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Clitoria ternatea Extract as Natural Antibiotic Growth Promoter (AGP) on Broiler Concerning Carcass Quality and Intestine Morphometric

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ABSTRACT

Background: This study aims to ascertain the optimal dose of Clitoria ternatea extract in relation to the quality of broiler carcasses and intestinal morphometrics.

Methods: The Clitoria ternatea extract was applied in this study in different doses, where control, 4 ml, 6 ml, 8 ml, and 10 ml per litre of drinking water at weekly intervals in broilers to know the effect on carcass quality and intestine morphometrics. Statistical Tool for Agricultural Research (STAR) Software/STAR was used to analyze the data Using the corrplot function of the corrplot package from RStudio 4.2.2.

Result: The results of variance analysis showed that the Clitoria ternatea extract treatment had a very significant effect (P<0.01) on Thigh Meat Cholesterol (TMC), Breast Meat Fat (BMF), low-density lipoprotein (LDL) of carcass broiler, and intestinal weight. In correlation, HDL Blood is positively correlated with intestinal weight (r=0.59) and blood cholesterol with LDL blood (r=0.77). The other parameters are positively correlated, such as abdominal fat is positively correlated with blood cholesterol, breast meat fat, and thigh meat cholesterol, where thigh meat cholesterol is positively correlated with breast meat cholesterol, breast meat fat is positively correlated with blood cholesterol and LDL blood, and these two parameters are positively correlated also with breast meat cholesterol and thigh meat fat. It can be concluded that *Clitoria ternatea extract*, as a natural antibiotic growth promoter for broilers, can improve the quality of broiler carcasses but does not have a substantial effect or correlation with intestine morphometrics.

Key words: Broiler, Carcass, *Clitoria ternatea,* Herbal, Natural AGP.

INTRODUCTION

Poultry feed plays a pivotal role in the efficiency and profitability of poultry production (Amrah *et al.,* 2024; Mujnisa *et al.,* 2019). In recent years, the use of antibiotic products as feed treatment in poultry production has been increasingly restricted across many countries due to growing concerns over antimicrobial resistance (AMR) and public health risks (Abreu *et al.,* 2023; Tiwari *et al*., 2022). The antibiotic ban in poultry production was not primarily implemented due to the risk of consumers accidentally consuming antibiotics. Instead, the ban on antibiotic growth promoters in poultry feed was primarily driven by concerns over antibiotic resistance, which poses a significant threat to poultry health and overall food safety. Antibiotic resistance can develop in bacteria present in poultry due to the routine use of antibiotics, potentially leading to treatment challenges in animals and humans (Chakrabarti *et al.,* 2020) Therefore, the restriction on antibiotic use aims to address this critical issue rather than consumer exposure to antibiotics (Roth *et al.,* 2019).

Currently, the use of locally sourced raw materials and herbs is becoming increasingly popular in livestock (Hasan *et al.,* 2020; 2024) especially in poutry feed. In fact, some herbs are also recognized for their antibiotic-like properties (Amrah *et al.,* 2024). Herbs are a beneficial strategy to reduce the use of antibiotics while maintaining poultry production (Mehdi *et al.,* 2018). Studies have demonstrated that herbal medical medications and plant bioactive substances can substitute antibiotic growth promoters in poultry feed (Rafiq *et al.,* 2022). One of the plants that can be used to reduce the use of antibiotics is *Clitoria ternatea* extract has antibacterial properties with bioactive substances such as flavonoids, alkaloids, tannins, saponins and a num he immune system (Neeraj *et al.,* 2023). According to Phillips *et al.* (2023), several natural active compounds have the potential to be added as additives to enhance immunity and improve blood profiles in poultry.

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Clitoria ternatea, commonly referred to as butterfly pea, has attracted significant interest as a potential antibiotic growth promoter (AGP) in broiler chickens, particularly due to its positive effects on carcass quality and intestinal morphometrics. The plant's extract is abundant in bioactive compounds such as flavonoids, saponins and phenolic compounds, which demonstrate antimicrobial properties, thereby contributing to improved gut health and overall growth performance in poultry (Jeyaraj *et al.,* 2020).

