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The Effect of Teak Leaf Extract (*Tectona grandis* Linn. *f*) on Feed to Intestinal Microflora of Laying Quails

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ABSTRACT : *The research was aimed to evaluate the effect of the addition of teak leaf extract (Tectona grandis Linn. F) to feed as an alternative antibiotic towards intestinal microflora of laying quails. The materials used were 240 laying quails (Coturnix coturnix japonica) aged 21 days old, divided into four treatments and six repetitions (10 birds of each repetition). The treatments applied were basal feed without teak leaf extract (P0), basal feed with teak leaf extract at 0.8% (P1), 1.2% (P2), and 1.6% (P3). The data were analyzed using Anova and continued with Duncan Multiple Range Test. The results of this research showed that teak leaf extract exerted a significant influence (P>0.05) on Lactic Acid Bacteria with the average of bacteria P0 (4.68 Log CFU), P1 (5.56 Log CFU), P2 (5.78 Log CFU), P3 (5.51 Log CFU). However, in the Salmonella sp case, it did not produce a significant difference with the average values at P0 (2.42 Log CFU), P1 (2.45 Log CFU), P2 (2.70 Log CFU), P3 (2.27 Log CFU) while in Escherichia coli, the average values are P0 (2.38 Log CFU), P1 (2.51 Log CFU), P2 (2.44 Log CFU), P3 (2.58 Log CFU). Thus, it was concluded that the use of teak leaf extract contains flavonoid influenced Lactic Acid Bacteria value, yet it had a contrast influence on Salmonella sp and Escherichia coli in intestinal microflora of laying quails.*
KEYWORDS *Teak leaf extract, Lactic Acid Bacteria, Salmonella sp, Escherichia coli.*

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I. INTRODUCTION

Teak leaf (*Tectona grandis* Linn. *f)* is a family of *Lamiaceae* that contains chemical flavonoids to have antioxidant and anti-bacterial effects. Teak leaf (*Tectona grandis* Linn. *f)* is a tropical plant that widely grows in Asia (Lacret et al., 2012). Teak leaves could be found in large quantities but have not been widely used as a feed additive. The teak leaf's bioactive compounds are alkaloid, tannin, sterol, saponin, protein, carbohydrates, calcium, phosphorus, raw fiber and β-carotene, flavonoid, anthocyanin, pheophytin, pelargonidin 3-glucoside, pelargonidin 3.7-diglucoside and chlorophyll [1].

Quail (*Coturnix coturnix* japonica) is an efficient livestock commodity capable of producing animal protein in eggs and meat. Therefore quail is called dual-purpose livestock. Several types of quail are scattered throughout the world, but only a few types of quail can be used as producers of food products. Quail is a livestock that has an aggressive nature and is easily stressed. This happens when the quail is exposed to a change in the environment or a factor that can trigger stress. Several stress factors in quail can be observed with changes in hematological, endocrinological elements of metabolism and behavior [2].

The addition of antibiotics in the feed is concerned with a resistant effect on bacteria, including *Salmonella*, *Escherichia coli*, *Enterococcus*, *Campylobacter*, which are in the digestive tract of livestock, transfer to humans and cause residues when consumed. With the prohibition on of antibiotics as feed additives, alternative replacements are needed, such as bioactive compounds found in plants. One of the efforts to maintain performance and increase immunity is by providing feed additives from herbal plants that function as antioxidants and anti-bacterial [3]. Therefore, the research was aimed to evaluate the effect of the addition of teak leaf extract in the feed is expected to to balance the intestinal microflora of quails by suppressing the number of pathogenic bacteria found in the intestines of laying quails.

II. MATERIALS AND METHODS

MATERIALS

This research used 240 laying quails (*Coturnix coturnix japonica*) at aged 21 days old. Feeding was conducted every day with 25g/bird/day, while drinking water was supplied ad libitum. Teak leaf extract with different levels was added to the feed with the composition of corn, wheat flour, soybean meal, meat and bone meal, corn glutter, wheat bran, DDGS, palm oil, methionine, lysine, methionine, and cystine. The result of proximate feed nutrient analysis applied in this research was arranged in Table 1.

