International Research Journal of Advanced Engineering and Science
ISSN (Online): 2455-9024

The Effect of Eugenol from Clove Oil on Bacterial Count and Nutrient Utilization in Broiler

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Abstract— *The purpose of this study was to determine the effect of eugenol levels in feed on bacterial populations and nutrient utilization in broiler. Eugenol used was extracted from clove leaves bough from farmers' gardens in Central Sulawesi. Bacterial tests and observations were carried out at the Health Laboratory Central Sulawesi Province, while the testing of nutrient utilization was carried out at the Experimental Farm of the Faculty of Animal Husbandry and Fisheries, Tadulako University. In the first experiment, there were 3 types of bacteria tested, Escherichia coli, Salmonella sp., Lactobacillus sp. by using several concentrations of eugenol, namely: 0%; 0.25%; 0.5%; 0.75%; 1.00%; 1.25%; 1.50%; 1.75%; 2.00%; 2.25%; 2.50%. While the second experiment was carried out by using 24 broiler chickens aged 6 weeks with a body weight ranged of 1.90 ± 0.08 kg. In this experiment, eugenol was added to the basal ration with 6 treatments, namely: E_0 = basal ration + 0g eugenol; E_1 = basal ration + eugenol 5g/kg ration; E_2 = basal ration + eugenol 10g/kg ration; E_3 = basal ration + eugenol 15g/kg ration; E_4 = basal ration + eugenol. 20g/kg ration; E_5 = basal ration + eugenol. 25g/kg ration. The variables observed were bacterial population and nutrient utilization values. The results showed that bacterial counts significantly decreased (P>0.01) with increasing concentration of eugenol in feed. Furthermore, the population of Escherichia coli and Salmonella sp at 2% eugenol and Lactobacillus sp in 1.75% eugenol decreased to zero, meaning all of these bacteria died. The treatment that gave the highest nutrient utilization was the use of eugenol essential oil 15g / kg ration. It is then concluded 15g/kg clove oil in broiler feed showed the optimum nutrient utilization.*

Keywords— *Bacteria, broiler, eugenol, cloves leaves, nutrient utilization.*

I. INTRODUCTION

Bioactive substances derived from plants can be used as an alternative to antibiotics. Cloves (*Syzygium aromaticum* L) is one of the plant containing essential oils that is commonly named eugenol. Clove contains essential oil which can be obtained from the extraction or distillation process of different plant parts (flowers, flower stalks and leaves). Essential oil contents of each part of clove differs, of which flower, stalk and leaf may contain 10-20%, 5-10%, and 1-4%, respectively. Among the essential oil, eugenol content is dominant (70-80%). Eugenol can function as a stimulant, local anesthetic, carminative, antiemetic, antiseptic, and antispasmodic [1, 2]

As an antimicrobial, eugenol in the clove leaf essential oil could expectedly act to suppress the growth of pathogenic bacteria. Thus, the use of eugenol as feed additive in the ration should then inhibit the growth of pathogenic bacteria in the digestive tract, with the preference to improve digestion and absorption of nutrients. The other function of essential oil component is to improve the stimulation of digestive enzyme secretions in the chickens [3, 4]. Therefore, the effective dose of eugenol in feed additive to kill pathogenic microorganism and improve performances of broiler remains unknown. This research was conducted on evaluating the use of eugenol essential oils of clove leaf by determining the population of 3 types of bacteria i.e. *Escherichia coli*, *Salmonella* sp. *Lactobacillus* sp, and determining nutrients utilization if used as feed for AGP replacer in broiler feed.

II. MATERIAL AND METHOD

2.1. Experiments Sites

The study was conducted in Health Laboratory the Central Sulawesi Province to test the killing power of eugenol on bacteria i.e. *Escherechia coli*, *Salmonella* sp. and *Lactobacillus* sp. Nutrient utilization testing was carried out at the Experimental Farm Animal Science Department, Faculty of Animal Husbandry and Fishery Tadulako University, Palu Central Sulawesi Province. Analysis of dry matter and crude protein, and energy of feed and excreta were held at the Laboratory of Animal Food Chemistry, Hasanuddin University, Makassar.