Fluctuations in antioxidant levels resulting from reduced feed intake directly impact intestine function and eventually adversely affect broiler carcasses' general health and quality (Azzam *et al.,* 2023). Research related to the effect of *Clitoria ternatea* extract on broilers has not been widely developed. The bioactive chemicals found in *Clitoria ternatea* extract can potentially decrease oxidative stress and enhance mucosal growth in broilers, hence impacting carcass quality. *Clitoria ternatea* extract is thought to be potential natural AGP. Hence, this study aims to determine the most effective dosage of *Clitoria ternatea* extract in terms of broiler carcass quality and intestinal morphometrics.

MATERIALS AND METHODS

The study was conducted from March to November 2022. The broiler-rearing activities were conducted at the Poultry Production Laboratory, Faculty of Animal Husbandry at Hasanuddin University. The small intestine histology and meat carcass preparations were conducted at the Pathology Laboratory of the Maros Veterinary Center (BB-Vet). The measurement of small intestine histology at Maros Pet Care. The extraction process of *Clitoria ternatea* was performed at the Biopharmaca Laboratory, Faculty of Pharmacy, Hasanuddin University.

Extraction of *clitoria ternatea*

The *Clitoria ternatea* flowers were washed and dried using an oven at 50°C for 24 hours (moisture content<10%), then pulverized using a blender. The sifted powder was then weighed. A total of 100 g of sympilia powder was added with 70% ethanol solvent. The ratio of material to solvent was 1:10 (b/v), then placed in a closed container for 24 hours at room temperature (28°C) with occasional stirring and filtered with a Whatman filter (No.1). The filtrate was concentrated in a vacuum rotary evaporator at 30°C to obtain a thick extract. The extraction was arranged into some treatments, *i.e*., T0 = drinking water without *Clitoria ternatea* extract (control); T1= drinking water + 4 ml of *Clitoria ternatea* extract; T2 = drinking water + 6 ml of *Clitoria ternatea* extract; T3 = drinking water + 8 ml of *Clitoria ternatea* extract; and T4 = drinking water + 10 ml of *Clitoria ternatea* extract.

In vivo treatment

This study utilized 80-day-old chicks (DOC) of the Lohman strain, with unsexed broilers, raised for 40 days. This study comprised a total of 20 experimental units. Each trial group included of 4 juvenile broilers. The rearing period was divided into two stages: The starter phase, 1 to 14 days and the finisher phase, 14 to 40 days. Additionally, a lengthy brooding phase lasted from 1 to 14 days. The 80 broilers were placed directly into iron cages, specifically postal cages (litter system), comprising 20 plots of 120×100×80 cm. Each plot consisted of 4 broilers and used incandescent lamps (40 watts) as heaters instead of brooders. Every plot was equipped with containers for both feed and water. Feeding was conducted ad libitum on a daily basis. The drinking water initially contained sugar (*ad-libitum*). The drinking water was supplemented with *Clitoria ternatea* extract. The different concentrations of *Clitoria ternatea* extract were added to 1 litre of drinking water. The treatment was provided at a frequency of once in every week, specifically between the second and fourth weeks of the maintenance period.

Sampling preparation

The sampling procedure involved selecting one male broiler from each experimental unit. Each treatment involved the slaughter of a total of 20 broilers. Sample preparation was performed on the 40th day of rearing, at the end of the finisher period. The carcass quality was analyzed based on the Liebermann and Burchard method (Naeemi *et al.,* 1995). Broiler breast meat and thigh meat fat meat samples were analyzed based on the Soxhlet Extraction method (AOAC, 1984). For blood cholesterol, HDL and LDL, samples were measured at the end of the observation period (week 5). Blood samples were taken through the vein (Vena brachialis) as much as three cc by disposable syringe, subsequently inserted into an anti-coagulant tube and analyzed by the CHODP-PAP KIT procedure, Boehringer Mannhaem EMBH Diagnostics., 1987 (Raitto, 1983). The abdominal fat percentage was calculated according to abdominal weight and live weight (Mihardja, 1981). The small intestine is first cleaned inside using distilled water for intestine morphometrics parameters. Fresh intestinal tissue for histological preparations (Gava *et al.,* 2015).

