

# The Effect of Turmeric, Ginger, and Teak Leaf Nanoparticles Extraction as Feed Additives on the Microbial of Broiler Chickens

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**Abstract.** This study evaluated the effect of using ginger, turmeric and teak leaf extracts in the form of nanoparticles on the total plate count of lactobacillus, *Escherichia coli* and *Salmonella* sp. in broiler chicken manure. 200 broiler chickens were divided into 5 groups allocated in a randomized block design with 5 treatments and 4 replications (10 broiler chickens per replication). Treatment consisted of control (T0) in the form of 100% commercial feed, (T1) in the form of the addition of 0.2% extract, (T2) in the form of the addition of 0.4% extract, (T3) in the form of the addition of 0.6% extract, and (T4) in the form of adding 0.8% extract. This research began with the extract making stage, nanoparticle synthesis stage, data collection and analysis. Samples of feed given, the amount of feed given, the remaining amount of feed given from each animal replication were recorded every day for data collection. Data from this study were tabulated using the Microsoft Excel software program. Data were subjected to ANOVA and followed by Duncan Multiple Range Test at 5% level. The results of the study showed that giving feed additives from ginger, turmeric and teak leaf extracts in the form of nanoparticles had a significant effect ( $P < 0.05$ ) on increasing the *Lactobacillus* population, reducing the population of *Escherichia coli* and *Salmonella* sp. In conclusion, giving feed additives from ginger, turmeric and teak leaf extracts in the form of nanoparticles up to 0.8% increased the population of lactic acid bacteria, reducing *Escherichia coli* and *Salmonella*.

Keywords: extract, nano particles, feed additive, *E. coli*, *Salmonella*, *Lactobacillus*

## 1 Introduction

Broiler chickens are chickens that are raised to produce meat in a short period, which can be raised within 21-35 days [1]. Farmers make various efforts to increase the productivity of broiler chickens, such as improving the management of housing and feed maintenance including the addition of feed additives such as antibiotics. However, due to the uncontrolled use of antibiotics in feed mixtures can cause residues on livestock products produced [2] and

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harm the health of humans who consume them, it is necessary to find other alternative ingredients to replace synthetic antibiotics by utilizing herbal plants that have antibiotic-like functions including ginger, turmeric, and teak leaves.

Ginger contains several effective compounds, such as *gingerol* and *gingerdione* that exert powerful antioxidant activity. In addition, it has antibacterial and immunomodulatory properties in laboratory animals. Additives derived from plants used in animal feed to improve production performance are known as phytogetic feed additives, and ginger is one such additive. Ginger powder has a lipid-reducing effect and can also be used as a growth promoter. When included in chicken feed, it has properties similar to antibiotics. This natural feed additive lowers the load of enteric pathogenic microbes and improves digestion and nutrient absorption, which improves poultry production and broiler performance [3].

Turmeric has a camphoraceous aroma and contains many functional compounds such as phenolics, flavonoids and various antioxidant enzymes making it possible to be used as a potential chemopreventive [4]. The active substance content in turmeric includes curcumin of  $1.89 \pm 0.10\%$  and total phenol  $51.56 \pm 2.4$  mg GAE/100g [5]. Turmeric has flavonoid compounds that can attach to immune cells and provide intracellular signals to activate the work of immune cells [6]. Turmeric has inhibitory activity against *Escherichia coli* and *Bacillus subtilis* bacteria [7]

Teak leaf extract contains several bioactives including *flavonoids*, *steroids* and *anthocyanins* and has antibacterial, antifungal, antioxidant activity [8]. Apart from the selection of ingredients used for additional feed, there is a process that can make it easier for additional feed to be absorbed in the chicken's body, the process is nano particles.

Nanoparticles are used in the delivery of liposome- and polymer-based. Nanoparticles are seen as excellent carriers for increasing bioavailability of biomolecules, as they have better diffusion and penetration capabilities into the mucus layer.

Based on the description above, this study was conducted to determine the effect of adding feed additives in the form of nano extracts of turmeric ginger extract and teak leaves on the Total Plate Count of *Lactic Acid Bacteria*, *Escherichia coli*, and *Salmonella sp.*

## **2 Materials and Methods**

### **2.1 Time and location of research**

This research was carried out in June 2023 - October 2023. Sample preparation and laboratory analysis was carried out at the Animal Nutrition and Forage Laboratory, Faculty of Animal Science, Universitas Brawijaya. Chicken rearing and treatment were carried out in Tumpang, Malang for 35 days.

### **2.2 Research material**

The ingredients used in this study were broiler chicken, commercial feed, ginger powder, turmeric powder, teak leaf powder, and 70% ethanol. The tools used in this study were microwave assisted extraction (MAE), beaker glass, Intelligent Ultrasonic Processor (IUP), jar, and filter paper.

