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Effect of the addition of fermented Lamtoro leaf water extract (*Leucaena Leucocephala*) in drinking water on the performance and histology of the broiler intestine

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Abstract---*This study aims to determine the productivity of broilers fed with water extract of lamtoro leaves (Leucaena leucocephala) fermented through drinking water. This study used a Complete Random Design (RAL) consisting of 4 treatments and 5 replicates, each replicate consisted of 3 broiler chickens. The treatment provided was drinking water without fermented lamtoro leaf water extract (P0), 2% fermented lamtoro leaf water extract in drinking water (P1), 4% fermented lamtoro leaf water extract in drinking water (P2), and 6% fermented lamtoro leaf water extract in drinking water (P3). The variables observed were the performance and histology of the broiler intestine. The results of the broiler study that was given fermented lamtoro leaf water extract at doses of 2%, 4%, and 6% had a real effect (P<0.05) on the final weight, body weight gain, villi height, and small intestine crypta depth. However, there was no significant difference (P>0.05) in ration consumption, drinking water consumption, and FCR. It can be concluded that the addition of fermented lamtoro leaf water extract in broiler drinking water can improve the performance and histology of broiler small intestine.*
Keywords---*broilers, lamtoro leaves, fermentation, and productivity.*

Introduction

The need for animal protein in the community from year to year is increasing along with the increase in population so the demand for meat is increasing. The broiler is a type of poultry that produces meat to meet the needs of animal protein with a fast maintenance period and high enough meat production (Anggitasari, 2016). To support broiler productivity, Antibiotic Growth Promoter (AGP) was added, 2018 Indonesia officially banned the use of antibiotics in animal feed because of the potential for chemical residues in meat if consumed will cause disease resistance (Kompiang, 2009; Escorpizo, 2008; Hur et al., 2004). Other alternatives are needed as a substitute for AGP sourced from natural antibiotics such as the lamtoro plant.

Lamtoro plant (Leucaena leucocephala) is a type of leguminous plant that has a high protein content of 15-38% (Zayed et al., 2014) and has phytochemical content such as phenols, flavonoids, alkaloids, saponins and other phenolic compounds that can act as antibacterial and antioxidant for livestock (Simbolon, 2021). Flavonoid compounds in lamtoro leaves have benefits as antibacterial and antioxidants to inhibit the growth of pathogenic bacteria to increase the absorption of nutrients in the digestive tract (Lestariningsih et al., 2015). Lamtoro plants contain crude fiber and antinutrients, so fermentation technology is needed to make it easier for broilers to digest

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(Prabayanti et al., 2024). The fermentation process can decompose coarse fiber into simpler forms and reduce antinutrients to increase nutritional value, one of the fermenters that can be used is EM-4 because there are lactic acid bacteria that can decompose coarse fiber into simpler forms (Nggena et al., 2019; Singhania et al., 2009). The results of Siti et al. (2021), showed that the administration of a combination of herbal leaf water extracts in broilers was able to improve performance and reduce the number of pathogenic bacteria in the digestion of broilers. Based on this description, further research is needed to determine the benefits of lamtoro leaves on broiler productivity (Taslim et al., 2023; Kusumawati et al., 2021).

Materials and Methods

Livestock, drinking water, and research drafts

This research was carried out at Farm Sesetan, Faculty of Animal Husbandry, Udayana University which is located on Jalan Raya Sesetan, Gang Markisa, Denpasar, Bali for 2 months. The research design used in this study is a Complete Random Design (RAL) with four (4) treatments and five (5) replicates, each experimental unit consists of 4 broilers so that the number of chickens used is 80 DOC broilers from PT. Charoen Pokphand Tbk. The treatment provided was drinking water without fermented lamtoro leaf extract (P0), drinking water with 2% fermented lamtoro leaf extract (P1), drinking water with 4% fermented lamtoro leaf extract (P2), drinking water with 6% fermented lamtoro leaf extract (P3). Lamtoro leaf extract is made by preparing fresh lamtoro leaves that have been washed until clean, the leaves that have been washed are then shaved which is separated between the leaf bones and the leaves, after being shaved add drinking water using a ratio of 1:1 then blend until smooth, the lamtoro leaves that have been blended are then filtered using coated gauze so that the extract is separated from the residue, Mix 25ml of EM-4 solution with 25ml of molasses in a bowl and let it sit for 5 minutes to activate bacteria, after 5 minutes add the activated fermenter solution to the lamtroro leaf extract and mix until evenly distributed, the well-mixed ingredients will be fermented in a silo anaerobically for 5 days and ready to be given to the broiler according to the dosage of each treatment. Rations are given twice a day, namely in the morning and evening (Ilham et al., 2015). Drinking water is given ad libitum.

