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Original Research Article

Encapsulation Affects Concentration of Liquid Smoke Active Substances and Intestinal Microflora of Broiler

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Abstract: The purpose of this research was to evaluate effect of encapsulated coconut shell liquid smoke as a replacement for antibiotics in broiler feed on active substances and intestinal microflora. The materials used in this research were 225 Day Old Chick from Cobb strains, which were allocated into 5 treatments and 5 replications. The treatments used in this research were T0 (basal feed) as a control treatment, T0 + (basal feed + antibiotic) as a positive control treatment, T1 (basal feed + 0.5% encapsulated coconut shell liquid smoke), T2 (basal feed +1 encapsulated coconut shell liquid smoke), T3 (basal feed + 1.5% encapsulated coconut shell liquid smoke). The variables measured were active substance contents and intestinal microflora population including *Lactic Acid Bacteria* (LAB), *Salmonella sp*, and *Escherichia coli*. The microflora data were analyzed by using One Way Analysis of Variance and if there is a significant difference, the different test was performed with Duncan's multiple range tests. The results showed that a slight decrease in the total phenol was found, but total acid and pH of the encapsulated coconut shell liquid smoke markedly changed. In addition, encapsulated coconut shell liquid smoke at 1.5% in broiler feed significantly ($P < 0.01$) increased LAB and decreased *Salmonella sp*. The conclusion of this research was the addition of encapsulated coconut shell liquid smoke 1.5% could be considered to replace the use of antibiotics in broiler feed.

Keywords: Encapsulation, coconut shell liquid smoke, active substances, intestinal microflora, and broiler.

INTRODUCTION

Coconut shell liquid smoke is widely traded and used in the food industry as a natural preservative. This preservative function is related to the content of phenolic compounds which have antibacterial and antioxidant properties, so that can control the growth of putrefactive bacteria dominated by gram-negative bacteria. Distribution of active substances in this liquid smoke are dominated by phenol (90.75%), carbonyl (3.71%), alcohol (1.81%), benzene (3.73%) (Hadanu and Apituley, 2016) having pH of 2.8 which makes it acid. Phenolic compounds are well known as an effective anti-microbial agent function as both bacteriostatic and bactericidal due to posses reactive hydroxyl groups. Attachment of this hydroxyl group to the cell wall could causes lysis of bacteria due to disruption of metabolism in bacterial cells. The use of liquid smoke in broilers has previously been studied by Widodo, et al. (2020) reported that the use of liquid smoke could suppress the growth of pathogenic bacteria and increase non-pathogenic bacteria in broilers due to the anti-microbial properties of phenolic compounds in coconut shell liquid smoke. However, it expected that encapsulation would improve effectiveness of liquid smoke to substitute the use of antibiotic in broiler feed.

Encapsulation is, therefore, could protect phenolic compounds to be easily oxidized because the character of hydroxyl groups is reactive to oxidative reactions. The purpose of encapsulation then protect the bioactive compounds by encapsulated them in the core part and save from environmental effects. It might then lengthen the storage time. Considering the potential benefits of this liquid smoke, current study was attempted to substitute the use of commercial antibiotic in broilers. The lack of information regarding the utilization of protected coconut shell liquid smoke on changes of active substances and intestinal microflora in broiler, this study was conducted.

MATERIALS AND METHODS

A. Materials

A food-grade coconut shell liquid smoke which locally produced was used. Maltodextrin was

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obtained in local chemical store. In addition, Day Old Chicks (DOC) were bought from local commercial hatchery, basal feed was formulated after purchasing some feed ingredients, water and other standard equipments were provided in this bioassay experiment.

B. Encapsulation Process

A part of the liquid smoke was encapsulated. The process of encapsulation was in accordance with Natsir, et al. (2017) by using Microwave-Assisted Encapsulation (MAE) method with some modification as follow: the liquid smoke was mixed with maltodextrin at ratio 1:1 (v/w), put in a beaker and stirred for 3 minutes by using an ultrasonic mixture. The mixture then was poured into a microwavable plate, inserted into a microwave set at stable temperature (45-60°C) controlled by using a thermostat for a period of 5-6 minutes. The product then was cooled down, collected and stored in a sealed plastic bag.