### **2.3 Production of extract of ginger, turmeric, and teak leaves**

The procedure to extract Ginger, Turmeric, and Teak Leaves begins with preparing ingredients, namely ginger powder, turmeric powder, teak leaf powder, and 70% ethanol. The next step is to weigh ginger powder, turmeric powder and teak powder (1:1:1) and add

ethanol in a ratio of 1:5. The next step is to macerate the sample for 24 hours in a closed jar. After that, the macerated sample is put into the kjeldahl flask. Then the sample was extracted using MAE in a controlled temperature of 50-60°C for 10 min. The extracted sample is cooled at room temperature, then filtered using a filter cloth to obtain the filtrate. The next process is to distillate the filtrate until all ethanol has evaporated using MAE at a controlled temperature of 50-60°C for 15 minutes with a medium low temperature setting. The next process, the nanoparticle process is carried out. Furthermore, nanoparticles extract [9].

## 2.4 Nano particle process

The nanoparticle extract procedure of a combination of Ginger, Turmeric, and Teak Leaves begins with a sample inserted into a 100 ml beaker glass. The next step is to insert beaker glass into the Intelligent Ultrasonic Processor, then the time is set for 10 minutes with a temperature of 27-33°C. The process is carried out twice with the same time but with different temperatures, namely 33-37°C. Furthermore, the combined extract of ginger, turmeric, and teak leaves is mixed into commercial feed to be given during the chicken rearing period.

## 2.5 Sampling

Chicken manure was obtained from chickens treated with T0 without addition, T1 with the addition of 0.2% extract, T2 with the addition of 0.4% extract, T3 with the addition of 0.6% extract, T4 with the addition of 0.8% taken from the ileum part of broiler chickens at the time of harvest.

## 2.6 Total plate count analysis

The Total Plate Count analysis procedure begins with making selective media for the growth of *lactic acid bacteria*, *E. coli*, and *Salmonella sp.* (MRSA, EMBA, and SSA). The next steps of sterilization of tools include; Petri dishes and selective media. After going through the sterilization process, petri dishes and selective bacterial growth media are removed from the autoclave.

At each treatment, 7 test tubes contain 9 ml of peptone. After that, weighing 1 gram of chicken manure homogenized with 1 ml of aquades. The next step is that the homogenized manure is put into a test tube that has been prepared and diluted until the 7th test tube. The sample in the test tube is put into a petri dish with selective media according to the dilution, for lactic acid bacteria at  $10^{-6}$  and  $10^{-7}$  with MRSA media, *E. coli* at dilutions of  $10^{-3}$ , and  $10^{-4}$  with EMBA media, *Salmonella sp.* at dilutions of  $10^{-3}$  and  $10^{-2}$  with SSA media. Furthermore, the sample is put into the incubator. The sample is incubated for 24 hours. After going through the incubation process, bacteria are counted using a colony counter.

## 2.7 Research method

This research was conducted by experimental method on broiler chickens who were given additional feed extracts in the form of a combination of ginger, turmeric, and teak leaves in the form of nanoparticles. The data collected were obtained from the chicken manure at the age of 35 days and the chicken manure was analyzed by Total Plate Count for the effect of treatment on the number of bacteria.

Chicken manure is taken at each treatment and repetition which includes; T0 without addition, T1 with the addition of 0.2% extract, T2 with the addition of 0.4% extract, T3 with the addition of 0.6% extract, T4 with the addition of 0.8% each one repeat. Each sample is

inserted in a film pot labeled with treatment and repeat. The collected samples were then analyzed for Total Plate Count of *Lactic Acid Bacteria*, *E. coli*, and *Salmonella sp.*

## 2.8 Research variables

Variables tested included Total Plate Count of *Lactic Acid Bacteria*, *E. coli*, and *Salmonella sp.* From the chicken manure fed with added ginger, turmeric, and teak leaf extracts in the form of nanoparticles

## 2.9 Data analysis

The research data were analyzed using Analysis of Variance (ANOVA) in a Complete Randomized Design because maintenance was carried out at one homogeneous cage location. The analysis aims to determine the effect of additional feed extracts of ginger, turmeric, and teak leaves in the form of nanoparticles on the Total Plate Count of *Lactic Acid Bacteria*, *E. coli*, and *Salmonella sp.* If the test results conclude that the treatment or group has a noticeable effect, continue with the duncan's test, by following this models [10]:

$$Y_{ijk} = \mu + \beta_1 X_{ij} + b_i X_{ij} + e_{ijk} \quad (1)$$

Where:  $Y_{ijk}$  = parameter observed,  $\mu$  = overall mean value,  $\beta_1$  = level given,  $X_{ij}$  and  $X^2_{ij}$  = continuous values of the predictor variable,  $b_i$  = random effect of the study  $e_{ijk}$  = residual value from unpredictable error.

## 3 Results and discussion

### 3.1 *Lactic Acid Bacteria* (LAB)

*Lactic Acid Bacteria* (LAB) is one type of normal microflora of the digestive tract of chickens, which has the potential as a probiotic by stimulating immune responses, preventing *enteropathogenic bacterial* infections, treating and preventing diarrhea.