Table 1
Nutrient Content of 511 Bravo Commercial Ration

Nutrient Content (%)	Power
Moisture content	Max 13
Protein	21 - 23
Fat	Min 5
Fiber	Max 5
Ash	Max 7
Calcium	Min 0.9
Phosphorus	Min 0.6
ME (kcal/kg)	2900 - 3000

Source: Feed brochure of PT. Charoen Pokphan Indonesia, Tbk.

Table 2
Results of Analysis of Phytochemical Compounds of Fermented Lamtoro Leaf Extract

Womb	Results of the analysis
Antioxidant Capacity (mg GAE/L)	345,01
Total Phenol (mg/100g) Levels	67,70
Flavonid (mg.100g	31,95
Tannin content (mg/100g)	17,61

Source: Integrated Service Laboratory, Faculty of Agricultural Technology, Udayana University (2024).

Intestinal histology analysis

The preparation of small intestinal histology preparations was carried out using paraffin and Hematoxylin-Eosin staining. Then the small intestine is to be fixed with 10% NBF solution and then put into the tissue cause, then the dehydration process is carried out using a solution of 70%, 80%, 95% ethanol, and absolute alcohol twice

transferred. The clearing process with the xylol solution is transferred three times, each for 60 minutes at room temperature. Paraffin infiltration by inserting tissue into liquid paraffin at 60ºC with three transfers for 45 minutes each. Next, the tissue is immersed in a mold containing liquid paraffin and cooled at room temperature until it becomes a block of paraffin. Next, the paraffin block is cut 5µm thick using a rotary microtome, the incision is placed on the surface of warm water with temperatures of 45ºC and 47ºC. Then it is attached to the glass of the object that has been coated with gelatin. Prepare the prepared equipment that has been drained and then placed vertically to be placed on the slide warmer until it sticks to the object glass. Pieces of tissue in paraffin to be stained with Hematoxilin Eosin are stored in racks for staining, and incubated at 60º C for 45 minutes. After that, it is placed at room temperature until cool.

Deparaffin is dissolved in xylol 3 times, then followed by a rehydration process in 100%, 95%, and 80%, 70% alcohol in each stage for 5 minutes. Then put in an aquaade for 10 dips or until the alcohol dissolves. The process of staining with hematoxilin by soaking the slide with hematoxylin solution for 5 minutes then washing it in running water for 5 minutes, and staining using eosin for 3 minutes. After being stained in eosin, the slides are inserted in a graded alcohol solution of 70%, 80%, 90%, to 100% each for 10 dips. Then the clearing process uses xylol twice for 2 minutes, after which the preparation is covered with glass covered with Canadian balsam media. The preparation is ready to be observed under a 10 x 10 or 10 x 40 eyepiece-objective magnification microscope. Observations were carried out in 5 fields of view and repeated 3 times. For intestinal histology observed were villi height and crypta height which were measured and processed with the Image Raster program.

Results and Discussion

Performance and histological broiler intestine

The results of the 4-week broiler study with the addition of fermented lamtoro leaf water extract through drinking water on the final body weight, ration consumption, drinking water consumption, body weight gain, FeeD Conversion Ratio (FCR), villi height, and crypta depth can be seen in tables 3 and 4. The administration of fermented lamtoro leaf water extract through drinking water was statistically significantly different ($P<0.05$) on final body weight, body weight gain, villi height, and crypta depth compared to the control. Meanwhile, ration consumption, drinking water consumption, and FCR were not significantly different ($P>0.05$).

The addition of fermented lamtoro leaf extract through drinking water was able to increase the body weight of the final broiler at 4 weeks of age and statistically showed significantly different results ($P<0.05$). The increased final weight was caused by the increase in the consumption of broiler rations during the study The highest final body weight of 1,567g was given fermented lamtoro leaf extract at a dose of 6%, this was due to phytochemical compounds that act as antioxidants and saponins as antimicrobials can maintain the body's immunity so that it is not susceptible to diseases and maintain the condition of broilers against oxidative stress (Astuti & Irawati, 2022). Antibacterial can suppress the growth of pathogenic bacteria in the intestines so that the feed consumed will be used more efficiently and increase the absorption of nutrients into the body through intestinal villi which is characterized by an increase in body weight. The increase in body weight will be accompanied by an increase in the carcass component, the higher the body weight, the higher the carcass produced (Nuriyasa et al., 2021). This is in line with the opinion of Hardiawan et al. (2021), that the addition of probiotics to drinking water can increase the final body weight of the broiler.

