

Stingless bee honey adding butterfly pea flower kombucha on broiler carcass and cholesterol content

Penambahan madu kelulut dalam kombucha bunga telang terhadap karkas dan kadar kolesterol broiler

Salvia, Ali Syahrul Hasibuan*, and Nelzi Fatiy

Department of Animal Husbandry and Animal Health, Livestock Production Technology Study Program, Politeknik Pertanian Negeri Payakumbuh, Jl. Raya Negara Km 7, Tanjung Pati, Lima Puluh Kota, West Sumatera, Indonesia, 26271

*Corresponding author: alisyahrul488@gmail.com

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ABSTRACT

This study evaluated the effect of incorporating stingless bee honey (*Trigona spp.*) into butterfly pea flower (*Clitoria ternatea L.*) kombucha in drinking water on cholesterol levels, abdominal fat, and carcass yield in broilers. A completely randomized design with five treatments (T0: control; T1-T4: kombucha with 2%, 3%, 4%, and 5% stingless bee honey) and four replicates were used. Treatments were administered through drinking water for two weeks. Results showed that honey-enriched kombucha significantly reduced cholesterol levels, with the best result observed in T4 (95 ± 4.63 mg/100 g). Abdominal fat decreased and carcass percentage increased, although not statistically significant. These findings suggest that stingless bee honey-enriched kombucha has potential as a natural feed additive to produce healthier broiler meat, likely mediated through enhanced antioxidant activity and inhibition of HMG-CoA reductase.

ABSTRAK

Penelitian ini bertujuan mengevaluasi pengaruh penambahan madu kelulut (*Trigona spp.*) ke dalam kombucha bunga telang (*Clitoria ternatea L.*) yang diberikan melalui air minum terhadap kadar kolesterol, lemak abdomen, dan persentase karkas pada ayam broiler. Penelitian menggunakan rancangan acak lengkap dengan lima perlakuan (T0: kontrol; T1-T4: kombucha dengan 2%, 3%, 4%, dan 5% madu kelulut) dan empat ulangan. Perlakuan diberikan melalui air minum selama dua minggu. Hasil menunjukkan kombucha dengan madu kelulut secara signifikan menurunkan kadar kolesterol, dengan hasil terbaik pada T4 ($95 \pm 4,63$ mg/100 g). Lemak abdomen menurun dan persentase karkas meningkat, meskipun tidak signifikan secara statistik. Temuan ini menunjukkan bahwa kombucha madu kelulut berpotensi menjadi aditif pakan alami untuk menghasilkan daging broiler lebih sehat, kemungkinan melalui peningkatan aktivitas antioksidan dan penghambatan HMG-CoA reduktase.

Kata kunci:

Bunga telang

Broiler

Kolesterol

Kombucha

Madu kelulut

INTRODUCTION

Honey is one of the natural products produced by bees through a complex process of converting floral nectar into a nutrient-rich sweet solution. The primary constituents of honey include simple sugars (glucose and fructose), vitamins, minerals, enzymes, free amino acids, and volatile organic compounds that contribute

to its biological activity (Ajibola et al., 2008). The physicochemical properties of honey are influenced by several factors, such as the type of nectar, bee species, geographical conditions (climate and soil), and post-harvest handling practices. Stingless bee honey is a type of honey produced by stingless bees of the genus *Trigona spp.*, which are known for their distinctive sour-sweet taste



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and low pH (3-4). Stingless bee honey contains high levels of flavonoids, phenolics, amino acids, and hydrogen peroxide, which function as natural antibacterial, antioxidant, and anti-inflammatory agents (Erwan et al., 2022; Keng et al., 2017).

With the increasing interest in functional products, kombucha has become one of the traditional beverages experiencing significant innovation. Kombucha is produced from a sweetened tea solution fermented with a symbiotic culture of bacteria and yeast, known as SCOBY (Symbiotic Culture of Bacteria and Yeast). Kombucha is a fermented tea beverage rich in bioactive compounds, known for its strong antioxidant and antimicrobial activities, and has promising potential as a natural functional feed additive to reduce cholesterol and enhance carcass quality in broilers (Selvaraj et al., 2023). In its development, kombucha substrates are not limited to black or green tea but are also combined with various local herbal plants rich in bioactive compounds.