**Table 1.** The average number of lactic acid bacteria in broiler chicken manure due to the addition of combination extract treatment

	T0	T1	T2	T3	T4
<b>LAB</b> <b>(10<sup>7</sup> CFU/g)</b>	158,5 ± 19,8 <sup>ab</sup>	172,5 ± 154,8 <sup>ab</sup>	115,5 ± 0,7 <sup>a</sup>	254 ± 112,4 <sup>b</sup>	318 ± 132,9 <sup>b</sup>
<b>LAB</b> <b>(10<sup>6</sup> CFU/g)</b>	327 ± 37,5 <sup>a</sup>	321,5 ± 21,9 <sup>ab</sup>	249,5 ± 0,7 <sup>a</sup>	591,5 ± 59,4 <sup>bc</sup>	592 ± 12 <sup>c</sup>

Different superscripts on the same line show a noticeable difference (p<0.05)

The results of the overall analysis showed that the addition of a combination of ginger, turmeric, and teak leaves in the form of nanoparticles gave a very noticeable difference in effect (P<0.05) on the number of lactic acid bacteria. LAB in the digestive tract is influenced by several factors, one of which is feed [11]. T4 with the addition of 0.8% extract has the highest *lactic acid bacterial* value. LAB is beneficial for livestock health and production, because it increases absorption of certain nutrients, eliminates symptoms of lactose intolerance, reduces serum cholesterol, improves intestinal motility, anti-cancer effects,

inactivation of *enterotoxin* from pathogenic microbes and stimulates the immune system [12].

### 3.2 *Escherichia coli*

In general, *E. coli* is a normal microflora in the intestines of humans and animals, but some strains are pathogenic [13].

**Table 2.** Average amount of *Escherichia coli* in broiler chicken manure due to the addition of combination extract treatment

	<b>T0</b>	<b>T1</b>	<b>T2</b>	<b>T3</b>	<b>T4</b>
<i>E. coli</i> (10 <sup>4</sup> CFU/g)	140 ± 4,2 <sup>b</sup>	71,5 ± 23,3 <sup>ab</sup>	96 ± 32,5 <sup>ab</sup>	60,5 ± 44,5 <sup>a</sup>	41 ± 1 <sup>a</sup>
<i>E. coli</i> (10 <sup>3</sup> CFU/g)	71,5 ± 7,7 <sup>b</sup>	25,5 ± 19,1 <sup>ab</sup>	46,5 ± 26,2 <sup>ab</sup>	27,5 ± 21,9 <sup>ab</sup>	22,5 ± 2,1 <sup>a</sup>

Different superscripts on the same line show a noticeable difference (p<0.05)

The results of the overall analysis showed that the addition of a combination extract of ginger, turmeric, and teak leaves in the form of nanoparticles gave a very noticeable difference in effect (P<0.05) on the amount of *Escherichia Coli*. T4 with the addition of 0.8% extract had the lowest amount of *Escherichia coli*. This is as expected because *Escherichia Coli* can cause several diseases such as *colibacillosis* in poultry [14].

### 3.3 *Salmonella sp.*

*Salmonella sp.* is one of the gram-negative bacteria that is pathogenic and is the agent that most often causes foodborne disease in the world.

**Table 3.** Average Amount of *Salmonella sp.* in broiler chicken manure due to the addition of combined extract treatment

	<b>T0</b>	<b>T1</b>	<b>T2</b>	<b>T3</b>	<b>T4</b>
<i>Salmolnella sp.</i> (10 <sup>3</sup> CFU/g)	23 ± 0 <sup>abc</sup>	40,5 ± 16,3 <sup>c</sup>	35 ± 4,2 <sup>bc</sup>	19 ± 2,8 <sup>ab</sup>	10,5 ± 2,1 <sup>a</sup>
<i>Salmolnella sp.</i> (10 <sup>2</sup> CFU/g)	3 ± 0 <sup>ab</sup>	9 ± 4,2 <sup>b</sup>	7,5 ± 0,7 <sup>ab</sup>	3 ± 2,1 <sup>ab</sup>	2,5 ± 1,4 <sup>a</sup>

Different superscripts on the same line show a noticeable difference (p<0.05)

The results of the overall analysis showed that the addition of a combination extract of ginger, turmeric, and teak leaves in the form of nanoparticles gave a very noticeable difference in effect (P<0.05) on the amount of *Salmonella sp.* T4 with the addition of 0.8% extract has the amount of *Salmonella sp.* Lowest. The less *Salmonella sp.* thus minimizing the ability of *Salmonella sp.* In animals and humans, it can cause salmonellosis which disrupts the gastrointestinal tract and many of them can cause death [15].

## 4 Conclusion

In the study, the addition of ginger, turmeric, and teak leaf extracts in the form of nanoparticles was found to suppress the growth of pathogenic bacteria such as *Escherichia coli* and *Salmonella sp.* Additionally, it increased the number of lactic acid bacteria with a

positive effect on the health and production of broiler chickens. The best treatment observed was T4 with the addition of 0.8% extract.

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