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Effects of fermented Ginger and *Coleus amboinicus* extracts on growth and immune response in broilers

Pengaruh ekstrak fermentasi jahe dan daun bangun bangun terhadap pertumbuhan dan respon imun pada ayam pedaging

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ABSTRACT

This study investigated the effects of fermented of ginger (*Zingiber officinale*) and *Coleus amboinicus* extracts administered via drinking water on broiler growth performance and immune response. A completely randomized design was employed with four treatments and five replications, totaling 100 broiler chicks divided into cages of five birds each. The treatments consisted of drinking water supplemented with ginger fermented extracts at concentrations of 0% (T0, control), 2% (T1), 4% (T2), and 6% (T3). Parameters measured included feed intake, body weight gain (BWG), feed conversion ratio (FCR), and relative weights of lymphoid organs (thymus, bursa of Fabricius, and spleen). The results showed that fermented extract supplementation significantly improved broiler performance. T1 had the highest BWG (1415.4±31.17g), while T3 showed the highest feed intake (2496.56±59.37g). The most efficient FCR was observed in T1 (1.75±0.045). In terms of immune response, T2 significantly increased spleen weight (0.15±0.02%) compared to the control (0.09±0.01%). Phytochemical analysis demonstrated that fermentation enhanced the bioactive content of the extract, increasing total phenols 20.64 ppm, flavonoids 163.08 ppm, and antioxidant activity 20.8% inhibition 5000. These findings suggest that administering 2% fermented extract via drinking water optimally improves broiler growth, while 4% enhances immune response. Fermented ginger and *Coleus amboinicus* extract show potential as natural feed additives and could serve as a sustainable alternative to antibiotics in poultry production.

ABSTRAK

Penelitian ini menyelidiki pengaruh ekstrak jahe (Zingiber officinale) dan daun bangun-bangun yang difermentasi dan diberikan melalui air minum terhadap performa pertumbuhan dan respons imun ayam broiler. Rancangan acak lengkap digunakan dengan lima perlakuan dan empat ulangan, melibatkan total 100 ekor anak ayam broiler yang ditempatkan dalam kandang berisi lima ekor per unit. Perlakuan terdiri dari air minum yang ditambahkan ekstrak fermentasi dengan konsentrasi 0% (P0, kontrol), 2% (P1), 4% (P2), dan 6% (P3). Parameter yang diukur meliputi konsumsi pakan, pertambahan bobot badan (BWG), rasio konversi pakan (FCR), dan bobot relatif organ limfoid (timus, bursa Fabricius, dan limpa). Hasil menunjukkan bahwa suplementasi ekstrak fermentasi secara signifikan meningkatkan performa broiler. Perlakuan P1 menghasilkan BWG tertinggi (1415,4±31,17 g), sedangkan P3 menunjukkan konsumsi pakan tertinggi (2496,56±59,37 g). Nilai FCR terbaik diperoleh pada P1 (1,75±0,045). Dalam hal respons imun, P2 secara signifikan meningkatkan bobot limpa (0,15±0,02%) dibandingkan kontrol (0,09±0,01%). Analisis fitokimia menunjukkan bahwa fermentasi meningkatkan kandungan senyawa

Kata kunci:
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Pertumbuhan
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bioaktif dalam ekstrak, yaitu peningkatan total fenol 20,64 ppm, flavonoid sebesar 163,08 ppm, dan aktivitas antioksidan 20,8% inhibition 5000. Temuan ini menunjukkan bahwa pemberian ekstrak fermentasi 2% melalui air minum secara optimal meningkatkan pertumbuhan broiler, sementara konsentrasi 4% meningkatkan sistem imun. Ekstrak fermentasi jahe dan Coleus amboinicus berpotensi menjadi aditif pakan alami yang berkelanjutan sebagai alternatif pengganti antibiotik dalam produksi unggas.

INTRODUCTION

The broiler chicken industry in Indonesia plays an important role in providing animal protein for the community. With the increasing population and changing consumption patterns, demand for broiler chicken meat continues to grow. inistry of Agriculture shows that broiler chicken meat production in Indonesia will reach 3.84 million tons in 2024, a significant increase from 3.26 million tons in 2020 (Kementerian Pertanian Republik Indonesia 2024). This growth demonstrates a positive trend in the national poultry sector, with broiler chicken meat production growing by an average of 4.51% annually from 2020 to 2024 (Kementerian Pertanian Republik Indonesia 2024). However, broiler farmers are faced with various challenges that can hinder the productivity of their livestock, especially in terms of chicken growth and health.