Observed parameter

The observed parameters in this study are broiler carcass quality and intestine morphometrics. The broiler carcass quality included blood cholesterol (mg/100 ml), breast meat cholesterol (mg/100 g), thigh meat cholesterol (mg/100 g), blood HDL (mg/100 ml), blood LDL (mg/100 ml), thigh meat fat (%), breast meat fat (%), abdominal fat (%) the intestine morphometrics included length (cm) and weight of small intestine (g).

Data analysis

Data was analyzed by Statistical Tool for Agricultural Research (STAR) Software/ STAR Vers 2.0.1 for analysis of variance and least significant difference (LSD) test. Pearson correlations between carcass quality parameters and intestine morphometrics were analyzed using the corrplot function of the corrplot package by RStudio 4.2.2.

RESULTS AND DISCUSSION

Caracas quality in broilers

The cholesterol and fat content post-treatment are presented in Table 1.

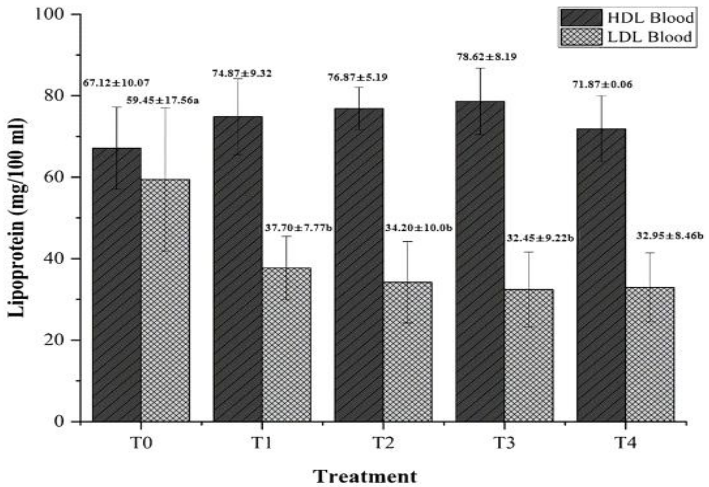
The results of variance analysis showed that the *Clitoria ternatea* extract treatment had a very significant effect (P<0.01) on Thigh Meat Cholesterol (TMC) and Breast Meat Fat (BMF) of carcass broiler but had no significant effect on Blood Cholesterol (BC), Breast Meat Cholesterol (BMC), Abdominal Fat (AF) and Thigh Meat Fat (TMF) of carcass broiler. The treatment on TMC shows a significant difference between Control and T1 and T4. The average of the TMC is from 5.67 mg/100 ml to 7.11 mg/100 ml. As we can see, the lowest and the highest TMC values appeared in the T4 and control treatments, respectively. The trend of the lowest and highest values appearing in T4 and control treatment is also confirmed in BC and TMF. The treatment on BMF also shows significant differences between T1 and T3 or T4. The average of the BMF is 1.40 % to 2.28 %. As we can see, the lowest and the highest TMC values appeared in T4 and T1, respectively. The trend of the lowest and highest values appearing in T4 and T1 is also confirmed in BMC.

Meanwhile, the lowest and the highest AF values appeared in T4 and T3, respectively. These findings

Table 1: The cholesterol and fat content post-treatment.