Table 1: The Result of Feed Nutrient Analysis

Nutrient	Basal Feed	Teak Leaf Powder
Dry Matter (%)	89.93	89.62
Crude Protein (%)	21.59	9.44
Ash (%)	10.17	12.97
Crude Fat (%)	4.32	3.80
Crude Fiber (%)	5.09	29.44

Notes: Proximate Analysis Value of Laboratory of Animal Feed, Department of Animal Husbandry and Fisheries, Blitar Regency

METHODS

The method applied in this data was analyse using Anova (Analysis of variance), in which data were divided into four treatments and six repetitions. Every repetition had ten quails with the level of teak leaf extract as follows:

P0 = Basal Feed
P1 = Basal Feed + 0.8% teak leaf extract
P2 = Basal Feed + 1.2% teak leaf extract
P3 = Basal Feed + 1.6% teak leaf extract

The observed variables in this research were the number of *Escherichia coli*, *Salmonella sp*, and Lactic Acid Bacteria took from the ileum and counting the TPC of bacterial colonies.

III. RESULTS AND DISCUSSION

Flavonoid and antioxidant compounds value in teak leaf extract used as a feed additive in the feed can be seen in Table 2.

Table 2: Bioactive compounds of teak leaf extract

Analysis	Results
Flavonoid	6.24 µg/ml
Antioxidant IC50	11.64 µg/ml

Notes: The analyzed value of State Polytechnic Malang Laboratory

Flavonoids are secondary metabolite compounds found in plants and function as antioxidants. Table 2 showed that the total flavonoid in teak leaf extracted using 70% ethanol was 6.24 µg/ml. Flavonoids act as antioxidants by donating their hydrogen atoms and contain glucose side chains. As an antioxidant, flavonoids can catch free radicals that may damage body cells [4].

An antioxidant is a compound that can inhibit oxidation reactions by binding to free radicals and highly reactive molecules [5]. Table 2 showed the analyzed value of the number of antioxidants in teak leaves extracted using 70% ethanol, 11.64 µg/ml. An antioxidant produced by the body is not sufficient to fight free radicals; therefore, there is a need for additional feed containing antioxidants [6].

Table 3. Inhibition of Microbial Antibiotics and Teak Leaf Extract (mm)

Treatment	Lactic Acid Bacteria	*Salmonella* sp.	*Escherichia coli*
P0 (+)	2.39 ± 0.28^{d}	3.62 ± 0.22^{d}	3.95 ± 0.05^{e}
P1	0.59 ± 0.06^{a}	0.95 ± 0.04^{a}	0.72 ± 0.04^{a}
P2	0.77 ± 0.02^{ab}	1.29 ± 0.09^{b}	1.02 ± 0.06^{b}
P3	0.95 ± 0.03^{b}	2.85 ± 0.06^{c}	1.34 ± 0.11^{c}
P4	1.27 ± 0.05^{c}	3.08 ± 0.13^{c}	1.69 ± 0.24^{d}

Notes: Different notation in the same column showed highly significant differences (P<0,01)

From the analyzed value shown in Table 4, it can be concluded that the most significant microbial inhibition power both on antibiotics and teak leaf extract was *Salmonella sp.* The results showed the inhibition zone *Salmonella sp*. antibiotics are better compared to teak leaf extract, but the use of antibiotics can cause new problems, namely resistance, especially in uncontrolled use of antibiotics [7]. It stimulated the significance of using phytobiotics as a substitute for antibiotics from plants. One of the plants that can be used and available in large quantities is teak leaves which contain flavonoids that function as anti-bacterial [3]. The inhibition zone's

large diameter indicated that the antimicrobial substances in the solution could inhibit microbial growth. The higher the concentration of an antimicrobial substance, the greater the diameter that is formed [8].

Table 3 showed that the lowest diameter of the *Escherichia coli* inhibition zone was P1, and the highest was P0 (antibiotics). The higher the number of teak leaf extract, the higher the ability to inhibit bacteria. It is because teak leaf extract contains flavonoids that can be used as an anti-bacterial agent [9]. *E. coli* becomes pathogenic when the number in the digestive tract is high so that its growth must be inhibited. The use of teak leaf extract containing flavonoids can cause total damage to bacterial cell membranes and precipitate cell protein. In contrast, in low concentrations, it can cause leakage of bacterial cells so that important metabolites are released from bacterial cells [10].

