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Effect of *Tamarindus indica* leaf meal in feed on the growth of pathogenic bacteria, intestinal histology, and blood lipid profile in broilers

Ni Wayan Siti[1#], I Gst. Nym. Gde Bidura[1] and Ni Wayan Sudatri[2]

[1#] Faculty of Animal Science, Udayana University, Denpasar-Bali, Indonesia

[2]Animal Physiologi Laboratory, Biologi Departemen, Udayana University, Denpasar, Indonesia

E-mail: wayansiti@unud.ac.id ; bidura_unud@yahoo.com ; wayan_sudatri@unud.ac.id

#Corresponding author: wayansiti@unud.ac.id

ABSTRACT

The purpose of this study was to examine the phytochemicals compound of *Tamarindus indica* leaf meal (TLM) to the bio-control of pathogenic bacteria and growth promotion in broilers. *In vitro* test of Tamarindus leaf inhibition was done using the diffusion well method, by using *E. coli* as the testing bacteria. The *in vivo* test were carried out using a completely randomized design by using 240 broilers day-old-chick with homogeneous body weight were randomized into four treatment groups, namely: broilers fed with 0%; 2%; 4%; and 6% TML, as treatment A; B; C; and D, respectively. The results showed that 2-6% TLM supplementation in feed had no significant effect ($P>0.05$) on broiler performance and intestinal crypto height. The number of *E. coli* bacteria in broilers Group B, C, and D decreased significantly ($P<0.05$) compared to group A. Likewise, total cholesterol and LDL levels in the serum of Groups C and D were significantly lower ($P<0.05$) compared to groups A and B. It can be concluded that the *Tamarindus indica* leaf meal in feed can not effect broiler performance, but can reduce the amount of *E. coli* bacteria in the intestine and reduce blood serum cholesterol levels in broilers. Conversely, it can increase the height of broiler intestinal villi.

Keyword: *Eschericia coli*, villi, cholesterol, broilers

INTRODUCTION

The broiler farm industry is very vulnerable to disease attacks caused by bacteria, viruses, fungi, parasites, and so on. One of the bacteria that often attacks broilers is *Escherichia coli* (*E. coli*). These bacteria are pathogenic and cause *Colibasilosis*. In broilers, *Colibasilosis* causes deterioration of livestock health during the maintenance period [1]. Efforts to cope with cholibasilosis *Colibasilosis* are to use antibiotics. Antibiotics in the livestock industry with the aim of treating livestock, so that the risk of death can be reduced and the condition of the livestock returns to health. In addition, giving antibiotics to the livestock industry is also intended as a feed additive to stimulate growth (antibiotic growth promoter/AGP), increase production and increase the efficiency of feed use [2,3]). However, the use of antibiotics that are not as recommended and not according to the set dose can cause residues in livestock products produced [4].

Tamarindus indica (*Ti*) may be a substitute for antibiotics, because it contains active compounds that function as antibacterials, including: phenolic compounds, glycosides, mallic acid, tartaric acid, sap, pectin, arabinose, xylose, galactose, glucose, and uronic acid [5]. The leaves of *Ti* have long been used by the public as herbal leaves, because of the properties of their phytochemical compounds, such as: alkaloids, steroids, terpenoids, phenolics, flavonoids, and tannins [6]. Giving herbal leaf extract (*Ti*) through drinking water to laying hens can significantly improve the digestibility of feed nutrients [7], increase egg production and reduce cholesterol content in egg yolks [8]. According to [9], every part of *Ti* plants, such as roots, stems, fruits and leaves has important benefits in traditional medicine. The sap from *Ti* seeds also has the potential to be used as a medicinal coating/capsule [10]. The hexane content of methanolic extract from *Ti* leaves, is also a natural bioactive source to form compounds that have antimicrobial abilities [5].

The use of TLM as a substitute for the use of antibiotics in broiler livestock has not been reported. Therefore, it is necessary to do research on TLM as a natural feed additive in broiler livestock. This study aims to determine the inhibition of TML against *E. coli* bacteria and its effect on intestinal villi, serum lipid profile, and broiler production performance.