Several factors that contribute to this problem include suboptimal feed quality, stress due to unsupportive rearing environments, and disease infections. These conditions can cause decreased growth rates, low feed conversion efficiency, and increased mortality rates (Mehdi et al. 2018). To overcome this problem, farmers often use synthetic antibiotics as feed supplements. However, uncontrolled use of antibiotics can cause resistance to pathogenic bacteria, which has the potential to endanger human health through consumption of contaminated broiler products (Mehdi et al. 2018). Since the enactment of Minister of Agriculture Regulation No. 14/2017 on January 1, 2018, the use of synthetic antibiotics has been prohibited in Indonesia, thus encouraging the search for safer and more environmentally friendly feed supplement alternatives (Kementerian Pertanian Republik Indonesia 2017).

Ginger (*Zingiber officinale*) and *Coleus amboinicus* extracts are one promising alternative which is the use of natural materials that have undergone fermentation processes. Ginger is known to have various health benefits thanks to its bioactive compound content, such as gingerol and shogaol, which can improve the immune system and growth performance of broiler chickens (Prasad et al., 2016; Suleria et al 2015). These compounds have antioxidant, anti-inflammatory, and antimicrobial properties that support livestock health. On the other hand, *Coleus amboinicus* leaves are also rich in bioactive compounds, such as rosmarinic acid and flavonoids, which function as antioxidants and immunomodulators (Maleki et al., 2019). Previous research shows that *Coleus amboinicus* leaf extract can improve growth performance and immune response in broiler chickens (Abdel-Wareth et al., 2012; Chayomchai et al., 2020).

The fermentation process on both natural materials can increase the activity of bioactive compounds, breaking down complex components into simpler compounds, making them easier to absorb by livestock bodies (Prasad et al. 2016). In addition, the administration of additive materials through drinking water also enables more even distribution of active substances and faster absorption compared to administration in feed. Fermentation plays an important role in increasing nutrient digestibility and reducing digestive tract viscosity, thus supporting increased consumption (Kurniawan et al., 2020; Wahyuni et al., 2022).

This study is believed to be the first to investigate the synergistic effects of fermented extracts of ginger (*Zingiber officinale*) and *Coleus amboinicus* when administered simultaneously through broiler chicken drinking water. To date, no prior research has specifically examined this particular combination, thus highlighting the

novelty and potential relevance of the current study. The primary objective of this research is to evaluate the impact of fermented ginger and *Coleus amboinicus* extracts on the growth performance and immune response of broiler chickens. These plant-based extracts are rich in bioactive compounds that are expected to improve feed intake, feed conversion efficiency, and immune modulation, thereby reducing disease risk and promoting sustainable productivity. This approach also aligns with current trends in antibiotic-free poultry production and offers an alternative strategy that may help the broiler industry comply with global restrictions on synthetic growth promoters while maintaining optimal animal health and protein yield.

MATERIALS AND METHODS

Time and Place

The research was conducted at the quality testing and analysis laboratory, nutrition and feed technology laboratory, and livestock production laboratory of Payakumbuh State Agricultural Polytechnic from February to March 2025.

Materials and Equipment

The materials used in the fermentation process of *Coleus amboinicus* leaves and ginger included *Coleus amboinicus* leaves, fresh ginger, EM4 (*Effective Microorganisms 4*), molasses, and well water as the solvent. These ingredients were selected for their bioactive properties and potential as natural feed additives. For the maintenance phase of the broiler chickens, the materials used consisted of day-old chicks (DOC) of broiler strain, commercial starter and finisher feed, corn, fish meal, soybean meal, palm kernel cake, rice bran, cooking oil, and mineral mix to ensure balanced nutrition during the rearing period.