2.2. First Experimental Procedure

2.2.1. Observation of Eugenol's Population Size on Bacteria

1. Making a bacterial suspension

A suspension was made to test the killing power of eugenol on tested bacteria, as follows:

- Bacterial colonies samples were taken and then put onto NaCl media
- Shake gently until homogeneous of the bacterial colony samples up to murky based on Macfarlan standards

2. Testing of population size

The stages of testing the population size of bacteria were as follows:

- Bacterial suspension tested was taken 1 ml and put into a test tube (according to the number of treatments)
- Eugenol extract was diluted according to the treatment concentration
- Each treatment were added 1 ml eugenol extract

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International Research Journal of Advanced Engineering and Science
ISSN (Online): 2455-9024

- Next stept was incubation for 24 hours at 37ºC

3. Calculation the number of bacteria
   Calculation of the number of bacteria in each treatment was done as follows:
   - Prepared a liquid PCA media for each treatment
   - Added 1 ml eugenol extract that had been incubated until thickened, then incubated for 24 hours at 37ºC
   - Then observed and calculated the number of bacterial colonies in each treatment

2.2.2. Second experimental procedure (nutrient utilization)

The nutrient utilization test was carried out in a single battery cage. Twenty four broilers of 6 weeks olds were randomly distributed in battery cages. The average body weight of broiler used was 1.90 ± 0.08 kg. Each cage were equipped with feeder and drinker. Water were provided any time of the day and placed regularly in each unit of the cage. A total of 24 cages were needed.

This experiment consisted of 2 periods, namely preliminary and collection period. Preliminary period was carried out for 3 days to allow chickens accustomed with the cage and other facilities. Then, in the day 4, all broilers were fasted for 24 hours to remove the remaining feeds from the digestive organs. The fecal collection period was then done for 4 days following the method of Sklan and Hurwitz [5] which was modified by Abun et al. [6]

Furthermore, during collection period feed was given as 200 g for each chicken and provided drinking water *ad libitum*. Basal diets of the treatments were shown in Table 1. In each collected excreta was sprayed 0.01 N H_2SO_4 every two hours to prevent nitrogen loss [7]. The collected excreta was then stored in the freezer. Before being analyzed, the excreta were towed, and dried in the oven for 24 hours with temperature of 50-70^{o}C. The dried sampels were weighed, then analyzed to determine the content of dry matter, crude protein and gross energy.

TABLE 1. Feed Ingredients and Basal Feed Chemical Composition

Feedstuffs	Composition %)
Yellow Corn grain	55.00
Rice bran	14.45
Soybean grain	15.75
Fish meal	14.55
DL-Methionine	0.10
Lysine	0.15
Total	**100**
Nutrient Contents*	
Metabolizable Energy (kcal/kg)	3,110
Crude Protein (%)	21.98
Crude Fat (%)	6.24
Crude Fibre (%)	3.91
Calcium (%)	1.00
Phosphor (%)	0.89
Methionine (%)	0.58
Lysine (%)	1.48

* Based of calculation of nutrients

The method of research was experiment by using a Completely Randomized Design (CRD) with 6 treatments and 4 replications. The treatments consisted of:

E_0 = Basal feed (0% eugenol)
E_1 = Basal feed + eugenol of clove essential oil 5 g/kg feed
E_2 = Basal feed + eugenol of clove essential oil 10 g/kg feed
E_3 = Basal feed + eugenol of clove essential oil 15 g/kg feed
E_4 = Basal feed + eugenol of clove essential oil 20 g/kg feed
E_5 = Basal feed + eugenol of clove essential oil 25 g/kg feed

2.3. Data Analysis

Data were analyzed using an analysis of variance based on Completely Randomized Design [8]. If the results of anova were found a significant effects, it will be continued with Duncan's Multiple Range Test (DMRT).

III. Result and Discussion

3.1. Population Size of Bacteria

The use of eugenol clove leaf essential oil at different concentrations should killed bacteria (both pathogenic and non-pathogenic ones). The results of the experiment as shown in Table 2. The table shows that the use of clove leaf oil eugenol could reduce the population number of pathogenic bacteria *E.coli* and *Salmonella* sp, and at 2.25% eugenol can kill all of the two types of bacteria. The use of clove leaf extract in a concentration of 1.25% bacterial growth was still detected, however, in a concentration of 2.5% - 20% all the bacteria died [9]. Whereas the use of 0.5% eugenol found an increase number of *Lactobacillus sp*. as apatogenic bacteria. However, when the level of eugenol to 0.75% the number of *Lactobacillus sp* started to decrease, and at 1.75% all of the *Lactobacillus sp* was killed. The current result shows that the use of eugenol with a concentration of 2.25% can kill all of bacteria (pathogenic and non-pathogenic, *E.coli* and *Salmoinella* sp, and *Lactobacillus* sp. The antibacterial activity of clove oil can inhibit the growth of pathogenic bacteria such as *Candida albicans*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus* [10].