MATERIALS AND METHODS

Plant Material and Reagents

The main ingredient of this research was *Ti* leaves. After extracting the material, the *in vitro* test of the extract material was carried out on *E. coli* bacteria to see the ability to inhibit the growth of *E coli* bacteria. To test the antibacterial properties of *Ti* leaf extract, an *in vitro* test was carried out using *E coli* bacteria culture.

Nutrient Agar (NA) Media: Weighed 7 g of NA with an electric scale, added 250 ml of aquadest, then heated it over low heat for 15 minutes. Then 10 ml of hot NA media were taken, put in a test tube and covered with

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cotton as tightly as possible. All NA media that have been put into test tubes along with petridishes and other tools used in *in vitro* testing were sterilized by using autoclap for approximately 1 hour at a temperature of 121^0C.

Plant Material and Extraction

Tamarindus leaves were obtained from gardens in the Tabanan Regency, Bali Province. *Tamarindus* leaves were washed with clean water, weighed 100 grams, then kneaded, added with aquadest until the volume was 100 ml and the water was filtered. The filter results obtained from the extract of *Ti* leaves were considered to be 100% *Ti* leaf solution. The concentration of *Ti* leaves solution were 1%; 2%; 3%; 4%; and 5% made by adding aquadest according to the desired concentration.

This research was carried out in two stages, namely: (i) Extraction of *Ti* leaves with the maceration method continued with *in vitro* tests using *E coli* bacteria as test bacteria and (ii) *in vivo* testing in the field used one day old (DOC) broilers. For the *in vitro* test, the type of extract consisted of aqueous extract of *Ti* leaves. The extract concentration consisted of the following concentrations: 1%; 2%; 3%; 4%; and 5%. Each was repeated 6 times, so that 5 x 6 = 30 petridishes were used. Tetrachlor 5% antibiotic was used as a positive control and aquadest as a negative control. For field testing, the experimental design used was a completely randomized design.

Measurement of blood images was carried out at the Laboratory of Animal Physiology, PS Biology, Faculty of Mathematics and Natural Sciences, Udayana University, and the making of histological preparations was carried out at the Pathology laboratory of the Center for Veterinary Medicine (BBVet) Denpasar.

Antimicrobial Activity

To test the antibacterial properties of *Ti* leaf extract, an *in vitro* test was carried out using *E coli* bacteria culture. Various dosage combinations of *Ti* leaf extract were tried on *E. coli* bacteria culture according to the method of [11]. The dosage combinations tested include:

- Negative control: *E. coli* + aquadest
- Positive control: *E. coli* + antibiotics (tetrachlor 5%)
- *Tamarindus* leaves: *E. coli* + *tamarind*: 1% dose; 2%; 3%; 4%; and 5%, repectively

The method used to test the inhibitory ability of *Ti* leaf water extract against *E coli* bacteria was the diffusion well method as practiced by [12]. The extract solution to be used, as well as the tools to be used, were put into a laminar flow except for *E. coli* culture, then sterilized with UV light for 15 minutes. NA (Nutrient Agar) media was heated at ± 40^0C. Each petridish was filled with 200 µl of *E. coli* obtained from culture stock at the Microbiology Laboratory of FMIPA, University of Udayana. Furthermore, 10 mL of NA medium was poured into the petridish and shaken horizontally until well blended and allowed to solidify. After the media solidified, 2 diffusion wells were made with a cork border (5 mm in diameter) for each petridish. Each well was filled with 20 µl of *Ti* leaf aqueous extract solution. Furthermore, NA media and *E. coli* bacteria that have been filled with the tested *Ti* water extract are incubated at a temperature of 27^0C for 18-24 hours.