The equipment used in the fermentation process comprised a knife for cutting the ingredients, a digital scale for accurate weighing, a blender for homogenizing the mixture, measuring cups for precise liquid volumes, a strainer, a plastic bucket, and a fermentation container in the form of a 20-liter capacity gallon. During the maintenance of the broilers, the equipment included 20 units of cages, each measuring 0.6 m × 1 m, feed and drinking containers, chick trays, analytical scales, a 10-kg capacity feed scale, electric lamps, heaters, electrical plugs, water measuring liters, cage partitions, and a thermometer to monitor environmental temperature. All tools and equipment were used to support the standard management and treatment conditions throughout the research.

Procedure for Making Ginger and *Coleus amboinicus* Extract

The extract based on fresh ginger (*Zingiber officinale*) and bangun-bangun leaves *Coleus amboinicus* was obtained through a fermentation process using *Effective Microorganisms* (EM4). Clean water (5 liters) was prepared as the fermentation medium. Each herbal ingredient was weighed at a dosage of 100 grams per liter of water, resulting in 500 grams of ginger and 500 grams of bangun-bangun leaves.

The fresh bangun-bangun leaves were washed thoroughly, thinly sliced, and homogenized using a blender. Fresh ginger was also washed, sliced, and blended until smooth. The blended herbal materials were combined into the water medium, then supplemented with 500 cc of molasses as a carbon source to support microbial activity, and 500 cc of EM4 as the fermentation starter.

The mixture was thoroughly homogenized, sealed tightly in a fermentation container, and stored at room temperature under anaerobic conditions for 14 days (2 weeks). The fermentation process was considered complete when gas production ceased, indicating a decline in microbial activity. After fermentation, the fermented solution was filtered through cheesecloth to separate the solids, and the extract was stored in a sealed container under cool, anaerobic conditions until use.

This fermented extract is applied as a natural feed additive by mixing it into the drinking water of broiler chickens to enhance growth performance and immune response naturally, without chemical residues (Haroen and Budiansyah 2018).

Research Method

Experimental design

This experiment used a Completely randomized design (CRD), which included four treatments and five replications. The number of livestock used in this study was 100 day-old broiler chickens (*Cobb strain*), with five broilers per cage unit, resulting in 20 cage units total.

Ingredient preparation and proportions

The fermented mixture consisted of ginger (*Zingiber officinale*) and *Coleus amboinicus* in a 1:1 ratio (w/w). The fermentation process was conducted for 14 days under anaerobic conditions at room temperature (28-30°C). The fermented mixture was then diluted in drinking water according to the treatment concentrations.

Treatment Groups

The doses of fermented ginger and *Coleus amboinicus* mixture addition in drinking water per liter for each treatment were as follows:

T0: 0% (Control - plain drinking water)
T1: 2% (Fermented mixture of ginger and *Coleus amboinicus)*
T2: 4% (Fermented mixture of ginger and *Coleus amboinicus)*
T3: 6% (Fermented mixture of ginger and *Coleus amboinicus)*

Rearing management

a. Housing System:

Chickens were housed in battery cage system with closed-sided housing to control environmental conditions. Each cage measured 60 cm × 70 cm × 40 cm, providing adequate space for five broilers.

b. Feed and Water Management:

Drinking water containing the respective treatment solutions was provided ad libitum using nipple drinkers. Water containers were cleaned and refilled daily at 08:00 hours to ensure freshness and prevent contamination. Feed was provided as commercial starter feed during the first week, while from the second week onward, basal feed was used according to broiler nutritional requirements. Feed was provided ad libitum and replaced with fresh feed twice daily at 07:00 and 17:00.

c. Environmental Control:

The housing temperature was maintained at 32-35°C during the first week, then gradually reduced to 24-26°C by week 5. Lighting was provided 24 hours daily using LED lights. Ventilation was controlled to maintain optimal air quality.