C. Chemical Analysis

Both encapsulated and non-encapsulated liquid smoke then were being analyzed for total phenol by using the UV-Vis spectrometric method (done in the laboratory of Instrumentation Analytical Chemistry, Department of Engineering Chemistry State Polytechnic of Malang). While total acid was tested by using the volumetric method and measurement of pH was with a pH meter (done in the Laboratory of Food Quality and Safety Testing, Faculty of Agriculture Engineering Brawijaya University). Encapsulation morphology using the Scanning Electron Microscope (SEM). SEM test was carried out in Central Laboratory of Biological Science of Brawijaya University.

D. Biological experiment

Biological test on intestinal microflora was carried out by using 225 DOC commercially produced and have been already vaccinated in the hatchery. The DOC used had an average initial body weight of 44.07±2.14 g with a variability coefficient of 4.38%. Twenty-five flock units were used; each had a size of 100 × 100 × 60 cm. The ingredients used to make basal feed composed of yellow corn, soy bean meal, meat and bone meal, rice bran, fish meal, coconut oil, methionine, lysine, salt and premix. The basal feed was formulated according to standard guidelines for nutritional needs for Cobb at starter and finisher phases.

E. Methods

The biological assay was designed with five treatments and five replications so that there were 25 experimental flocks. Each experimental unit consisted 9 chicks, and they were reared for 35 days of age. Feed and drinking water were provided by *ad libitum* during the raising period. The research treatments were as follows:

T0: Basal feed
T0+: Basal feed + antibiotic
T1: Basal feed + 0.5% encapsulated coconut shell liquid smoke
T2: Basal feed + 1,0% encapsulated coconut shell liquid smoke
T3: Basal feed + 1.5% encapsulated coconut shell liquid smoke

F. Microflora Measurements

At the end of experiment, samples for microflora were taken from the ileal digesta of broilers. The taken samples were kept in a film pot and transferred to cool box and immediately brought to laboratory for analysis. The variables observed in this study were intestinal microflora, which included determination of *Lactic Acid Bacteria (BAL), Escherichia coli* and *Salmonella sp*. Observation of the bacterial population was by method of Hernandez, et al. (2004) using the pour plate method and calculated by the rule of Total Plate Count (TPC).

G. Statistical Analysis

The data obtained on microflora measuent were analyzed with a one-way analysis of variance (ANOVA) and if significant effect appeared then it is continued with Duncan's Multiple Range Test.

RESULT AND DISCUSSION

A. Effect of Encapsulated on Coconut Shell Liquid Smoke

The coconut shell liquid smoke was encapsulated using maltodextrin encapsulant in the same ratio as the Microwave-Assisted Encapsulated (MAE) method. This process could transform liquid smoke in the form of powder. The results of the analysis of changes in the content of coconut shell liquid smoke bioactive compounds and those that have been encapsulated are shown in Table 1.

Table-1: Testing of liquid smoke and its encapsulation

Observation parameters	Liquid smoke	Encapsulated coconut shell liquid smoke
pH	2.88	3.36
Total acid (%)	9.55	3.25
Total phenol (µg/mg)	26.876	25.354

The test results on pH value showed an increase pH of encapsulated coconut shell liquid smoke, this is because of dilution with maltodextrin during encapsulation process will increase the pH value. Accordingly, the total acid content decreases as well due to encapsulation process. Therefore, encapsulation

has a negative correlation with the content of acidic compounds. Decreasing total acid or increasing pH might adversely affect digestion process in animal digestive tract. It might be worthy if when encapsulation is necessary then additional acidifier needed to avoid pH decreases.

The value of total phenol and acid content in coconut shell liquid smoke showed a small decrease after undergoing the encapsulated process. The actual total phenol of liquid smoke without encapsulation might be higher than 26 µg/mg, because there was delay in analysis due to pandemic covid-19. The possibility of total phenol loss during storage due to exposure to environmental condition. Logically, encapsulation should reduce total phenol concentration attributed to dilution of maltodextrin. This decrease largely occurred because maltodextrin was added at the same amount with liquid smoke. Experiment of Saloka, et al. (2004) reported that encapsulated with combination of chitosan and maltodextrin found that total phenol was more than 70% because of smaller amount encapsulant addition.

B. Morphological form of encapsulated liquid smoke

The morphological form of encapsulated coconut shell liquid smoke can be seen in Figure 1.