The addition of fermented lamtoro leaf extract through drinking water was able to reduce the consumption of broiler rations at 4 weeks of age and statistically showed no significant difference in results ($P>0.05$). This is caused by the rapid metabolic rate that occurs in the body as a result of increased digestive activity. Leaf extracts fermented using EM-4 solution can also increase the nutrient content contained in lamtoro leaves, one of which is the protein content which is good for supporting broiler growth (Putra et al., 2021), so that fewer rations are needed for production and maintenance needs. Decreased ration consumption can also be affected by livestock physiology and environmental conditions. Fattah et al. (2023), stated that broilers are homeothermic livestock that will maintain their body temperature relatively constant by reducing ration consumption and increasing drinking water consumption to reduce heat accumulation in the body (Mehdi et al., 2018; Landoni & Albarellos, 2015). The results of research by Prabadewi & Nuryanto (2015), stated that the administration of fermented herbal extracts through drinking water reduced the consumption of broiler rations.

The addition of fermented lamtoro leaf extract through drinking water was able to increase the drinking water consumption of 4-week-old broilers and statistically showed no significant difference in results ($P>0.05$). This is because lamtoro leaves fermented using *EM-4 solution* have a sweet and sour smell so that it will affect the taste of

broiler drinking water. Aprisa et al. (2024), said that the EM-4 solution contains *Lactobacillus bulgaricus* bacteria which plays a role in the formation of aroma while *Streptococcus thermophilus* plays a role in the formation of acidity levels and taste of fermented products. An increase in drinking water consumption can also occur due to the high temperature of the cage environment which causes broilers to drink more than ration consumption to maintain body temperature balance so that heat stress does not occur which can trigger oxidative stress (Fattah et al., 2023). The results of this study have an average drinking water consumption of 175ml/e/h lower than the results of the Astuti and Irawati study, 2022 of 190ml/e/h.

Table 3
Effect of Fermented Lamtoro Leaf Extract through Drinking Water on Broiler Performance

Variable	Treatment1)				SEM2)
	P0	P1	P2	P3	
Final weight (g)	1454a	1539 [b]	1553 [b]	1563b	12,34
Feed consumption (g/28 days)	Unrated 1993a	2420A	2378a	2340A	23,63
Drinking water consumption liters/28 days)	4858 [A]	4864 [A]	4958 [A]	4974 [A]	31,05
Body weight gain (g)	1402 [A]	1487 [b]	1501 [b]	1512 [b]	12,09
FCR	1.78 [A]	1.63 [A]	1.59 [A]	1.55 [A]	0,27

Information:

1. Drinking water treatment is:
   P0: Broiler is given drinking water without fermented lamtoro leaf extract
   P1: Broiler is fed drinking water with 2% fermented lamtoro leaf extract
   P2: Broiler is fed drinking water with 4% fermented lamtoro leaf extract
   P3: Broiler is fed drinking water with 6% fermented lamtoro leaf extract
2. SEM: *Standard Error of Treatment Means*
3. Values with the same letter on the line that show a real difference ($P<0.05$)

The body weight gain of 4-week-old broilers who were treated with fermented lamtoro leaf extract showed a significant difference in results ($P<0.05$) with the administration of a dose of 6% fermented extract through drinking water had the highest average value. Increased body weight gain is accompanied by a high final body weight produced. Yuniharni et al. (2021), stated that flavonoid and tannin compounds have antioxidant and antibacterial properties that can prevent oxidative stress and reduce the occurrence of infections in broilers. Healthy livestock will have a more optimal growth rate (Junaedi & Amin, 2024). In addition, the higher the dose of fermented lamtoro leaf extract given, the higher the digestibility of broiler feed caused by increased nutritional value and low crude fiber content. In line with the opinion of Lie et al. (2024), argues that in *EM4 solution* there are cellulolytic bacteria that can convert coarse fiber into an energy source, while lactic acid bacteria can lower antinutrients and increase the value of nutrients absorbed into the body.