Treatment	Cholesterol (mg/100 g)			Fat (%)		
	BC	BMC	TMC	AF	BMF	TMF
T0	137.80±7.72	5.80±0.54	7.11±0.72a	3.76±0.58	2.22±0.32ab	2.84±0.98
T1	125.05±16.74	5.83±0.89	6.83±0.64a	3.32±0.50	2.28±0.37a	2.74±0.67
T2	121.30±9.83	5.06±0.48	6.47±0.37ab	3.22±0.45	1.72±0.39bc	2.40±1.30
T3	123.05±12.34	5.06±0.40	6.35±0.44ab	3.83±0.50	1.65±0.34c	2.17±0.35
T4	115.30±11.91	4.72±0.39	5.67±0.41b	2.89±0.80	1.40±0.20c	1.66±0.40
Significance	ns	ns	**	ns	**	ns

Description: Means ± Deviation Standard; Means with a similar superscript letter in the same column are not significantly different. Least Significant Difference (LSD) Test level of significance= 1%.*significant at p(α) 0.01; ns non significant; Blood cholesterol (BC); Breast meat cholesterol (BMC); Thigh meat cholesterol (TMC); Abdominal fat (AF); Breast meat fat (BMF); Thigh meat fat (TMF); T0= Drinking water without *Clitoria ternatea* extract (control); T1= Drinking water + 4 ml of *Clitoria ternatea* extract; T2= Drinking water + 6 ml of *Clitoria ternatea* extract; T3= Drinking water + 8 ml of Clitoria ternatea extract and T4= Drinking water + 10 ml of *Clitoria ternatea* extract.



Means ± Deviation Standard; Means with a similar superscript letter in the same column are not significantly different. Least Significant Difference (LSD) Test level of significance = 1%.*significant at p(α) 0.01; T0 = Drinking water without Clitoria ternatea extract (control); T1 = Drinking water + 4 ml of Clitoria ternatea extract; T2 = Drinking water + 6 ml of Clitoria ternatea extract; T3 = Drinking water + 8 ml of Clitoria ternatea extract; and T4 = Drinking water + 10 ml of Clitoria ternatea extract.

Fig 1: Lipoprotein in broiler's carcass post treatment.

demonstrate that supplying *Clitoria ternatea* extract treatment reduces cholesterol and fat content in broiler carcasses. The higher the level of *Clitoria ternatea* extract in the drinking water given during broiler rearing, the lower the cholesterol and fat content in broiler carcasses. The lipoproteins, including HDL and LDL, related to carcass quality post of *Clitoria ternatea* extract treatment is presented in Fig 1.

The results of variance analysis showed that the *Clitoria ternatea* extract treatment had a very significant effect ($P<0.01$) on the low-density lipoprotein (LDL) of the broiler but had no significant effect on the high-density lipoprotein (HDL) of the broiler. The treatment of LDL on control treatment shows significant differences from the treatment with *Clitoria ternatea* extract. The average LDL is from 32.95 mg/100 ml to 59.45 mg/100 ml. As we can see, the lowest and the highest LDL values appeared in the T3 treatment and the control treatment. The average HDL is from 67.12 mg/100 ml to 78.62 mg/100 ml, where the lowest and the highest HDL appeared in the control treatment and T3 treatment, respectively.

Particularly at specific levels have been shown to improve broiler growth performance and feed conversion ratio (Hassan *et al.,* 2023). Specifically, the cholesterol levels in broiler breast meat were found to be 47.11 mg/100 g (Ponte *et al.,* 2008), 66.79 mg/100 g, 45.96 mg/100 g (Oliveira e Vieira *et al.,* 2007) and 93.6 mg/100 g (Salma *et al.,* 2007). Breast meat from conventional broilers showed higher lipid (1.47%) and cholesterol (34.13 mg/100 g) concentrations. Moreover, thigh meat from free-range broilers had higher lipid (7.53%) and cholesterol (45.55 mg/100 g) concentrations (Giampietro-Ganeco *et al.,* 2020). Vlaicu *et al.* (2021) suggested that the herbal extracts in feed significantly reduced cholesterol concentration in breast meat.