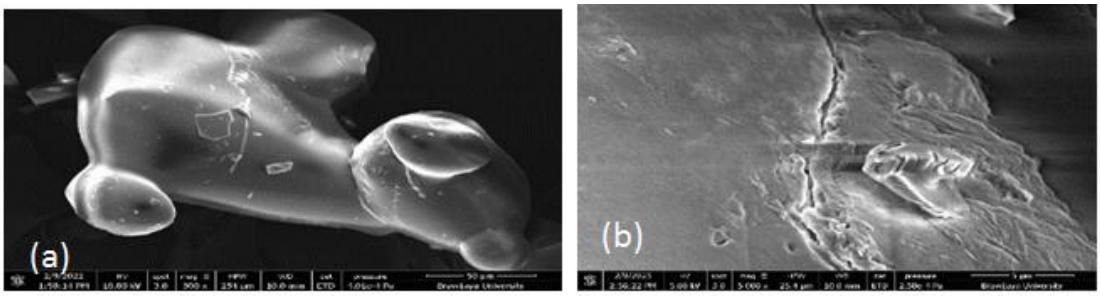

Fig-1: Morphological form of coconut shell liquid smoke encapsulation (a) 500x magnification and (b) 5000x magnification

The morphological shape of the capsule that protects the core material can be seen in Figure 1. Figure 1a showed an example of the capsule image in the complete coconut shell liquid smoke encapsulation with a round, wavy elongated shape, while Figure 1b shows a crack in the capsule. The crack is thought to trigger the release of volatile compounds so encapsulation process needs to be improved. According to Ali, et al. (2014), the treatment of coconut shell liquid smoke which was given nano-encapsulation technology with a maltodextrin channel as a control treatment showed a crack in the capsule morphology which triggered the release of bioactive in the core material.

C. Effect of Encapsulated Coconut Shell Liquid Smoke on Intestine Microflora

Intestinal microflora observation aims to determine the balance of the microflora population in the intestine. The balance of microorganisms in the gut is closely related health and productivity of broiler. The effect of adding encapsulated coconut shell liquid smoke on broiler intestinal microflora can be seen in table 2.

Table-2: Effect of encapsulated coconut shell liquid smoke on intestinal microflora

Variable	Treatment				
	T0	**T0 +**	**T1**	**T2**	**T3**
LAB	7.55 ± 0.47^{a}	7.88 ± 0.69^{ab}	8.56 ± 0.34^{b}	8.59 ± 0.25^{b}	8.65 ± 0.45^{c}
Escherichia coli	4.45 ± 0.77	4.29 ± 0.74	4.57 ± 0.42	4.29 ± 0.44	3.78 ± 0.19
Salmonella sp	$6,08\pm0.09^{d}$	5.98 ± 0.08^{cd}	5.29 ± 0.30^{bc}	5.04 ± 0.55^{b}	3.70 ± 0.57^{a}

Note: Different superscripts in the same row indicated significantly different (P<0.01)

Based on table 1 Indicated no changes in *Escherichia coli* but increasing level of encapsulated liquid smoke significantly reduced (P <0.01) population of *Salmonella sp*. On the other hand, population of LAB also significantly increased (P <0.01) with increasing level of encapsulated liquid smoke in feed. The higher the level of encapsulated liquid smoke related with higher level of total phenol which had an antibacterial effect. In fact, level of encapsulated liquid smoke could reduce pathogenic bacteria as indicated by decreasing *Salmonella sp* population. Interestingly, the population of LAB increases even with increasing level of encapsulated liquid smoke. Kailaku, et al. (2016) reported that the total phenol plays a dominant role in antimicrobial activity in liquid smoke. According to Turgis, et al. (2009) hydroxyl group (-OH) in phenol compounds works to inhibit bacterial growth by adhering to the cell wall which causes lipid dissolution and disrupts the performance of the cytoplasmic membrane by inhibiting ATP-ase bonds which cause cells to lysis. The increase population of LAB which produces lactic acid could reduce the digesta pH which

might also suppress or kill pathogenic bacteria in the digestive tract, and therefore maintaining the good balance of the microflora population in the broiler intestines. It might be though that such a better intestinal environment may have good impact toward performance of broiler.

CONCLUSION

Based on the results of the study, it could be concluded that the encapsulation process of liquid smoke change the bioactive substances in particular total acid and pH with no marked change in total phenol. The addition of encapsulated coconut shell liquid smoke at 1.5% in broiler feed increases the population of lactic acid bacteria, but it reduces the population of *Salmonella sp*. Therefore, encapsulated coconut shell liquid smoke might be considered to substitute antibiotic in broiler feed.

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