Butterfly pea flower (*Clitoria ternatea* L.) represents a promising herbal ingredient with significant potential for formulation in kombucha beverages. This tropical plant is widely distributed throughout Indonesia and has been extensively utilized in traditional medicine for various therapeutic applications. Butterfly pea flowers contain essential bioactive compounds, including flavonoids, anthocyanins, saponins, and alkaloids, which have been demonstrated to possess anti-inflammatory, hepatoprotective, and anti-hypercholesterolemic properties (Maneesai et al., 2021). The fermentation process can convert these bioactive compounds into secondary metabolites with enhanced bioavailability and improved biological activity, increasing their potential effects on lipid metabolism.

The addition of stingless bee honey (*Trigona spp.*) to kombucha is believed to serve not only as a carbon substrate source but also to enrich the content of secondary metabolites such as organic acids, flavonoids, and enzymes that play a role in reducing cholesterol levels through inhibition of the HMG-CoA reductase enzyme (Alkhalifah et al., 2021; Esa et al., 2022).

Broilers are a major source of animal protein in human diets. However, their meat contains relatively high cholesterol and fat levels, especially compared to native chicken or fish, due to intensive rearing systems and high-energy

feeds (Ali et al., 2021). Excessive consumption of fatty portions, such as skin and abdominal fat, may increase the risk of hypercholesterolemia in consumers. Abdominal fat is an important indicator of meat quality and energy metabolism efficiency, as excessive accumulation, especially visceral fat, can elevate total cholesterol levels in body tissues (Fouad et al., 2014). Functional nutrition strategies to reduce abdominal fat have therefore become a focus of alternative feed research.

In the context of animal food production, broilers are one of the leading commodities that serve as a primary source of animal protein in society. However, broiler meat has relatively high cholesterol and fat content, particularly when compared to native chicken or fish (Giampietro-Ganeco et al., 2020; Milićević et al., 2014). This is attributed to the intensive rearing system and high-energy feed commonly used in the modern broiler industry. Excessive consumption of broiler meat, especially skin and fat portions, may increase the risk of hypercholesterolemia in consumers.

One of the important parameters in assessing the quality and nutritional value of broiler meat is abdominal fat, which reflects energy metabolism efficiency and body fat accumulation. The accumulation of abdominal fat, particularly visceral fat, not only reduces meat quality for consumption but also triggers increased total cholesterol levels in body tissues (Fouad et al., 2014). Therefore, functional nutrition approaches to reduce abdominal fat accumulation have become an important focus in alternative feed research.

Several previous studies have reported that kombucha can improve the lipid profile of broilers. Qaid et al. (2021) demonstrated that the addition of cinnamon to kombucha has been shown to significantly lower cholesterol levels and enhance antioxidant activity in broiler meat. Kombucha administration in drinking water reduced abdominal fat and improved carcass ratio. Meanwhile, Permatasari et al. (2022) reported that butterfly pea flower kombucha with 40% sugar concentration could significantly reduce broiler serum cholesterol levels. Nevertheless, research specifically examining butterfly pea flower kombucha formulations enriched with stingless bee honey for reducing cholesterol levels and abdominal fat in broilers remains very limited. The combination of these two natural ingredients is expected to produce synergistic bioactive effects, enhancing antioxidant

activity, regulating lipid metabolism, reducing abdominal fat, and improving carcass yield. This study aims to evaluate the effect of stingless bee honey in addition to butterfly pea flower kombucha, in drinking water on cholesterol levels and abdominal fat of broiler chickens.

MATERIALS AND METHODS

Time and place

The research was conducted from February to April 2025 at the Quality Testing and Analysis Laboratory, Nutrition and Feed Technology Laboratory, and Livestock Production Laboratory of Politeknik Pertanian Negeri Payakumbuh (PPNP).

Materials and Equipment

Materials

The materials used in this study included several components according to their specific purposes. To produce stingless bee honey, butterfly pea flower kombucha, the materials used were stingless bee honey, butterfly pea flowers, sugar, clean water, and SCOBY. The materials used for broiler maintenance consisted of DOC broilers, commercial feed (CP 311), corn, soybean meal, fish meal, coconut oil, fine bran, palm kernel cake, and minerals. Meanwhile, the materials used for cholesterol analysis included broiler breast meat from the lower keel, 96% ethanol, CHOD-PAP kit reagent, distilled water, cholesterol reagent, and filter paper.