Table 1. Formulation and nutritional content of basal feed based on calculations

Broiler feed age 1 week to 5 weeks	%
Corn	53
Bran	2
Soybean meal	34
Fish meal	7
Mineral	0
Palm kernel cake	1
Vegetable oil	3
Total	100
Nutritional content	
Crude protein (%)*	21.924
Crude fat (%)*	2.494
Crude fiber (%)*	5.574
Ca (%)*	0.560
P (%)*	0.480
Metabolizable energy (kcal/kg)*	3099.820

Note: *Calculated values based on proximate analysis of individual feed ingredients conducted at Politeknik Pertanian Negeri Payakumbuh (2025). CP (crude protein), CF (crude fat) and CF (crude fiber)

Application of Fermented Ginger and *Coleus amboinicus Extract*

Treatment with addition of fermented ginger and *Coleus amboinicus* began when chickens were 2 days old. Broilers were given commercial feed from 1 to 7 days old. The commercial feed 311 given contained crude protein 21-22% and metabolizable energy 3000 kcal. Chickens aged 8 days to 5 weeks were given feed formulated with protein content of 21.92% and metabolizable energy 3099.8 Kcal. Materials used in making basal feed mixtures included fish meal, yellow corn, minerals, palm oil, coconut cake, and soybean meal. The formulation and content of basal feed and phytochemical content can be seen in Tables 1 and 2.

Table 2. Phytochemical results of ginger and *Coleus amboinicus* before and after fermentation

Content	Before fermentation	14th day fermentation
Total Phenol (ppm)	128.07	148.71
Flavonoid (ppm)	289.34	452.42
Antioxidant (%)	46% inhibition 10,000	20.8% inhibition 5,000

Note: Based on quality test analysis Politeknik Pertanian Negeri Payakumbuh 2025

Observed Variables

Feed consumption

Feed consumption (kg) was obtained from the difference between the amount of feed given and the remainder (Suryati et al., 2022).

Feed consumption = feed given - remaining feed

Body weight gain

Body weight gain is the difference between final body weight and initial body weight (Suryati et al., 2022).

BWG= final body weight (kg) - initial body weight (kg)

Feed conversion

Feed conversion is calculated by dividing feed consumption by body weight gain (Suryati et al., 2022).

$$\text{Feed conversion} = \frac{\text{Consumption}}{\text{BWG}}$$

Relative weight of immune organs

Relative weight of immune organs (bursa of Fabricius, thymus and spleen) was calculated using the calculation formula from (Rotiah et al., 2019)

$$\text{Relative weight of immune organs} = \frac{\text{Immune organ weight}}{\text{Body weight}} \times 100\%$$

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. Fermented mixture of ginger and *Coleus amboinicus.*

RESULTS AND DISCUSSION

The results of research on the addition of natural feed additives using ginger and *Coleus amboinicus* on growth response and immune response of broilers for 35 days, namely BWG, consumption, and feed conversion can be seen in Table 3.

Table 3. Mean growth performance parameters of broilers fed natural additives of fermented ginger and *Coleus amboinicus* over a five-week period

Treatment	Body weight gain (kg)	Feed consumption (kg)	Feed conversion
T0	1.29 ± 0.042^{a}	2.35 ± 0.077^{a}	1.82 ± 0.062
T1	1.41 ± 0.031^{b}	2.47 ± 0.037^{b}	1.75 ± 0.045
T2	1.39 ± 0.046^{b}	2.48 ± 0.039^{b}	1.78 ± 0.060
T3	1.40 ± 0.059^{b}	2.49 ± 0.059^{b}	1.78 ± 0.065

Note: Values are presented as mean ± standard deviation.

Different letters indicate significance ($P<0.05$). T0: 0% fermented ginger + *Coleus amboinicus*, T1: 2% fer- mented ginger + *Coleus amboinicus*, T2: 4% fermented ginger + *Coleus amboinicus*, and T3: 6% fermented ginger + *Coleus amboinicus.*

Broiler Body Weight Gain (BWG)

Statistical analysis results showed that the addition of natural feed additives in the form of fermented ginger and *Coleus amboinicus* had a significant effect (P<0.05) on broiler chicken body weight gain. Based on Table 3, the highest average BWG was obtained in treatment T1 (1.41±0.031 kg) followed by T3 (1.40±0.059 kg) and T2 (1.39±0.046 kg), which were statistically not significantly different from each other, but all were significantly different compared to T0 as control (1.29±0.042 kg). Treatment T1 demonstrated 9.3% higher BWG compared to the control group, indicating that the provision of natural feed additives can significantly improve broiler chicken growth performance.