Table 3 showed that the diameter of the zone of inhibition against lactic acid bacteria was low. It showed that flavonoids did not inhibit lactic acid bacteria's growth and indicated that flavonoids' inhibition was selective, which is vital in certain bacteria. Besides that, the teak leaf extract can also maintain the population of lactic acid bacteria compared to antibiotics. Lactic acid bacteria are beneficial bacteria in the digestive tract of poultry, so the use of phytobiotics should not inhibit the growth of lactic acid bacteria [11].

Table 4: The effect of treatment on populations of Lactic Acid Bacteria, *Salmonella sp*, and *Escherichia coli*

Treatment	Total Bacterial Colonies (Log CFU)		
	Lactic Acid Bacteria	*Salmonella* sp	*Escherichia coli*
P0	4.68 ± 0.57[a]	2.47 ± 0.45	2.49 ± 0.27
P1	5.56 ± 0.58a	2.45 ± 0.42	2.51 ± 0.30
P2	5.78 ± 0.24b	2.62 ± 0.31	2.44 ± 0.43
P3	5.51 ± 0.34[a]	2.27 ± 0.20	2.58 ± 0.21

Notes: Different notation in the same column showed highly significant differences (P<0,01)

The results of the observations in Table 4 indicated that the number of Lactic Acid Bacteria colonies in 1 g of quail intestinal digesta was significantly different (P <0.05) because it was influenced by treatment. The number of Lactic Acid Bacteria colonies in P2 treatment was higher than in other treatments. It indicated that the use of 1.2% teak leaf extract increased the number of Lactic Acid Bacteria colonies. [12] stated that LAB is grouped into several genera, including *Streptococcus, Leuconostoc, Pediococcus*, and *Lactobacillus sp*. LAB is a beneficial probiotic bacteria because it can help improve the intestinal defense system by forming colonies in the intestinal mucosa and helping absorb nutrients. The increase in the number of LAB colonies can produce organic acids which can suppress pathogenic bacteria's growth in the intestine so that pathogenic bacteria are only in the lumen and will be excreted with feces [13].

Table 4 showed that the TPC test result for quail intestines on *Salmonella* bacteria were not significantly different (P> 0.05), but there was a decrease in the number of bacterial colonies at P3 with 1.6% teak leaf extract. It indicated that the more addition of teak leaf extract to the feed can suppress *Salmonella* bacteria. *Salmonella* is a gram-negative bacteria that are parasitic and pathogenic for living things, rod-shaped, mesophilic, facultatively anaerobic, motile and does not form spores found in the intestine and is grouped in the *Enterobacteriaceae* family [14]. Teak leaf extract contains flavonoids that have an anti-bacterial effect of reducing the number of *Salmonella* bacterial colonies. It is following [15] that the decrease in the number of *Salmonella* bacterial colonies in quail intestines is due to flavonoids and phenols.

The TPC test results on Escherichia coli were not significantly different (P> 0.05), shown in Table 4 but showed a decrease in the number of *E. coli* bacteria colonies on P2, that is, the addition of 1.2% teak leaf extract in the feed. *E. coli* bacteria are gram-negative bacteria from the *Enterobacteriaceae* family. It is aerobic, in the form of peritric flagellum, motile, and non-spore [16]. The numbers of LAB and *E. coli* in the intestine are influenced by feeding and competition between bacteria, so that the possibility of a decrease in *E. coli* bacteria is due to the high number of LAB, thereby minimizing the number of *E. coli* bacteria and the influence of active flavonoid ingredients [17].

IV. CONCLUSION

Teak leaf extract contains flavonoid active substances which function as anti-bacterial. The addition of teak leaf extract (*Tectona grandis* Linn. *f*) as a feed additive of 1.2% quail feed affected bacteria, such as increasing the number of Lactic Acid Bacteria (LAB) and reducing the number of *E. coli*, while the *Salmonella* bacteria has decreased with the addition of teak leaf extract by 1.6%.

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