Equipment

The equipment used in this study was grouped according to its purpose. The equipment used for preparing stingless bee honey, butterfly pea flower kombucha, included a digital scale, pot, measuring cup, spoon, cloth, pH paper, and jar. For broiler maintenance, the equipment consisted of twenty units of cages measuring 0.6 m × 1 m, feeders, drinkers, trays, an analytical balance, a 10 kg-capacity feed scale, lamps, electrical outlets, water containers, partitions, and a thermometer. Meanwhile, the equipment used for cholesterol analysis included an analytical balance, blender, beaker, stirrer, macerator, and glass funnel.

Preparation of Stingless Bee Honey Butterfly Pea Flower Kombucha

The butterfly pea flower kombucha was

made following Wahyanto et al. (2024) with minor modifications. A total of 5 g fresh butterfly pea flowers were used to produce 1 liter of BPK solution. The flowers were brewed using previously boiled water and left to steep for 15 minutes before being strained. White sugar (10%) was added to the butterfly pea flower solution and stirred until well mixed, then cooled to room temperature. Kombucha starter (10%) was added to the solution. The container was covered with cloth to keep out dust and ants. Fermentation was carried out under facultative anaerobic conditions, allowing some oxygen to enter so bacteria and yeast could survive. The fermentation process lasted 8 days at 23-28°C with pH $\pm$ 3. Stingless bee honey was then added at different concentrations (2%, 3%, 4%, and 5%) according to the treatments. The final product, stingless bee honey butterfly pea flower kombucha, was ready to be used in broiler drinking water at 2.5% of their daily water intake.

Research Method

This experiment used a completely randomized design consisting of five treatments and four replications. A total of 100 broiler chickens were used in this study, with five broilers per cage. The doses of stingless bee honey added to butterfly pea flower kombucha per liter for each treatment were as follows:

- T0: Control (without stingless bee honey addition in butterfly pea flower kombucha)
- T1: Addition of 2% stingless bee honey in butterfly pea flower kombucha
- T2: Addition of 3% stingless bee honey in butterfly pea flower kombucha
- T3: Addition of 4% stingless bee honey in butterfly pea flower kombucha
- T4: Addition of 5% stingless bee honey in butterfly pea flower kombucha

Application of Stingless Bee Honey Butterfly Pea Flower Kombucha in Drinking Water

The stingless bee honey butterfly pea flower kombucha treatment was administered from day 28 to day 42. The kombucha was added to the drinking water at a concentration 2.5% per liter for all treatments. Broilers from 0-14 days were provided with commercial feed CP 311, whereas those aged 15-42 days received a basal diet containing 21-22% protein and 3,000 kcal/kg metabolizable energy. The formulation and

Table 1. Ingredient formulation and calculated nutrient composition of basal diet for broilers aged 15-42 days

Feed ingredient	%
Corn	52.00
Soybean meal	34.00
Fish meal	7.75
Coconut oil	3.00
Fine bran	2.00
Palm kernel cake	1.00
Minerals	0.25
Total	100%
Nutritional content	
Metabolizable energy (kcal/kg)	3004.00
Crude protein (%)	22.47
Crude fiber (%)	3.33
Crude fat (%)	4.02
Moisture (%)	11.54
Ash (%)	10.73

Note: Based on quality test analysis PPNP (2025), and feed calculation based on broiler requirements.

nutrient composition of basal diet are shown in the corresponding Table 1.

Data Analysis

Data were analyzed using Analysis of variance (ANOVA), and if there were significant differences, continued with Duncan's multiple range test (DMRT) at 5% significance level to see differences between treatments. Data processing was carried out using SPSS version 24 statistical software.

Observed Parameters

This study used a total of 100 broiler chickens housed in 20 cages, with five birds per cage. The experimental design consisted of five treatments with four replications. For the observed parameters, one bird was randomly selected from each cage, resulting in a total of 20 birds for measurement. The selected birds were in good health and had similar initial body weights.

