Effect on encapsulated liquid smoke in combination with formic acid on intestinal development and microbial counts in broiler

E Widodo¹, A A R Pranibilan², Y N N Ardilla², M H Natsir¹ and I H Djunaidi¹

¹Brawijaya University, Malang, Indonesia
²Student, Brawijaya University, Malang, Indonesia

Email: eko.widodo@ub.ac.id

Abstract. This research aimed to evaluate the effect of a combination between encapsulated coconut shell liquid smoke and formic acid on intestinal development and microflora population in the broiler. This study was carried out with 140 day old chicks (DOC) of strain Cobb allotted to 5 treatments and 4 replications, namely T0= basal feed added without addition of antibiotic, T1= basal feed added with 0.1% zinc bacitracin, T2=basal feed + 0.5% liquid smoke + 0.5% formic acid, T3=basal feed + 1% liquid smoke+0.5% formic acid, and T4=basal feed + 1.5% liquid smoke+0.5% formic acid. The variable of this research consists of length and weight of small intestine, pH and viscosity of ileal, and the total population of microorganism. Data were analyzed using one-way analysis of variance (ANOVA). If the data analyses have a significant effect continued with Duncan’s Multiple Range Test (DMRT). The results showed that there was no significant differences (P>0.05) effect of treatment on intestinal development and characteristics, but it significantly increased (P<0.01) Lactobacillus and decreased Salmonella sp population. It can be concluded that the combination of encapsulated coconut shell liquid smoke 1.5 % with 0.5% formic acid in the broiler diet did not affect small intestinal development and characteristics, however improved the population of lactobacillus and decrease Salmonella sp. Based on the results might suggest that the combination of encapsulated liquid smoke and formic acid could be replaced the use of antibiotics in broiler feed.

1. Introduction
During pandemic Covid-19, all businesses have difficulties experienced, especially businesses in the broiler chicken farming had to face challenges such as uncertainty of the broiler price, increasing-price of broilers’ input i.e., feed, day-old chicks (DOC), equipment, vaccines, and medicines. Consumers are willing to pay more attention to buy healthy meat. The government has issued a regulation to save farmers from going bankrupt. On the other hand, most of the government expenditures were allocated to eradicate the covid-19 pandemic. In fact, since January 2018 the government has implemented a policy to control the use of antibiotic growth-promoter (AGP) in animal feed, to ensure that animal products are more healthy and safe to consume. Antibiotics can only be used to cure diseases, under the prescription of a veterinarian. Since then, more researches were directed toward the improvement of broiler performance on an antibiotic-free basis. The use of natural products which have antibiotic
properties is extensively elaborated. One such product is liquid smoke, an in-conventional waste product, which has been reported to exhibit antibiotic activities.

Studies regarding the bioactive compounds of liquid smoke in Indonesia to reduce the antibiotic use in animal feed were reported extensively, however, the use of liquid smoke from coconut shells was still limited. Liquid smoke has been widely used as an antimicrobial agent in food products and other preservation technological aspects. Liquid smoke from coconut shells consists of several bioactive substances 3.13 % [1] or 0.73 % [2] phenol and 9.30% carboxyl with pH value of 3.2. In another research [2] indicated that liquid smoke can decrease pathogenic microbes. *In vitro* experiments showed the antimicrobial effect of liquid smoke on the population of *Lactobacillus plantarum, Salmonella, Escherichia coli,* and *Saccharomyces cerevisiae* [3].

The phenol and carbonyl compounds of liquid smoke from coconut shells might improve small intestinal characteristics and total non-pathogenic bacteria, thus improve the broiler performance. However, limited research was conducted to assess the liquid smoke properties as an antimicrobial agent in poultry feed. One of the possible reasons is the unpleasant smell. Therefore, the common encapsulation by the use of spray dryer is not possible due to the relatively high temperature during the process.

In the previous experiment, it has been reported that microwaving broccoli would decrease flavonoid content, and the decrease in flavonoid concentration is negatively correlated with time [4]. Alternatively, we use a simple microwave oven of medium power to reduce the loss of flavonoid. We previously invented that encapsulation of herb in combination with acidifier could improve intestinal development and microflora population [5]. Another attempt was also made by adding formic acid, as an acidifier to enhance the performance of liquid smoke. Therefore, current research aims to evaluate the development of small intestinal, characteristic and total count microbial under *in vivo* experiment by the addition of the combination encapsulated liquid smoke and formic acid on broiler feed.