All treatments were repeated 5 times and the inhibition of *Ti* leaf water extract can be seen by measuring the diameter of the clear zone formed with a caliper. Based on the formed inhibition zone, the antibacterial activity can be classified into three groups, namely: (i) weak antibacterial activity (inhibition zone <5 mm); (ii) moderate antibacterial activity (inhibition zone 5-10 mm); and (iii) strong antibacterial activity (inhibition zone >20 mm) [13].

Feeding trial on broilers

After the *in vitro* antibacterial test of *Ti* leaf water extract was carried out using *E. coli*, then *Ti* leaves were tested on broilers in the form of flour (TLM) mixed in the feed. The experimental design used was a completely randomized design with five levels of TLM in the feed, namely: 0%; 2%; 4%; and 6% as treatments A, B, C, and D, respectively. Each treatment was repeated 6 times and in each experimental unit there were 10 broilers aged one day with homogeneous body weight, so that the total DOC of broilers used was 4 x 6 x 10 = 240 birds.

The day before the treatment ended, blood was drawn from the branchial vein with a 5 mL 3 ml syring for testing blood lipid levels and complete blood profiles. The blood obtained was placed in a tube containing EDTA or anticoagulant, then to separate blood cells and plasma, centrifugation was carried out at a speed of 1500 rpm for 10 minutes. Blood that has been separated from the plasma was taken serum by pipette, put into a cuvette and labeled, stored in a refrigerator at -20^0C until ready to be tested. At the end of the study, the intestines of the broiler cecum were sterile, put in a plastic and cooling box, then the *E. coli* and *Coliform* bacteria populations were tested by the Most Probable Number (MPN) method according to the procedure used

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at the Laboratory of the Medan Environmental Health Engineering Center [14]. For the preparation of intestinal histological preparations, the digestive tract was washed with 0.9% NaCl solution, and put in a collection bottle that had been filled with 10% NBF fixative, then made histological preparations.

Blood profile

Blood was drawn from the broiler wing branchial vein. Blood was collected in a tube that contains antiquagulants. The counting of red blood cells, white blood cells was done by the dilution method. Red blood cells were taken as much as 0.51 ml, then diluted with Hayem's solution until the volume became 101 ml while shaking, then poured into a glass object Neuber Chamber counting room and covered with a cover glass, then observed under an electric microscope that has been connected with Opticlab and Laptop cameras. The number of blood cells was counted by a counter according to the dilution formula. The same procedure was performed to count white blood cells, but the diluent used was Turk's solution. Meanwhile, to measure blood Hb, a stick for measuring Hb was used which was already sold in health equipment stores. Hematocrit levels were measured using a hematocrit pipette [15].

Blood lipid profiles analyzed were total cholesterol, triglycerides, LDL and HDL. The test was carried out at the Bali Provincial Health Laboratory according to the method used by [16].

Intestinal histological preparations

Histological preparations were made by the paraffin method, and the Hematoxylin-Eosin staining according to [17]. Pieces of small intestine that have been fixed in 10% NBF solubility, were put into a tissue casse, then the dehydration process was carried out with a solution of ethanol with a grade of 70%, 80%, 95%, and absolute alcohol twice removed, then continued with the clearing process, with three xylol solutions. Each stage lasts 60 minutes at room temperature. The next process, namely paraffin infiltration by inserting the tissue in liquid paraffin (temperature 60ºC) with three transfers each for 45 minutes. Furthermore, the network was immersed in a mold containing liquid paraffin, then cooled at room temperature, so that it became a paraffin block.

Paraffin blocks were sliced 5µm thick using a rotary microtome, then the incision was placed on the surface of warm water with a temperature of 45^0C and attached to a glass slide that has been coated with gelatin. The preparation was dried by placing it vertically, then placing it on the slide warmer until it sticks to the object glass. The tissue pieces on paraffin were to be stained with Hematoxilin-Eosin, put on a shelf for staining, then incubated at 60ºC for 45 minutes, then placed at room temperature until cold. Furthermore, deparaffinization was carried out through the stages of dissolving paraffin in xylol 3 times, then followed by a rehydration process in 100% alcohol; 95%; 80%; and 70%. Each stage lasts 5 minutes, then put it in distilled water for 10 dips or until the alcohol dissolves.