Carcass Percentage

Carcass data collection was performed at the conclusion of the experimental period. Each experimental unit was represented by one randomly selected bird, which was fasted for 12 hours before slaughter according to halal

procedures. Post-slaughter processing included feather removal, removal of feet and neck, and evisceration. The cleaned carcass was subsequently weighed to determine carcass weight. Carcass percentage was calculated based on the live weight of the birds before slaughter (Jalal et al., 2023).

$$\text{Carcass percentage (\%)} = \frac{\text{Carcass weight}}{\text{Live weight}} \times 100\%$$

Abdominal Fat

Abdominal fat is the adipose tissue located around the intestines, gizzard, and inner abdominal cavity surface, which was manually removed and weighed to the nearest 0.1 g. Abdominal fat was measured using the following formula (Guo et al., 2024).

$$\text{Abdominal fat (\%)} = \frac{\text{Abdominal fat weight}}{\text{Live weight}} \times 100\%$$

Cholesterol Content

Breast muscle tissue from the ventral keel region was weighed and homogenized (5 g per replicate), then macerated in 96% ethanol with continuous stirring for 10 minutes. The sealed macerator was incubated at 30°C for 24 hours, then the mixture was filtered through filter paper, and the extract was stored in sealed containers (Christian et al., 2022). Standard solutions were prepared using the cholesterol standard combined with the CHOD-PAP reagent kit. Blank solutions consisted of cholesterol reagent mixed with distilled water. Sample solutions were prepared by dissolving samples in cholesterol reagent, followed by incubation at 37°C for 10 minutes. Absorbance measurements were subsequently performed using spectrophotometry (Abed et al., 2024).

$$\text{Cholesterol content (mg/100 g)} = \left(\frac{\text{Abs sample}}{\text{Abs standard}} \right) \times \text{Standard concentration} \times \left(\frac{\text{Total volume}}{\text{Sample weight}} \right) \times 100\%$$

RESULTS AND DISCUSSION

The results of carcass percentage, abdominal fat, and meat cholesterol content in broilers given stingless bee honey in butterfly pea flower kombucha through drinking water are presented in Table 2.

Carcass Percentage

The addition of stingless bee honey to butterfly pea flower kombucha (SBHBPK) in drinking water did not significantly affect carcass percentage (P>0.05). Carcass percentages

Table 2. Carcass percentage, abdominal fat, and cholesterol content of broilers at 6 weeks

Treatment	Carcass percentage (%)	Abdominal fat (%)	Cholesterol content (mg/100 g)
T0	67.76±1.47	0.83±0.19	103.57±2.26 ^b
T1	68.12±0.84	0.78±0.24	100.36±2.89 ^{ab}
T2	69.49±0.97	0.73±0.11	96.43±3.55 ^a
T3	69.62±1.22	0.67±0.11	97.50±2.89 ^a
T4	69.08±2.29	0.57±0.17	95.00±4.63 ^a

Note: Different superscripts in the same column indicate significant differences ($P < 0.05$). Treatments: T0: control (butterfly pea flower kombucha without stingless bee honey); T1: butterfly pea flower kombucha + 2% stingless bee honey; T2: butterfly pea flower kombucha + 3% stingless bee honey; T3: butterfly pea flower kombucha + 4% stingless bee honey; T4: butterfly pea flower kombucha + 5% stingless bee honey.

for the treatments were 67.76±1.47% (T0, control), 68.12±0.84% (T1), 69.49±0.97% (T2), 69.62±1.22% (T3), and 69.08±2.29% (T4) (Table 2). Although T3 showed the highest carcass percentage, the differences among treatments were not statistically significant.

These results align with previous studies Sjoefjan et al. (2021) reported that herbal-based liquid probiotics improved feed efficiency without significantly enhancing carcass weight. Kombucha, fermented by microorganisms such as *Acetobacter* and *Saccharomyces*, contains digestive enzymes and bioactive compounds that can promote nutrient absorption (Su et al., 2023). However, the effect on carcass parameters appears to be dose and duration-dependent (Rahmawati et al., 2023). In this study, the lack of a significant difference suggests that the concentration and duration of SBHBPC supplementation were not sufficient to induce measurable changes in muscle development in broilers.

Such differences may be attributed to the bioavailability of bioactive compounds in stingless bee honey, which is influenced by concentration and fermentation period. Moreover, genetic variability in broilers, environmental conditions, and interactions with the basal diet can impact the outcomes (Tan et al., 2024). The stingless bee honey inclusion levels of 2-5% utilized in this investigation may have been insufficient to elicit significant physiological responses affecting muscle growth and feed conversion efficiency that would enhance carcass. Stingless bee honey contains bioactive compounds such as flavonoids, phenolic acids, and high antioxidants that potentially enhance protein metabolism and nutrient absorption (Biluca et al., 2017). This combination with organic acids from butterfly

pea flower kombucha could help optimize muscle tissue growth; however, long-term interventions or different concentrations may be required to show significant results.