Sapsuha *et al.* (2023) stated that *Clitoria ternatea* extract concentrations of 0.5 ml, 1 ml, 1.5 ml and 2 ml/kg feed showed significant effects on body weight gain, consumption and feed conversion compared to untreated *Clitoria ternatea* extract in feed. However, there was no difference in the broiler's blood profile, internal organ weight, or carcass percentage. There are still limited reports about the impact of *Clitoria ternatea* extract in broiler drinking water on high-density lipoprotein (HDL) and low-density lipoprotein (LDL) levels. Nevertheless, certain herbal extracts in drinking water showed HDL and LDL lipoprotein levels in the broiler (Bhortakur *et al.,* 2025). According to this study, the herbal drink may have raised HDL levels by boosting Apolipoprotein A1 levels, as a cofactor of lecithin cholesterol acyltransferase and as a lipoprotein receptor ligand by inhibiting LDL oxidation in the body (Thompson *et al.,* 2022).

HDL and LDL levels in broilers can be influenced by dietary factors that can reduce fat and cholesterol levels in broilers, which may contribute to an improved lipid profile (Bogusławska-Tryk *et al.,* 2016). Rahmah *et al.* (2016) found that Herbal with additional turmeric, up to a concentration of 1.5%, did not show any impact. The small intestine is essential for the development of broilers as it is the primary site where the absorption of food substances occurs with the help of digestive enzymes (Svihus and Itani, 2019). Pajarillo *et al.* (2021) stated that the small intestine is an organ with several enzymes that facilitate the absorption process and accelerate and streamline the breakdown of carbohydrates, proteins, or fats. Supplementation with *Clitoria ternatea* extract can enhance the relaxation of the small intestine, reducing peristaltic movement. As a result, the digesta remains in the small intestine longer, allowing for more efficient absorption of dietary ingredients (Sjofjan *et al.,* 2020).

Histomorphometry of the small intestine in broilers

Histomorphometry of the small intestine, such as intestinal length (cm) and intestinal weight (g) in broilers, is presented in Fig 2.

The results of variance analysis showed that the *Clitoria ternatea* extract treatment had a significant effect ($p<0.05$) on the intestinal weight of the broiler but had no significant effect on the intestinal length of the broiler. The treatment of intestinal weight in the control treatment shows significant differences in the treatment with *Clitoria ternatea* extract. The average internal length is from 33.09 cm to 33.42 cm. As we can see, the lowest and the highest intestinal length values appeared in the control treatment and T4 treatment, respectively. The average intestinal weight is from 32.51 grams to 33.44 grams, where the lowest and the highest intestinal length values appeared in the control treatment and T2 treatment, respectively. In this investigation, the *Clitoria ternatea* extract at a concentration of up to 1% did not impact the length and weight of the small intestine in broilers.

The histomorphometry of the small intestine in broilers, specifically about its length and weight, is affected by the type of feed. Related to small intestine morphometric, there is a positive correlation between small intestine morphometric and body weight. Body weight and small intestine morphometrics also change (Biesek *et al.,* 2022). This indicates that the small intestine also has a significant impact on determining body weight.

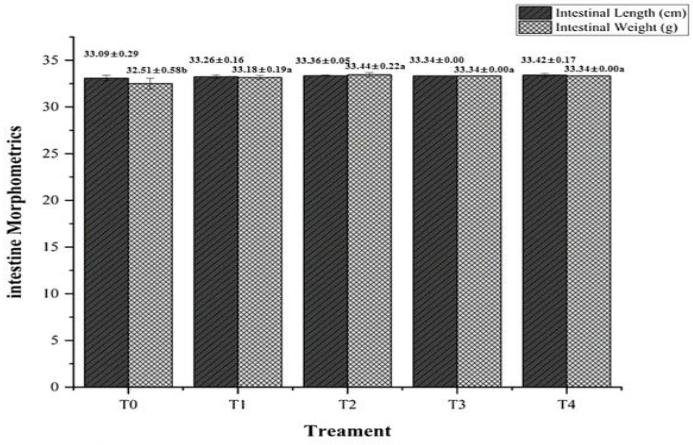
Correlation of each parameter

The Pearson correlation matrix of broiler carcass quality parameters and intestinal morphometrics is presented in Fig 3.