The superior performance of T1 treatment resulted in final body weights of 1.455 kg compared to 1.335 kg in the control group, representing a commercially significant improvement. However, this enhancement in BWG cannot be attributed solely to fermentation effects, as feed consumption patterns significantly influenced growth outcomes. Treatment T1 achieved optimal feed conversion efficiency (1.75±0.045) compared to control (1.82±0.062), representing a 3.8% improvement in feed utilization, while consuming 2.47±0.037 kg of feed compared to 2.35±0.077 kg in the control group.

This increase in BWG can be associated with the active role of bioactive compounds in ginger, such as gingerol, shogaol, and zingiberene which are antioxidant and stimulate digestive enzyme secretion (Zhang et al., 2020; Zhou et al., 2016). In addition, fermented *Coleus amboinicus* leaves are known to contain high phenolic compounds and flavonoids that function as immunostimulant and antimicrobial agents, and support gut microbiota balance (Fati et al., 2018; Fitriani et al., 2021). Fermentation also increases soluble protein content and enriches probiotic enzymes, thereby increasing nutrient digestibility (Rahman et al., 2021).

The dose-response relationship observed in this study revealed a non-linear pattern, where the 2% inclusion level (T1) achieved maximum efficiency, while 4% inclusion (T2) showed reduced performance, possibly due to palatability issues or nutrient interference. The 6% inclusion (T3) partially recovered performance but required higher feed consumption (2.49±0.059 kg) to achieve similar BWG (1.40 kg) as T1, indicating less efficient nutrient conversion at elevated dosages.

This research aligns with quantitative findings from previous studies. (Nasution et al., 2021) reported that fermented herbal mixtures increased broiler body weight gain from 1.28 kg in control groups to 1.42 kg in treated groups, representing a 10.9% improvement, which closely matches our T1 treatment results of 9.3% improvement over control. Similarly, (Winarno et al., 2019) demonstrated that herbal feed additives improved feed conversion ratios from 1.85 to 1.73, a 6.5% improvement, which is comparable to our T1 treatment's 3.8% improvement in feed efficiency. (Lubis et al., 2024) found that the addition of natural feed additives to drinking water achieved body weight gains ranging from 1.43-1.74 kg in broilers, with our T1 treatment (1.41 kg) falling within the lower range of their reported values, though direct comparisons are limited due to different experimental durations and initial body weights.

Feed Consumption

Broiler chicken feed consumption during five weeks showed significant differences between treatments (P<0.05). Treatment T3 showed the highest consumption of 2496.56±59.37 g, followed by T2 (2481.32±39.10 g), T1 (2470.2±37.31 g), and the lowest P0 (2344.28±77.97 g). The addition of natural fermented ginger and *Coleus amboinicus* extract in drinking water increased appetite and chicken acceptance of feed, which had positive implications for productivity.

This increase in consumption can be explained by the sensory effects of ginger which stimulate appetite through increased production of saliva and digestive enzymes (Mekbungwan et al., 2017). Ginger is also known to accelerate gastric emptying and reduce intestinal tension (Sa'aci et al. 2018). Meanwhile, fermented *Coleus amboinicus* leaves have a distinctive aroma and contain secondary metabolites such as eugenol and tannins in controlled amounts, which stimulate poultry taste receptors and support healthy gut microflora (Fati et al., 2018; Fitriani et al., 2021).

Research by (Haroen and Budiansyah 2018) showed that the use of fermented ginger extract in drinking water significantly affected (P<0.05) feed consumption of broiler chickens. Similarly, Suryatni (2021) reported that the addition of herbal concoctions in drinking water significantly increased broiler chicken feed consumption. Other research by (Lestari et al., 2014) also noted that the use of fermented herbal extract fermentation in drinking water can increase feed consumption and feed conversion efficiency in broiler chickens.

Feed Conversion

Feed conversion ratio (FCR) values during the study were not significantly different (P>0.05) between treatments, but numerically, T1 had the best value (1.75±0.045), followed by T2 and T3 (1.78±0.060 and 1.78±0.065), while T0 showed the highest FCR (1.82±0.062). This means that broiler chickens in natural feed additive treatments tend to utilize feed more efficiently although the difference is not statistically significant.