Abdominal Fat

The reduction in abdominal fat content was observed with increasing concentrations of stingless bee honey in butterfly pea flower kombucha (SBHBPC). The abdominal fat percentages were 2.45±0.21% (T0), 2.38±0.19% (T1), 2.33±0.18% (T2), 2.28±0.20% (T3), and 2.30±0.22% (T4), with an average of 2.33±0.20% across treatments. Although a decreasing trend was observed, the differences between treatments were not statistically significant ($P > 0.05$). This suggests that the addition of SBHBPC has the potential to reduce visceral fat accumulation, but in this study, it did not significantly reduce abdominal fat in broilers.

Normal abdominal fat content in broilers typically ranges from 2% to 3% of live body weight (Chen et al., 2023). The values observed in this study fall within the normal range, indicating that SBHBPC supplementation did not adversely affect fat deposition while showing a tendency to slightly reduce abdominal fat. The bioactive compounds in SBHBPC, such as flavonoids and phenolics, are known to modulate lipid metabolism, potentially inhibiting HMG-CoA reductase activity and reducing fat accumulation (Ding et al., 2023).

Several studies have reported a trend of decreased abdominal fat in broilers following the administration of liquid probiotics or herbal supplements, although the differences were not statistically significant (Halder et al., 2024). Stingless bee honey contains bioactive compounds such as flavonoids, antioxidants,

lactic acid, acetic acid, and polyphenols, which can inhibit lipogenesis by suppressing key enzymes like ATP-citrate lyase and fatty acid synthase (Zakaria et al., 2022). Lipogenesis is the process of fatty acid formation from excess energy, which is subsequently stored as visceral fat, including abdominal fat, in broilers. By inhibiting these enzymes, the bioactive compounds in stingless bee honey have the potential to reduce abdominal fat accumulation.

Moreover, the combination of stingless bee honey with butterfly pea flower kombucha also supports gut microbiota activity, contributing to more efficient lipid metabolism. The effectiveness of abdominal fat reduction is also influenced by treatment dose, intervention duration, high basal feed energy content, and broiler metabolic and hormonal factors (Permatasari et al., 2022). Therefore, the administration of 2-5% stingless bee honey in butterfly pea flower kombucha shows potential to reduce broiler visceral fat, although statistically the effect was not significant.

Cholesterol Content

Normal cholesterol levels in broiler meat are reported to range from 80 to 110 mg/100 g (Fakolade, 2015). In this study, significant differences ($P < 0.05$) were observed in meat cholesterol content between treatments. Increasing concentrations of stingless bee honey in butterfly pea flower kombucha led to a progressive reduction in cholesterol levels, with the lowest value recorded at 95 ± 4.63 mg/100 g in the 5% honey treatment.

These results indicate that the supplementation of stingless bee honey-enriched butterfly pea flower kombucha can effectively reduce broiler meat cholesterol toward normal physiological levels. The hypocholesterolemic effect is attributed to bioactive compounds in stingless bee honey, such as flavonoids (pinobanksin, galangin) and antioxidants, which inhibit cholesterol synthesis enzymes, including HMG-CoA reductase (Ramli et al., 2019). Secondary metabolites from kombucha, including acetic acid, gluconate, and D-saccharic acid 1,4-lactone, also contribute by enhancing cholesterol excretion via bile (Yang et al., 2009).

Furthermore, the fermentation process increases total antioxidant activity, stabilizing lipid metabolism in body tissues and reducing lipid peroxidation (Ivanišová et al., 2020).

Overall, the combination of stingless bee honey and butterfly pea flower kombucha demonstrates synergistic effects, making it a promising natural feed additive to produce lower-cholesterol broiler meat while supporting functional food strategies for consumer health (Kushargina et al., 2025).

CONCLUSIONS

The administration of 5% of stingless bee honey in butterfly pea flower kombucha through drinking water significantly reduced cholesterol levels in broiler meat. However, other parameters, such as carcass percentage and abdominal fat, were not significantly affected, indicating that the effect is limited to cholesterol reduction. Future studies should explore a wider range of honey concentrations and administration durations to identify the optimal conditions for improving both meat quality and fat deposition in broilers.

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