The next process was staining with hematoxylin by immersing the slides with a solution of hematoxylin for 5 minutes, then washing it in running water for 5 minutes, and then staining it with eosin for 3 minutes. After staining in eosin, the slides were put in an alcohol solution with a grade of 70%, 80%, 90%, to 100% for 10 dips each, then followed by the clearing process using xylol twice for 2 minutes. After that, the preparations were covered with a cover glass with a Canadian balsam medium. The preparations were ready to be observed under a microscope connected to an opticlab camera and a laptop.

The histological preparations were observed under a light microscope connected to a laptop and an opticlab camera with an ocular-objective magnification of 10 x 10 or 10 x 40. For intestinal histological observations, the height of the villi and the height of the crypts were observed. Observations were made in 5 visual fields and repeated 3 times and the results were presented as percentages.

Statistic analysis

The qualitative data obtained were presented in the form of images and descriptions, while the quantitative data obtained were analyzed statistically using SPSS (Statistical Product and Service Solutions) software. If the data distribution was homogeneous, then the data was tested with the one way ANOVA test and if there was a significant effect ($P<0.05$), it will be followed by the Duncan test.

RESULTS AND DISCUSSION

Inhibition of *Tamarindus* leaf extract against *E. coli* bacteria.

To see the antibacterial ability of *Ti* leaves, an *in vitro* test was carried out on the *E. coli* bacteria culture. The positive control used was tetrachlor 5%, while the negative control was aquadest. The clear zone formed as a result of the treatment given by *Ti* leaf extract was a growth inhibition zone for *E. coli* (Figure 1). The results of

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the inhibition zone test for *Ti* leaf extract on the growth of *E. coli* were presented in Table 1 and Figure 1. The aquadest inhibition zone (negative control) for *E. coli* was 0 mm, while the 5% Tetrachlor inhibition zone (positive control) was 50.56 mm. More details are presented in Table 1.

The growth of *E. coli* in NA media was inhibited by the high content of flavonoids and tannins in *Ti* leaf extract, which have antibacterial properties. Flavonoids are polyphenolic compounds that have various effects, including antioxidant, antibacterial, anti-tumor, antiviral and anti-inflammatory effects [18]. The ability of flavonoid compounds from *Ti* leaves as antibacterial by denaturing the lipids in the bacterial cell membrane, through hydrogen bonds, so that the cell membrane was damaged, the formation of new cell membranes was not formed, and bacterial growth was inhibited [19]. While tannins are polyphenolic compounds found in plants, they taste bitter and can coagulate proteins. Tannins are also antibacterial and anti-diarrheal [12]. Diethyl Phthalate is a major compound in both *Ti* leaf extracts that act as antioxidants and antimicrobials [20,21,22].

Table 1.Average diameter of the inhibition zone of *Ti* leaf extract on the growth of *E coli* (mm)

Concentration	Inhibition zone diameter (mm)
Concentration 1%	5.92±0.59a
Concentration 2%	12.07±0.78b
Concentration 3%	14.17±0.76b
Concentration 4%	18.54±0.41c
Concentration 5%	18.99±0.65c

Means with different superscripts within column values are significantly different (P<0.05)

The *Ti* extract concentration tested had a significant effect (P<0.05) on the *E. coli* growth inhibition zone. *Tamarindus* leaf extract at a concentration of 4% and 5% showed the best inhibition zone compared to other concentrations. The increasing the concentration of *Ti* leaf extract, the greater the inhibition zone for *E. coli*. Comparison of the inhibition zone against *E coli* bacteria between 5% aqueous extract of *Ti* leaves and 5% Tetrachlor is presented in Figure 1. According to [23], from the results of the GC-MS (Gass Chromatography-Mass Spectroscopy) test, *Ti* leaves contain Diethyl Phthalate compounds which act as antibacterials.