Lower FCR values in treatment groups reflect better feed utilization efficiency. This is thought to be influenced by increased digestive enzyme activity and gut microflora balance due to bioactive compounds in ginger and fermentation effects from *Coleus amboinicus* (Rahmawati et al., 2021; Zhang et al. 2020). Fermentation is known to reduce anti-nutritional compounds such as saponins and tannins and increase cellulase and protease enzyme levels, thereby improving nutrient utilization (Rahmawati et al., 2020; Wulandari et al., 2023).

These results are in line with research by Astika and (Astika and Yulianto 2023) who reported that the administration of probiotic fermentation with turmeric mixture in drinking water produced the lowest FCR value of 1.32 in broiler chickens. (Yuliani et al., 2023) also reported a decrease in FCR values in broilers given a combination of phytobiotics and fermentation probiotics. Similar findings were also reported by (Putra et al., 2019) and (Firmansyah et al., 2022) who confirmed that fermented natural materials can reduce FCR by increasing energy conversion and improving intestinal villi structure.

Broiler Immune Organ Response

The relative weight of broiler lymphoid organs during research using ginger and Coleus amboinicus extracts for 35 days tested using SPSS 24 can be seen in Table 4.

Table 4. Relative weight of broiler lymphoid organs at 35 days of age

Treatment	Thymus (%)	Bursa of Fabricius (%)	Spleen (%)
T0	0.18±0.05	0.23±0.03	0.09±0.01[a]
T1	0.21±0.03	0.21±0.05	0.08±0.04[a]
T2	0.23±0.05	0.22±0.06	0.15±0.02[b]
T3	0.25±0.08	0.24±0.05	0.11±0.02[ab]

Note: Values are presented as mean ± standard deviation.
Different letters indicate significance (P<0.05). T0: 0% fermented ginger + *Coleus amboinicus*, T1: 2% fer- mented ginger + *Coleus amboinicus*, T2: 4% fermented ginger + *Coleus amboinicus*, and T3: 6% fermented ginger + *Coleus amboinicus*.

Thymus

Based on statistical analysis results, the relative weight of thymus of 35-day-old broiler chickens in all treatments showed no significant difference (P>0.05). It tended to increase in treatment T3 (0.25±0.08%), followed by T2 (0.23±0.05%), T1 (0.21±0.03%), and lowest in T0 (0.18±0.05%). Although not statistically significantly different, there was a trend of increased thymus weight in groups receiving ginger and *Coleus amboinicus* extracts (T1-T3) compared to control (T0).

The thymus is a primary lymphoid organ that functions in the maturation process of T lymphocytes. The increase in thymus weight in treatment T3 is possibly influenced by the activity of bioactive compounds in ginger (*Zingiber officinale*) such as gingerol and shogaol, as well as flavonoids in *Coleus amboinicus* leaves, which have immunostimulant effects. Although

not significant, the increase in average values shows that the combination of these two herbal plants may be able to increase cellular immune response through stimulation of thymus organ development, especially if administration is carried out for longer durations or at different doses.

Research by (Satrian et al., 2022) showed that ginger extract administration can increase broiler chicken thymus weight, with thymus relative weight ranging from 0.47% to 53% although the effect is not always statistically significant (P>0.05) and is greatly influenced by dose and form of application. (Amanda 2025) reported that the administration of fermented ginger and African leaf combinations for 35 days resulted in thymus weight ranging from 0.16%±0.02 to 0.26%±0.08 of body weight. This is reinforced by findings by (Fadilah et al., 2021) which mentioned that phenolic compounds and antioxidants in herbs can support the development of primary lymphoid tissue, including thymus. In addition, (Putra et al., 2020) emphasized that the effectiveness of feed additives on immune organ growth is also influenced by the timing of administration during the rearing period. Thus, the results of this study are in line with previous studies that show a positive trend towards increased thymus weight, with the current study achieving a more substantial improvement (38.9% vs 6.25% in Arifa et al., 2021), although statistically it has not shown a significant difference (P>0.05).

Bursa of Fabricius

The relative weight of bursa of Fabricius of 35-day-old broiler chickens showed no significant difference between treatments (P>0.05). The average bursa of Fabricius weight values ranged from 0.21±0.05% to 0.24±0.05%, with inter-group variations still within normal physiological limits. Although numerically there were differences between treatments, statistically this fluctuation was not strong enough to show a significant effect from the administration of ginger and *Coleus amboinicus* extracts during 35 days of rearing.