The *Feed Conversion Ratio* (FCR) value of 4-week-old broilers who were treated with fermented lamtoro leaf extract through drinking water showed no significant difference in results ($P>0.05$) however, had a lower FCR value than the control treatment. The decrease in FCR value is due to the more efficient use of rations for growth, according to Amir et al. (2022), the lower the FCR value of broiler, the more efficient the use of rations to produce meat. The administration of fermented lamtoro leaf extract at a dose of 6% produced the lowest FCR value, showing that it has the best feed efficiency for livestock because feed consumption in the P3 treatment has the lowest average value but has the highest final body weight. According to Fanani et al. (2023), probiotic bacteria such as lactic acid bacteria in the fermentation process will change complex compounds into simpler ones with the help of the enzymes produced so that they will be easier to digest. Lactic acid bacteria produce lactic acid which can lower pH, this acidic condition will weaken the growth of pathogenic bacteria in the broiler intestines and increase beneficial microorganisms so that the nutrients absorbed are more efficient for growth (Kumalasari et al., 2020). The results of this study have an FCR value of 1.7 – 1.56 in line with the results of Mohamad et al. (2023), with the administration of fermented herbal medicine has an FCR value of 1.7 – 1.6.

Table 4
Effect of administration of fermented lamtoro leaf extract through drinking water on broiler small intestine histology

Variable	Treatment1)				SEM2)
	P0	P1	P2	P3	
Vili Height (µm)	516.7a	608.1b	612.8b	623.5b	102
Depth of Crypt (µm)	89.4a	186.6b	141.1b	90.5a	928

Information:
1) Drinking water treatment is:
   P0: Broiler is given drinking water without fermented lamtoro leaf extract
   P1: Broiler is fed drinking water with 2% fermented lamtoro leaf extract
   P2: Broiler is fed drinking water with 4% fermented lamtoro leaf extract
   P3: Broiler is fed drinking water with 6% fermented lamtoro leaf extract
2) SEM: *Standard Error of Treatment Means*
3) Values with the same letter on the line that show a real difference (P<0.05)

The addition of fermented lamtoro leaf extract through drinking water was able to improve the small intestinal histology of 4-week-old broilers and statistically showed no significant difference in results (P>0.05). The histological small intestine of 4-week-old broilers fed fermented lamtoro leaf extract showed significantly different results (P<0.05). Intestinal histology consists of increased villi height and crypta depth compared to no treatment. The increased height of intestinal villi is caused by fermented probiotic bacteria in lamtoro leaf extracts such as *lactobasillus.sp* which maintains the health of the broiler small intestine (Abdel-Rahman et al., 2013; Di Cagno et al., 2013). Hartono et al. (2016), said that probiotic bacteria that help in the fermentation of fiber that will produce short-chain fatty acids include acetate, propionate, and butyrate. Short-chain fatty acids will stimulate the number of intestinal epithelial cells because *SCFAc* is a phospholipid component in the epithelial membrane (Harimurti et al., 2009; Sahraei, 2012).

The increased height of the intestinal villi is accompanied by an increase in the depth of the crypt where the regeneration of intestinal epithelial cells occurs to multiply new epithelial cells (Yatalaththov et al., 2021). The increased depth of the crypt indicates that the more epithelial cells are produced, the higher the intestinal villi will be and the health of the intestines will be maintained. Satimah et al. (2019) that taller intestinal villi will expand the absorption surface of nutrients, taller villi can absorb nutrients faster than shorter intestinal villi. Following Hartono et al. (2016), probiotic bacteria can make the nutrient absorption surface wider because it affects the development of intestinal villi so that the nutrients absorbed are more optimal. The results of this study have an average height of 517-623µm higher than the results of Nurliana et al. (2022), which provides fermentation of soybean pulp and palm kernel meal has a broiler villi height of 363-606µm, and the depth of crypta in this study is 89-189µm higher than the results of Harimurti et al. (2009), which gave probiotics to a single strain of bacteria had a klipta depth result of 92-130µm.

Conclusion

Based on the results of the study obtained, it can be concluded that the administration of fermented lamtoro leaf water extract through drinking water up to a dose of 6% can improve small intestinal histology, increase final body weight, and increase body weight at 4 weeks of broiler age.

Suggestion

Based on the results of the research obtained, it can be suggested to the community, especially broiler farmers, to increase broiler productivity by utilizing alternative natural ingredients such as the provision of 6% fermented lamtoro leaf water extract through drinking water. The results of this study can also be suggested for further research on the use of fermented lamtoro leaf water extract in other types of livestock. It can also be suggested to students as a reference in writing the final project.

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