Tamarindus leaf extract with a concentration above 5% had a significantly higher inhibitory ability to *E coli* growth (P<0.05) compared to *Ti* leaf extract with a concentration of 1%, 2% and 3%, and was not significantly different (P>0.05) compared to a concentration of 4%. The *Ti* leaf extract had fairly good antibacterial activity against *E. coli* compared to 5% tetrachlor (positive control). Figure 2. shows the inhibition zone and the ratio of the growth inhibition zone of *E. coli* formed by the treatment of Ti leaf extract at a concentration of 5%.

The size of the *E. coli* bacteria growth inhibition zone that was formed was strongly influenced by the concentration of the extract of *Ti* leaves. The higher the concentration, the higher the diameter of the inhibitory power, because the increasing concentration of phenol compounds which can inhibit the growth of *E. coli*.

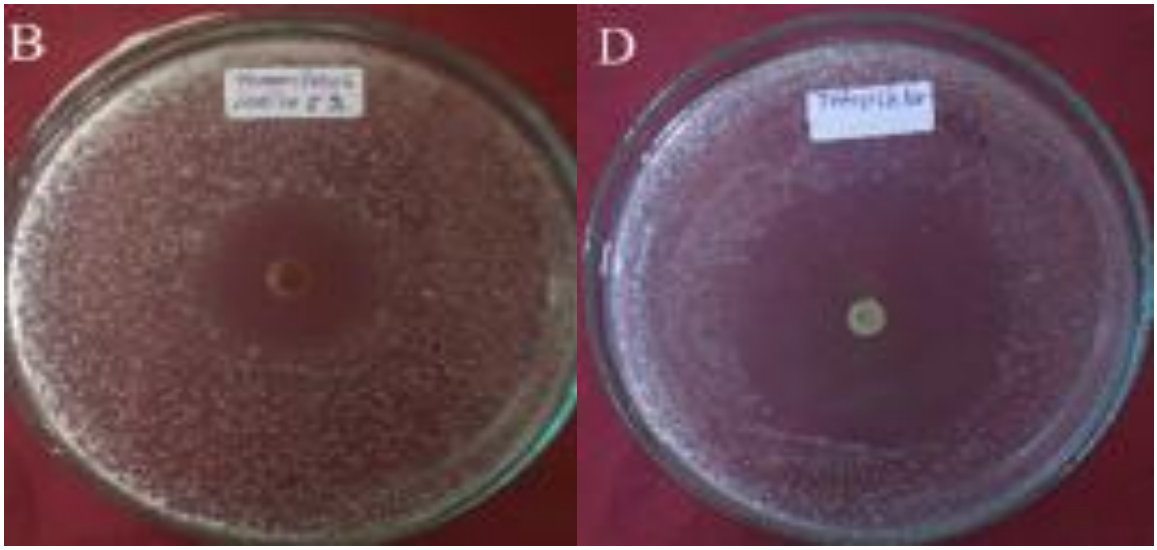

Figure 1. Comparison of the inhibition zone diameter of *E. coli* colonies due to treatment of 5% Ti leaf extract with (B) and 5% antibiotic tetrachlor (D)

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The antibacterial activity of the water extract of *Ti* leaves with a concentration of 1% to 5% (*in vitro*), is classified as moderate to strong, because the inhibition zone formed ranges from 6 to 18 mm. According to [24], the antibacterial activity of a substance is classified as weak, if the formed inhibition zone is <5 mm; moderate activity, if the zone of inhibition is between 5- 10 mm; vigorous activity, if the zone of inhibition is 10-20 mm; and very strong, if the formed zone of inhibition is >20 mm. In this study, the water extract of *Ti* leaves at a concentration of 4-5% had the best antibacterial activity against *E. coli* compared to levels of 1-3%, but was still lower than the 5% Tetrachlor antibiotic (Figure 1).