2. Materials and methods

2.1. Materials

The materials of this research were used 140 unsexed DOC strain Cobb that had been vaccinated. The average initial body was weight 43.07±2.44g. Cages were divided into twenty-four of 1 m² size and equipped with feeder, drinker, and lamp. During the brooding period, the room was heated by using a gaseous heater. The basal feeds were divided into 2 types: starter feed (1–20 days) and finisher feed (21–35 days). Preparation of feed formulations as a basal diet following the requirements nutrient of broiler chickens. The formula and nutrient content of the basal diet was depicted in table 1.

| Table 1. Feed formula and nutrient content of the basal diet. |
|-----------------------------|----------------|----------------|
| Raw materials (%) | Starter | Finisher |
| Yellow corn | 56.00 | 56.10 |
| Soybean meal | 22.00 | 21.00 |
| Meat bone meal | 8.50 | 6.00 |
| Rice bran | 5.00 | 9.29 |
| Fish meal | 4.76 | 4.00 |
| Palm Oil | 3.00 | 3.00 |
| Lysine | 0.22 | 0.21 |
| Methionine | 0.17 | 0.05 |
| Salt | 0.05 | 0.05 |
| Premix | 0.30 | 0.30 |
| Nutrient content | | |
| Moisture (%) | 11.36 | 11.54 |
| Crude protein (%) | 21.67 | 19.88 |
| Crude fiber (%) | 5.31 | 5.14 |
Crude Fat (%)  
7.01  
6.36

Metabolizable energy (kcal/kg)*  
3,082  
3,086
*calculated value

2.2. Method
The birds were reared for 35 days in an experimental research design which was divided into 5 treatments and 4 replications, each replication used 7 birds. The experimental treatments were T0= basal diet + no antibiotic, T1 = basal diet + 0.1% zinc bacitra cin, T2 = basal diet + 0.5% encapsulated liquid smoke + 0.5% formic acid, T3 = basal diet + 1% encapsulated liquid smoke + 0.5% formic acid T4 = basal diet + 1.5% encapsulated liquid smoke + 0.5% formic acid.

Feed and water were provided *ad libitum* during the raising period. The liquid smoke was obtained commercially as a product from the coconut shell liquid process. At 35 days, 1 chicken from each replication was selected and slaughtered, then the examination of small intestinal parts was performed. The intestine was cut into different parts, namely the duodenum, jejunum, and ileum. The ileal content (digesta) was collected, and directly send to the laboratory just after collection for microbial analysis, and another part was stored in a deep freezer for pH and viscosity measurements.

2.3. Encapsulation process
Encapsulation equipment (microwave oven and other apparatus) and process or condition employed was done based on the procedure of [5], except only one encapsulant was used, which was replaced with maltodextrin. The mixture of maltodextrin and liquid smoke was set at a ratio of 1:1.

2.4. Variables observed
The variables observed in this study were a) the length and weight (filled and empty weights) of each small intestine portion (duodenum, jejunum, and ileum), b) measurement of ileal content pH and viscosity according to Lee at al (2015) the previous method [6], c). Calculated total population of *Escherichia coli*, *Salmonella* sp. and *Lactic Acid Bacteria* (LAB) according to Hernandez et al (2004) the Total Plate Count (TPC) method [7].

2.5. Statistical analysis
Data were analyzed using one-way-anova. If the data analysis showed a significant effect, it was continued with Duncan’s Multiple Range Test to determine the differences among experimental units.

3. Results and discussion
3.1. Effect of liquid smoke and acidifier on intestinal development and characteristics
Tables 2 and 3 show the summary results of the effect of coconut shell liquid smoke and acidifier combination in broiler feed on the small intestinal development and characteristics.