Based on Fig 3, some parameters show a positive correlation ($0<r\leq1$), where HDL Blood is positively correlated with intestinal weight (r=0.59), as well as blood cholesterol with LDL blood (r=0.77). The other parameters are positively correlated, such as abdominal fat is positively correlated with blood cholesterol, breast meat fat and thigh meat cholesterol, where thigh meat cholesterol is positively correlated with breast meat cholesterol, breast meat fat is positively correlated with blood cholesterol and LDL blood and these two parameters are positively correlated also with breast meat

cholesterol and thigh meat fat. However, some of these parameters only show a weakly positive correlation with r value of 0.27-0.49. Some parameters are negatively correlated ($0 < r \leq -1$), such as intestinal length, intestinal weight and HDL blood, with the parameters of blood cholesterol, breast meat cholesterol, thigh meat cholesterol, abdominal fat, breast meat fat and thigh meat fat.

Most previous studies have shown positive effects on body fat accumulation in poultry (Wen *et al.,* 2019). Other studies have also demonstrated the effect of fatty acid profile of dietary fat supplements on the body fat composition of poultry (Skøivan *et al.,* 2018). Hassan *et al.* (2007), in their research on broilers, showed that total blood cholesterol was positively correlated with high-density lipoprotein (HDL) and low-density lipoprotein (LDL). In addition, another study reported a direct correlation between abdominal fat deposits and LDL cholesterol levels found in broiler chickens (Navidshad *et al.,* 2010). The feed can also influence the presence of correlation between parameters.



Means ± Deviation Standard; Means with a similar superscript letter in the same column are not significantly different. Least Significant Difference (LSD) Test level of significance = 5%.*significant at p(α) 0.05; T0 = Drinking water without Clitoria ternatea extract (control); T1 = Drinking water + 4 ml of Clitoria ternatea extract; T2 = Drinking water + 6 ml of Clitoria ternatea extract; T3 = Drinking water + 8 ml of Clitoria ternatea extract; and T4 = Drinking water + 10 ml of Clitoria ternatea extract.

Fig 2: Histomorphometry of the small intestine in boilers.

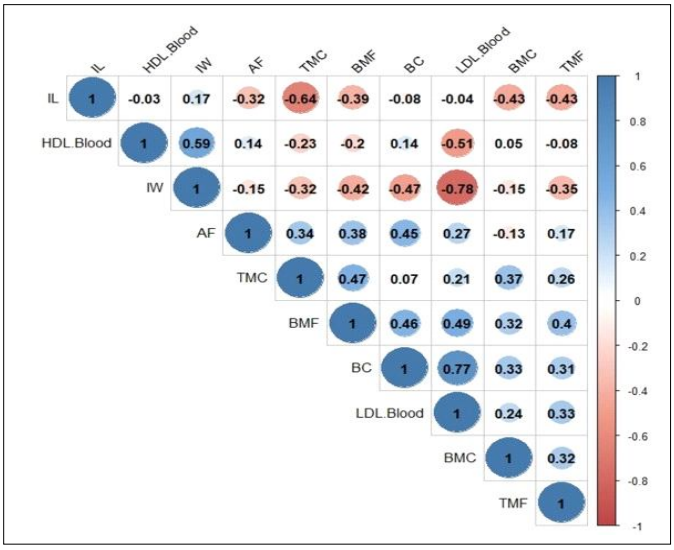


Fig 3: Pearson correlation matrix of broiler carcass quality parameters and intestinal morphometric.

CONCLUSION

Applying herbals as natural antibiotic growth promoter (AGP) is an efficient way to decrease the need for antibiotics for poultry. *Clitoria ternatea* extract as a broiler AGP can improve broiler carcass quality, but has no substantial influence or correlation with intestinal morphometrics. Further research is required to determine the effectiveness of the optimal dose of *Clitoria ternatea* extract in AGP on carcass-quality intestinal histomorphometrics.

Conflict of interest

All authors declared that there is no conflict of interest.

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