Total Coliform and *E. coli* bacteria in the intestine

Coliform and *E. coli* bacteria are Gram negative bacteria which are normal flora in the intestine, but if the number exceeds the normal threshold, they can be pathogenic. *Coliform* and *E. coli* populations in the intestines of the broiler group fed with TLM until 35 days of age are presented in Table 2. Population means of total *Coliform* and total *E. coli* in the intestines of the broiler group fed with TLM were significantly different (P<0.05) compared to the control. The population mean total *Coliform* and total *E. coli* in the control broiler intestine was higher, namely: $8.98x10^6$ CFU/g and $6.75x10^5$ CFU/g and these numbers were still within normal limits.

Table 2. The average number of *Coliform* and *E coli* populations in the broiler intestines which were fed with TLM

Level of TML in feed	Bacteria in the intestines of Broilers	
	Total *Coliform* (CFU/g)	**Total *E. coli* (CFU/g)**
TLM 0%	$8.98x10^6 \pm 2.13x10^6$a	$6.75x10^5 \pm 1.39x10^5$a
TLM 2%	$4.51x10^6 \pm 1.08x10^6$b	$2.71x10^5 \pm 1.67x10^5$b
TLM 4%	$3.26x10^6 \pm 1.35x10^6$c	$2.64x10^5 \pm 1.73x10^4$c
TLM 6%	$4.17x10^6 \pm 2.03x10^6$ c	$2.39x10^5 \pm 1.65x10^4$ c
Normal	$4.0\ x10^6 – 9.4\ x10^6$	10^4 - 10^5

Note: Means with different superscripts within row values are significantly different (P<0.05)

E. coli bacteria live in the intestines of humans and other animals as normal flora, but there are some strains of *E. coli* that produce toxins and cause diarrhea. *E. coli* bacteria are commensalism in broilers and their presence in chicken feces is very high which can be a disease transmission agent [25]. *E. coli* bacteria are pathogenic and cause colibasilosis in poultry [26].

The results of the total population test for *Coliform* and *E. coli* in the broiler intestine showed that the total population of *Coliform* and total *E. coli* in Group A broiler intestines was higher than that of broilers fed TLM, but the range was still within normal limits. Essential oil as antibacterial found in TLM can reduce intestinal pH and inhibit bacterial growth, so that the number of *Coliform* and *E. coli* bacteria decreases [23].

Blood profile

The mean blood profile test results are presented in Table 2. From the results of the blood profile test, there was no significant difference (P>0.05) between the blood profiles of broiler groups A, B, C, and D, namely red blood cells (RBC) and white blood cells (WBC), hemoglobin (Hb), monocytes, eosinophils, basophils, and neutrophils. Whereas in the PVC (pack volume cell) and lymphocytes variables there was a significant difference (P<005) between group A compared to Groups B, C, and D.

Table 2. Effect of TLM in feed on blood profile in broilers

Variable	**Groups**[1)]				**Normal**
	A	**B**	**C**	**D**	
WBC ($x10^3/\mu L$)	38.91±5.63a	37.85±5.72a	39.05±6.17a	38.45±5.38a	17- 40
RBC ($x10^6/\mu L$)	1.95±0.38a	2.16±0.15a	1.98±0.09a	1.94±0.27a	2 - 2,3
Hb (g/dL)	9.13±0.74a	9.73±0.49a	9.92±0.35a	8.97±0.63a	8,5-10,8
PVC (%)	25.91±2.75a[2)]	27.16±1.83b	28.85±1.63b	28.72±1.08b	24,7-29,5
Monocytes (%)	7.69±3.67a	8.27±2.48a	9.35±3.52a	8.93±2.75a	-
Eosinophils (%)	0	0	0	0	-
Basophils (%)	0	0	0	0	-
Lymphocytes (%)	67.93±7.52a	68.27±8.15a	59.36±5.75	61.49±5.48a	24 - 84
Neutrophils (%)	27.48±6.35a	25.18±5.91a	27.04±7.38a	26.93±6.37a	9 - 50

Note:

1. Four levels of TLM in the feed, namely: 0%; 2%; 4%; and 6% as treatments A, B, C, and D, respectively

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2. Means with different superscripts within row values are significantly different (P<0.05)

Blood PVC/hematocrit content in group A was significantly (P<0.05) lower compared to broilers in group B, C, and D. Meanwhile, between groups B,C, and D was not significantly different (P>0.05). The number of red blood cells, white blood cells, hemoglobin levels showed no significant difference (P>0.05) between broiler Group A and broiler Group B, C, and D. The percentage of red blood cells or hematocrit can be used as a reference for abnormalities in blood such as anemia, dehydration or other conditions [27]. Meanwhile, the number of lymphocytes in broiler group C was the lowest compared to broiler groups A, B and D. An increase in lymphocyte levels in the blood was an indication of inflammation caused by viruses or bacteria. The lowest lymphocyte levels were found in the broiler Group C, because the TLM has strong antibacterial properties, so it can suppress the growth of E coli and *Coliform* bacteria.

Intestinal Histology

The mean villi heights and crypt heights from broiler intestinal histology as measured by the Image Raster program are presented in Table 4. The results showed that TLM supplementation in the feed had a significant effect (P<0.05) on the height of broiler intestinal villi, while the height of crypts showed no significant difference (P>0.05). Supplementation of 2-6% TLM in feed significantly (P<0.05) increased the height of broiler intestinal villi.

Table 4. Effect of TLM in the feed on the height of villi and crypts in broiler intestines

Level of *Tamarindus* leaf meal in feed	Intestinal Histology	
	Villi height (µm)	Cripto height (µm)
TML 0%	8106.39± 378.27a	2495.06 ± 518.39a
TML 2%	10958.74 ±504.83b	2372.81 ± 473.46a
TML 4%	11792.03 ±694.71b	2673.59 ± 501.95a
TML 6%	12036.71 ± 491.35b	2795.08 ± 603.73a

Means with different superscripts within column values are significantly different (P<0.05)

The histology of the broiler intestine stained with Hematoxylin-Eosin dye, was observed with a microscope connected to an opticlab camera and a laptop with a 50x magnification, and the measurement of villi and crypt height using the Image Raster program as shown in Figure 2.

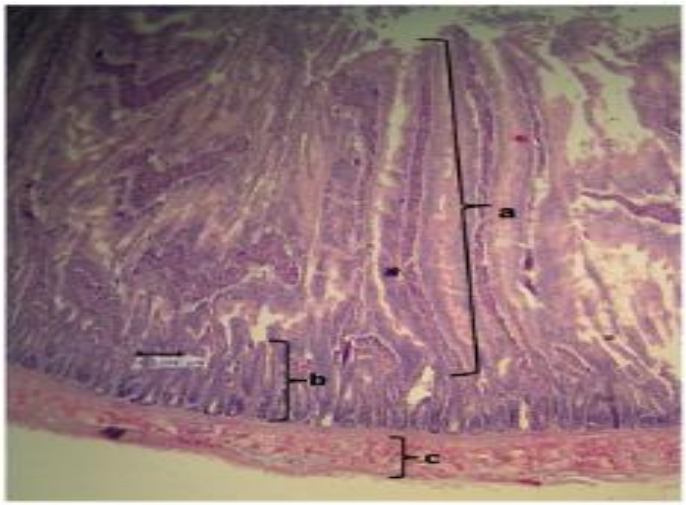

Figure 2. Histology of the broiler intestine stained with Hematoxylin-Eosin dye (observed with a microscope connected to an opticlab and laptop camera with 50x magnification, and measurement of villi and crypt height using the Image Raster program. - a = villi height, b = crypt height, c = smooth muscle)

In intestinal histology, villi and crypt are parts of the small intestine that function in the absorption of ingested food substances. The higher the villi and crypts in the small intestine, the wider the field of nutrient absorption, so that more nutrients will be absorbed.

