significant effect (p >0.05) on the height of the ileal villi of the broilers in the study (Table 4) (Figure 1 and 5). Crypto has an important role in villous epithelial cell turnover. The increase in the depth of the crypts indicated that the broiler that given the liquid smoke encapsulation (LSE) and antibiotics had received adequate nutrition. Intestinal growth is related to the number of cells in the crypt, the number of cells along the villi, and the surface area of the segments (Cengiz et al., 2012). The increase in crypt depth plays a role in repairing the damage that occurs in the intestinal mucosa and the increase in enterokinase which functions as a precursor for trypsin production leads to an increase in the availability of amino acids. Adeniji et al. (2015) stated that crypt depth is considered to be the producer of villi and deeper crypt depth indicates rapid tissue turnover to allow villi renewal as needed in exfoliation or inflammation from pathogens or their toxins and high demand for tissues.

The results showed that the mean surface area of the villi ranged from 1330.175 μm - 1828.600 μm. The results of statistical analysis showed that the treatment had no significant effect (p >0.05) on the surface area of the villi of the broilers in the study (Table 4) (Figure 2) (Figure 6). The surface area of the villi in the intestine can indicate the absorption of nutrients in the intestine by looking at the area of absorption, the wider the absorption area, the more nutrients that can be absorbed by the body. The expansion of the villi surface indicates that the level of digestion that occurs in the chicken's body is getting better, with the expansion of the area of absorption of nutrients in the intestine. Improved digestive function and intestinal absorption due to increased absorption surface area, expression of brush border enzymes, and nutrient transport systems (Awad et al., 2009). The increase in villi height in the small intestine of broilers is closely related to the increase in digestive function and absorption function because the absorption area expands and is an expression of a smooth nutrient transport system throughout the body that benefits the host. The large surface area of the villi for the absorption of nutrients that enter through the bloodstream.

The results of the study of the effect of adding coconut shell liquid smoke encapsulation in drinking water to pathogenic bacteria identified in *Escherichia coli* bacteria were significantly different (p <0.01) in T_4 treatment. Streptococcus bacteria were not found, presumably because the media used was MHA (Mueller Hinton Agar). the level of selectivity is less, thus affecting the growth of bacteria. The results showed a decrease in the number of bacteria along with an increase in the level of encapsulation given to broilers. Level 1.5% encapsulation of liquid smoke obtained identified bacteria *Escherichia coli* 6.0 x 10^3 CFU/g. Liquid smoke consisted organic compounds such as acetic acid, alcohols, phenols, and other compounds that can in

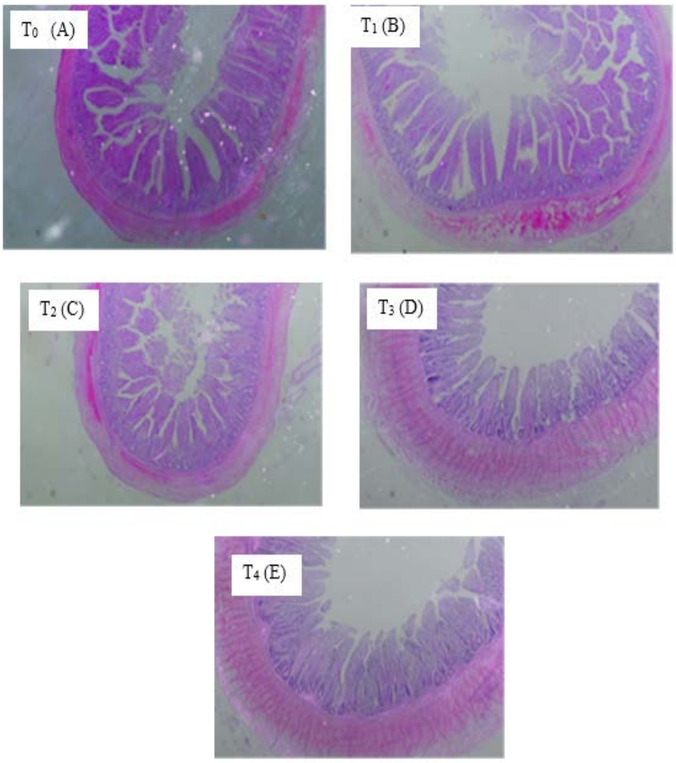

Figure 1-4: T_0 = control, T_1 = basal diet + acidal, T_2 = basal diet + 0.5% encapsulation of liquid smoke, T_3 = basal diet + 1.0% encapsulation of liquid smoke, T_4 = basal diet + 1.5% encapsulation of liquid smoke. BWG – Body weight gain; FCR – Feed conversion ratio.

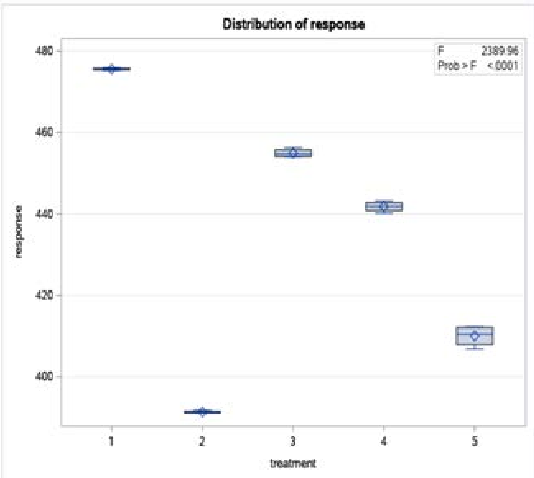

Figure 5: Distribution response of villus height

hibit the growth of pathogenic bacteria such as *Escherichia coli*, Staphylococcus aureus, and *Candida albicans* (Jiang, 2005), functions as a natural antibiotic (Yamauchi et al., 2010). *Escherichia coli* is a short rod-shaped Gram-negative bacteria that have a length of about 2 m, a diameter of 0.7 m, and a width of 0.4-0.7 m and is facultatively anaerobic. *Escherichia coli* in the digestive tract produce endotoxins that can increase the secretion of fluids and electrolytes, this can cause dehydration and electrolyte imbalance resulting in the collapse of the circulatory system followed by