**Table 2.** Effect of liquid smoke and acidifier on duodenum and jejunum measurements.

| Treatments | Duodenum length (cm) | Duodenum weight (g) | Duodenum empty weight (g) | Jejunum length (cm) | Jejunum weight (g) | Jejunum empty weight (g) |
|------------|----------------------|---------------------|--------------------------|---------------------|-------------------|-------------------------|
| T0         | 32.47±2.83           | 17.67±0.68          | 11.76±1.46               | 71.77±5.47          | 46.55±2.46        | 20.04±1.30             |
| T1         | 36.42±4.53           | 19.46±3.50          | 12.34±1.26               | 78.80±13.30         | 45.15±2.69        | 18.02±2.58             |
| T2         | 35.05±2.51           | 21.49±4.61          | 14.42±4.23               | 76.70±1.95          | 53.84±3.84        | 23.06±5.29             |
| T3         | 32.25±4.57           | 16.67±4.20          | 11.41±2.35               | 77.45±4.05          | 47.81±7.88        | 18.36±2.01             |
| T4         | 34.22±3.55           | 18.11±1.39          | 11.66±1.33               | 77.95±3.18          | 46.88±5.96        | 21.34±3.27             |

**Table 3.** Effect of liquid smoke and acidifier on the ileum measurements.

| Treatments | Ileal length | Ileal weight | Ileal empty | pH value | Digesta |
|------------|--------------|--------------|-------------|----------|---------|
| T0         |              |              |             |          |         |
| T1         |              |              |             |          |         |
| T2         |              |              |             |          |         |
| T3         |              |              |             |          |         |
| T4         |              |              |             |          |         |
The effect of liquid smoke and acidifier on intestinal microflora

Chicken health is influenced by the role of microflora in the small intestine. The effect of the addition of a combination coconut shell liquid smoke and acidifier on the population of intestinal microflora (Escherichia coli and Salmonella sp.) of the broiler is shown in Table 4.

**Table 4.** The effect of liquid smoke and acidifier on the total population of intestinal microflora.

| Treatments | Lactobacillus (log CFU/ml)* | Escherichia coli (log CFU/ml) | Salmonella sp. (log CFU/ml)** |
|------------|-----------------------------|-------------------------------|-------------------------------|
| T0         | 7.54±0.54^A                 | 4.47±0.87                    | 6.04±0.07^A                  |
| T1         | 7.76±0.73^AB                | 4.49±0.68                    | 5.99±0.09^A                  |
| T2         | 8.49±0.34^B                 | 4.66±0.41                    | 5.29±0.34^B                  |
| T3         | 8.67±0.19^B                 | 4.42±0.38                    | 4.91±0.54^B                  |
| T4         | 8.66±0.51^B                 | 3.76±0.21                    | 3.85±0.52^C                  |

Notes: *different superscripts in the same row showed significant differences (P<0.05).
**different superscripts in the same row showed highly significant differences (P<0.01).

Based on the data show that the addition of combination between liquid smoke and acidifier did not affect significantly (P>0.05) the Escherichia coli population. However, this combination significantly changes (P<0.05) the Lactobacillus and Salmonella sp population. The combination of coconut shell liquid smoke and formic acid as a feed additive was expected to increase the non-pathogenic bacteria and adversely reduced the number of pathogenic bacteria. Average data show that the increasing level of combination liquid smoke and acidifier leads to an increase in total Lactobacillus, similar to that reported by [4]. In addition, population of Salmonella sp decreased with increasing level of liquid smoke, without changing Escherichia coli count. A similar previous research reported within in vitro condition using Salmonella choleraeaus [2] and Salmonella sp [4]. Current research results indicated that the use of encapsulation effectively protects flavonoid loss during processing that implies under in vivo experiments. The combination of encapsulated coconut shell liquid smoke and formic acid indicated a positive effect on small intestinal by improved Lactobacillus population and reduce population of Salmonella sp as pathogenic bacteria.

4. Conclusion

It can be concluded that the combination of encapsulated coconut shell liquid smoke 1.5 % in with 0.5% formic acid in broiler diet did not affect small intestinal characteristics. However, it could improve the population of the beneficial bacteria (Lactobacillus) and decrease the pathogenic one (the Salmonella sp.). It might be suggested that the combination of encapsulated liquid smoke and formic acid could be replaced the use of antibiotics in broiler feed.
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