Broiler performance

Supplementation of 2-6% TLM into the ration did not have a significant effect (P>0.05) on broiler performance (Table 5). However, there was a tendency to increase broiler performance with the increasing use of TLM in rations.

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Table 5 shows that 4-6% supplementation of TML in feed has a significant effect (P<0.05) on the blood serum lipid profile in broilers. Total cholesterol concentrations in broiler blood serum groups C and D were significantly different (P<0.05) lower than groups A and B. Likewise, the LDL content in broilers group C and D was significantly (P<0.05) lower compared with groups A and B. TML supplementation in feed was not significantly affected (P>0.05) in the triglyserides and HDL content in broiler blood serum.

Table 5. Effect of TLM supplementation in the ration on broiler performance aged 0-5 weeks

Variable	Groups			
	A	**B**	**C**	**D**
Initial body weight (g/head)	41.72±0.31a	41.19±0.25a	41.42±0.41a	41.39±0.63a
Final body weight (g/head)	2147.4±95.39a	2168.6±84.73a	2198.5±79.35a	2207.9±81.53a
Live weight gains (LWGs) (g/head/35days)	2189.1±95.14a	2127.4±83.98a	2157.1±79.14a	2166.5±80.97a
Feed consumption (g/head/35days)	3392.2±74.31a	3105.8±92.75a	3127.4±85.32a	3142.9±95.92a
FCR (feed consumption:LWGs)	1.55±0.031a	1.46±0.027a	1.45±0.039a	1.45±0.041a
Blood lifida profile				
Total cholesterol (mg/dL)	121.86 a	122.07a	113.49b	115.92b
Triglyserides (mg/dL)	145.82a	139.57a	144.63a	147.05a
HDL (mg/dL)	75.91a	78.37a	77.54a	81.65a
LDL (mg/dL)	26.72a	24.38a	13.81b	14.09b

Note:
1. Four levels of TLM in the feed, namely: 0%; 2%; 4%; and 6% as treatments A, B, C, and D, respectively
2. Means with different superscripts within row values are significantly different (P<0.05)

Provision of 2-6% TLM in the ration can reduce total cholesterol and LDL levels in broiler blood serum. The results of this study are in line with the results of research by [28] who reported that giving *Ti* fruit extract could reduce blood cholesterol levels in laying hens, even though the egg cholesterol levels did not decrease. *Ti* affects cholesterol metabolism through its effect on the release of ENO1, ApoA-I TTR, and GDI-2, as well as proteins involved in cholesterol metabolism [29]. Giving *Ti* also causes a decrease in FAS 16, which plays a role in inhibiting the formation of fatty acids.

The flavonoids contained in *TLM* act as antioxidants and are able to protect cells from free radicals that cause damage to cells [30]. According to [31], the use of natural ingredients for treatment in the long term is much safer without side effects. *Tamarindus indica* itself is known to contain xylose 3,4 compounds, which are polysaccharides and dietary fiber that can bind cholesterol in the digestive tract, thereby reducing cholesterol absorption in the intestine [32]. This study is also in line with research conducted by [30] stated that giving a crude extract of *Ti* fruit to hamsters for 10 weeks significantly reduced levels of total cholesterol, triglycerides and LDL, and increased HDL levels. This is due to the ability of *Ti* extract as an antioxidant due to the activity of the enzymes superoxide dismutase, catalase, and glutathione peroxidase. Hamsters that were fed high cholesterol and supplemented with *Ti* fruit extract for 45 days showed increased lipolytic activity [33].

CONCLUSION

It was concluded that the Tamarindus indica leaf meal in feed can not effect broiler performance, but can reduce the amount of *E coli* bacteria in the intestine and reduce blood serum cholesterol levels in broilers. Conversely, it can significantly increase the height of the intestinal villi of broilers.

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AUTHORS' CONTRIBUTIONS

NWS and IGNGB were the principal investigator of this project, and the writer of the manuscript. NWS and NWS designed the project, and were involved in specimen collection (sampling), and lab works.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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