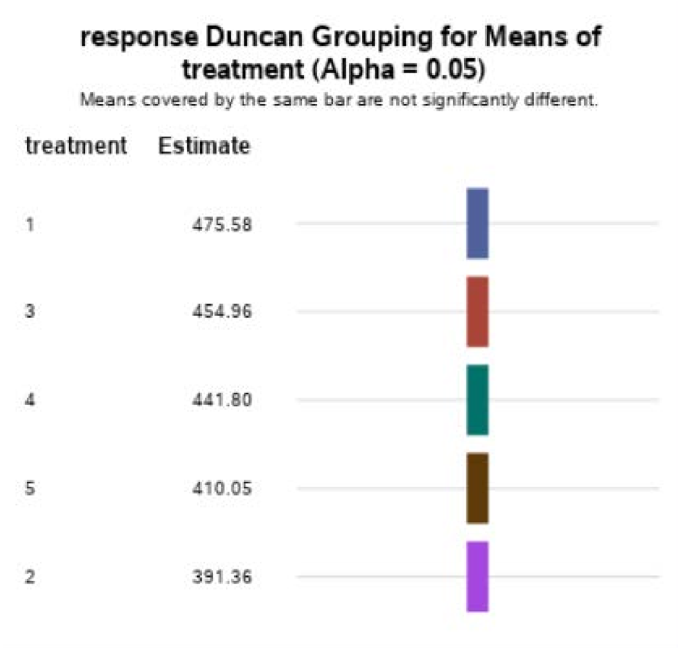

Figure 6: Distribution response of villus height

stress and death (Adil et al, 2012). *Escherichia coli* bacteria are the main cause of intestinal infections, especially diarrhea. *Escherichia coli* is a gram-negative facultative anaerobic rod-shaped bacterium. Escherichia coli is a bacterium that is normally found in the intestines and plays a role in the process of decaying food debris. However, if its presence is above the normal amount and has moved from its normal habitat, namely the large intestine, it can endanger health. The inhibition of the growth of pathogenic bacteria occurs because these bacteria cannot tolerate a decrease in pH in the digestive tract, the growth of *Escherichia coli* bacteria is inhibited when in an environment with a pH below 6. The addition of liquid smoke encapsulation treatment in drinking water on the observation of Escherichia coli bacteria showed a decrease in the number of bacteria along with the addition of treatment levels. The decrease in the number of bacteria was influenced by the antibacterial properties of phenol compounds in the encapsulation of liquid smoke. According to Kailaku et al. (2016) antimicrobial activity in liquid smoke is played by the dominant phenolic compound content. Phenolic compounds at low concentrations can act as bacteriostatic, while at high concentrations can act as bactericidal.

Secondary metabolism produces bioactive molecules in the form of phenol compounds that have a hydroxyl group, this group is the reactive part working on antimicrobial properties. Antimicrobials are chemical compounds including their derivatives and their analogous structures capable of inhibiting the life processes of microorganisms (Khameneh et al., 2019). The hydroxyl group (-OH) in phenolic compounds works to inhibit bacterial growth by attaching to the cell wall causing lipid dissolution and disrupting the performance of the cytoplasmic membrane by inhibiting

ATP-ase binding which causes cells to lysis (Turgis et al., 2009). The process of protein denaturation by phenolic compounds causes cell death. The structure of the bacterial cytoplasmic membrane containing proteins and lipids dissolves after penetration with hydroxyl groups, causing unstable metabolism in bacterial cells. Instability in the cell wall and cytoplasmic membrane causes the function of selective permeability, active transport function, and control of bacterial cell structure to be disturbed. The results of the study on the effect of liquid smoke given through encapsulated trough drinking water on the appearance of production, histology of broiler intestine and pathogen presumptive bacteria, can be concluded as follows: The encapsulation of coconut shell liquid smoke gave a significant effect on body weight gain and feed conversion ratio (FCR), while the treatment of feed and drinking water consumption variables had no significant effect. The encapsulation of coconut shell liquid smoke had a significant effect on the depth of the villous crypts, while the treatment had no effect on the height and surface area of the intestinal villi of broiler. The encapsulation of coconut shell liquid smoke has a significant effect on the population of *Escherichia coli* bacteria, at the level of 1.5% has the ability to replace the role of antibiotics because it has a better production appearance, has a balance of intestinal microflora, presumptive pathogenic bacteria.

CONCLUSIONS

The study presented LSE at a level of 1.5% to confirm the role of antibiotics because it has a better production performance and a balance of intestinal microflora and pre-bacteria. The addition of LSE showed a better value for Body weight gain, FC, crypt depth, and the population of *Escherichia coli* bacteria. Meanwhile feed and drinking water consumption, height, and villi surface area did not affect by treatment.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported

NOVELTY STATEMENT

The liquid smoke rarely used as an additive for animal. To enhance the performance, the encapsulation was added in order to protect the barrier before entering each organ of the poultry. This combination was attached to fulfilled the quality of novelty, since there is limited information using of this combination on growth performance and physiological health of poultry.

AUTHOR'S CONTRIBUTION

AA conceptualization, drafting the original manuscript, collecting data, RM conceptualization, supervision, SP conceptualization, supervision, HMA conceptualization, supervision, TLA drafting the original manuscript, collected data, DNA drafting manuscript, revise grammatically, creating illustration, and validation.

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