TABLE 2. Results of Bacterial Counts

Eugenol concentration (%)	Kinds of Bakteria		
	Salmonella **(cfu/ml)**	***E.Coli*** **(cfu/ml)**	***Lactobacillus*** **sp (cfu/ml)**
0	$1.61x10^{11}$	$1.33x10^{11}$	$1.17x10^{5}$
0.25	$1.02x10^{11}$	$1.04x10^{11}$	$1.40x10^{5}$
0.50	$9.9x10^{10}$	$9.8x10^{10}$	$1.25x10^{5}$
0.75	$7.3x10^{10}$	$7.1x10^{10}$	$7.9x10^{4}$
1.00	$5.9x10^{10}$	$6.2x10^{10}$	$2.8x10^{4}$
1.25	$5.2x10^{10}$	$6.0x10^{10}$	$2.2x10^{4}$
1.50	$4.4x10^{10}$	$5.7x10^{10}$	$1.7x10^{4}$
1.75	$1.8x10^{10}$	$1.3x10^{10}$	0
2.00	$1.0x10^{10}$	$4.0x10^{9}$	0
2.25	0	0	0
2.50	0	0	0

The mechanism by which eugenol inhibits bacterial growth was by penetrating the cytoplasmic membrane and interfering with the permeability of the bacterial cell wall. In addition, the hydropobic nature of eugenol makes it easier to enter the lipopolysaccharide portion of the bacterial cell membrane,

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especially gram negative bacteria and change the structure of the cell wall. This would change cell wall structure, then causes intracellular leakage [11, 12].

Clove oil is a plant part that contains eugenol, when tested on several types of bacteria showed antibacterial properties and inhibited *L. monocytogenes*, *Campylobacter jejuni*, *S.enteridis*, *E.coli* and *S.aureus* growth [13, 14, 15]. Clove flower essential oil is more active against *B. subtilis* and *B. cereus*, so it has more potent antibacterial effect. However, the antibacterial activity of clove flower is lower than amoxicillin and clove oil because the percentage of active compound in clove oil is lower [16].

3.2. Nutrient Utilization

Digestibility values of feed is the amount of nutrients in feed which are degraded in the digestive tract. The digestibility values of dry matter, protein and energy metabolism of the diets containing different levels of eugenol are shown in Table 3.

TABLE 3. The digestibility values of dry matter, protein and energy metabolism of the feed

Treatments	Level of Eugenol (g/kg)	Protein Digestibility (%)	Dry Matter Digestibility (%)	Metabolizable Energy (kcal/kg)
E0	0	72,54±0,18^{a}	56.36±1,25^{a}	3155,85±42,92^{a}
E1	5	72,69±0,55^{b}	59.35±0,93^{b}	3346,49±31,58^{b}
E2	10	80,11±1,11^{c}	60.45±1,32^{b}	3460,25±52,99^{c}
E3	15	80,31±1,28^{c}	60.52±1,72^{b}	3377,28±62,94^{bc}
E4	20	69,65±1,45^{d}	55.07±1,16^{a}	2974,92±54,97^{d}
E5	25	63,95±1,81^{e}	49.98±1,36^{c}	2800,20±66,82^{e}

Note: Numbers followed by different superscrip in the same column show highly significant difference (P <0.01)

Based on the data in Table 3 shows that the ration treatment with different levels of eugenol in the diets gives a highly significant effect (P <0.01) on dry matter and protein digestibility, and energy metabolism. The use of clove leaf eugenol of 10-15 g/kg ration could increase the digestibility values of protein and dry matter, and of energy metabolism. However, the further increase use of eugenol higher than 15g / kg ration reduced the digestibility values of protein and dry matter and, similarly, reduced energy metabolism.

The increasing of protein, dry matter, and metabolizable energy could be due to the presence of antibacterial properties in eugenol, which can suppress pathogenic *E. Coli* and *Salmonella sp* . These changing bacteria population in the digestive tract might cause an increase in digestion, probably also absorption, of nutrients. In addition, the use of optimum eugenol level of 15g/kg of ration still leaves the non-pathogenic *Lactobacillus sp* to grow and help break down the carbohydrates. The decrease of digestibility of nutrients and metabolizable energy at higher level of eugenol (more than 15g/kg ration) might also be relate to decrease palatability of the ration, increase drinking water consumption, and also the absence *of Lactobacillus sp*. This bacterium functions to break down carbohydrates into lactic acid which will then become energy and also improve digestive environmental condition. So, with no *Lactobacillus sp* in the digestive tract, the breakdown of carbohydrates into energy becomes low [5].

IV. CONCLUSION

The conclusion of this study:

1. The minimum killing power of eugenol cloves leaves essential oil on bacteria of *E. Coli* and *Salmonella sp* is at a concentration level of 2.25%.
2. The use of eugenol essential oils of cloves leaves 15g/kg ration gives the highest nutrient utilization, in particular protein and dry matter digestibilities. Whereas at the level of 20g/kg ration and 25g / kg ration tends to decrease the digestibilities of protein and dry matter.

The suggestion is that the use of 15g eugenol cloves leaves essential oil/kg broiler feed might be advised to replace AGP.

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Muhammad Tahir, Siti Chuzaemi, Eko Widodo, and Hafsah, "The Effect of Eugenol from Clove Oil on Bacterial Count and Nutrient Utilization in Broiler," *International Research Journal of Advanced Engineering and Science*, Volume 4, Issue 4, pp. 218-221, 2019.