The bursa of Fabricius is a primary lymphoid organ typical of poultry that plays an important role in B lymphocyte differentiation.

The absence of significant differences is possibly due to the level of bursa sensitivity to bioactive stimulation that requires more specific time and concentration. In addition, the bursa of Fabricius has developmental characteristics that are highly dependent on chicken age and initial immune status, so the effects of herbal extracts given for 35 days may not be sufficient to provide significant changes in organ size.

The results of this study are in line with findings by (Amanda 2025) who reported that the percentage of bursa of Fabricius weight was in the range of 0.20% to 0.22% after administration of feed additives in the form of ginger and African leaves. (Mulyani et al., 2020) also noted that herbal extract administration can stimulate humoral immune activity, although it does not always directly impact the increase in bursa of Fabricius weight. Conversely, (Sutrisno et al., 2018) found that the bursa of Fabricius tends to be more stable compared to other secondary lymphoid organs when facing immune stimuli, especially if there is no pathogen infection as the main trigger. In addition, (Phillips et al. 2023) added that the mechanism of bursa modulation by natural feed additives is more indirect, through increased antibody production and B lymphocyte activation. Overall, these findings support the results of this study which show that the relative weight of bursa of Fabricius did not undergo significant changes due to the treatments given.

Spleen

Unlike thymus and bursa, the relative weight of spleen showed significant differences (P<0.05) between treatments. Treatment T2 produced the highest relative spleen weight (0.15±0.02%), which was significantly different from T0 (0.09±0.01%) and T1 (0.08±0.04%). Treatment T3 produced moderate values (0.11±0.02%) which were not significantly different from other groups but showed a tendency to increase. This indicates that the administration of a combination of ginger and C*oleus amboinicus* extracts synergistically can increase the development of secondary lymphoid organs such as the spleen.

The spleen serves as a blood filter and site of secondary immune response through T and B cell activation and antigen phagocytosis. The increase in spleen weight in T2 shows more intensive immune activity, most likely due to the synergistic effect between active compounds

from both herbal plants that can stimulate immune cell proliferation. Ginger extract contains gingerol and zingiberene, while *Coleus amboinicus* leaves contain tannins and flavonoids known to function as natural immunostimulants. This combination appears to successfully increase the workload and cellular activity in the spleen, thereby increasing the mass of the organ.

The increase in broiler chicken spleen weight can be associated with the administration of herbal plant combinations that function as natural immunostimulants. Research (Satrian et al., 2022) demonstrated that broiler chickens given betel leaf decoction (Piper betle) in drinking water exhibited relative spleen weights ranging from 0.172% (T3) to 0.230% (T0/ control), with intermediate values at 0.198% (T1) and 0.184% (T2). Although statistically non-significant (P>0.05), these findings indicate that herbal treatments influence spleen development through antioxidant and antimicrobial actions. This is reinforced by findings by Sulistiyanto et al. (2019) who noted that broiler chickens given processed wheat pollard-based feed showed spleen weight percentages ranging from 0.11% to 0.17%, showing variations influenced by composition and characteristics of feed additives. Other research by Wijayanti et al. (2020) showed that herbal-based feed additive administration can increase lymphocyte activity, which contributes to spleen enlargement as a center of secondary immune activity. In line with that, Lee et al. (2019) stated that phenolic compound content in herbal plants has a role as natural adjuvants capable of stimulating cytokine expression in spleen tissue, thereby supporting increased volume and activity of lymphoid tissue. Overall, these results strengthen the findings in this study, where treatment T2 showed a positive trend in increasing immune response, as shown by the increase in relative spleen weight although not significantly different statistically.

CONCLUSIONS

The addition of 2% fermented ginger (*Zingiber officinale*) and bangun-bangun leaf (*Coleus amboinicus*) extract in broiler drinking water is proven safe and effective in improving performance, as shown by increased body weight gain, better feed efficiency, and higher feed intake without reduced efficiency. Immune organs remained normal, with a significant but still normal increase in spleen weight. Therefore, this combination has strong potential as a natural feed additive to enhance broiler performance and immunity, serving as a safe alternative to synthetic antibiotics.

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