









Lymphoid Organ Response Of Broilers With Addition Of Fermented Castor Leaves (*Jatropha Curcas Lin*) And Ginger (*Zingiber Officinale Var*) In Drinking Water

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ABSTRACT

This study aims to evaluate the response of the lymphoid organs of broilers with the addition of fermented castor leaves (Jatropha curcas Lin) and ginger (Zingiber officinale Var) in drinking water against the immunity of broilers, measured through the weight of lymphoid organs (bursa fabricius, thymus, and spleen) and final weight. The method used is an experiment with a Completely Randomized Design (CRD) consisting of 5 treatments and 4 replications. The treatments consist of: A (control), B (Addition of 0,5% fermented castor leaves and ginger in 1 liter of water), C (Addition of 1% fermented castor leaves and ginger in 1 liter of water), D (Addition of 1,5% fermented castor leaves and ginger in 1 liter of water), E (Addition of 2% fermented castor leaves and ginger in 1 liter of water). The observed parameters include the weight of lymphoid organs (bursa fabricius, thymus, spleen) and final weight. The data obtained were analyzed using analysis of variance (ANOVA). The research results show that the addition of fermented castor leaves and ginger has nonsignificant effect (P>0,05) on the weight of the bursa of fabricius, thymus, spleen and final weight. The conclusion of this study is that the addition of fermented castor leaves and ginger in drinking water is acceptable without producing negative effects that significantly influence the percentage of lymphoid organ weight, and thus can be considered as a feed additive in drinking water for broilers to enhance body resistance.

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Introduction

The poultry farming industry, particularly broilers, is one of the sectors that contributes significantly to the supply of protein. Broilers are known for their rapid growth and have advantages such as tender meat, large body size, and high feed efficiency. However, rapid growth comes with risks, one of which

is increased vulnerability to disease attacks. The immune system plays an important role iin maintaining the health of broilers to avoid various diseases.

All this time, maintaining the livestock's immunity is generally done through the administration of antibiotics. However, the continuous use of synthetic antibiotics is feared to create residues in meat products that negatively impact consumer health. Therefore, safer alternatives need to be developed, such as the utilization of herbal plants containing antioxidants and antibacterials to reduce the need for vaccines and supplements [1]. Two plants that serve as alternatives to commercial feed additives are castor leaves (*Jatropha curcas* Lin) and ginger (*Zingiber officinale*, Var).

The utilization of medicinal plants such as casto leaves and ginger as a natural alternative to enhance the health and performance of broilers is quite promising. Castor leaves are known to contain various active compounds such as flavonoids, saponins, alkaloids, streoids, terpenoids, tannins, and phenols that have antibacterial and antioxidant functions [2]. Meanwhile, ginger contains essential oils that have antimicrobial, antiseptic, and antioxidant properties, which can boost livestock's immunity and reduce dependence on antibiotics as antibiotic growth promoters (AGP)[3].

The efficiency of feed usage is also significantly influenced by the availability of quality drinking water. However, the utilization of Castor leaves as an additive in broiler drinking water has not been widely practiced by farmers. Castor leaves contain antinutritional compounds such as phorbol esters that are toxic to livestock. The fermentation process using microbes has been proven to reduce these toxic compounds, making them safe for animal consumption [4]. In addition, ginger contains gingerol and shogaol, which have antimicrobial, antioxidant, and anti-inflammatory properties; fermentation enhances these compounds through biotransformation, making their effectiveness in improving the performance and health of chickens more optimal [5].

Fermentation can also be a solution to enhance the utility of castor leaves and ginger, while simultaneously extending the shelf life of these materials. Research on the fermentation of castor leaves and ginger as a natural antibiotic substitute for broilers is still very limited. Therefore, the addition of fermented castor leaves and ginger to drinking water is expected to improve the immune resistance of broilers, which can be measured through the weight of lymphoid organs such as the bursa of fabricius, thymus, spleen, and final weight. This lymphoid organ is an important indicator in assessing the immunity of poultry, where the bursa of fabricius functions as an antibody producer. A sustained increase in antibody activity can lead to depletion of lymphoid follicles and a decrease in the number of lymphocytes, thereby reducing the ability to produce antibodies [6], [7].

Based on the description, this study aims to observe the response of the lymphoid organs in broilers with the addition of fermented castor leaves and ginger in drinking water towards the percentage of lymphoid organs that functions as a natural antibiotic, as well as to determine the optimal dosage of fermented castor leaves and ginger on the percentage of the weight of lymphoid organs and final weight.

Materials and methods

Time and place

This research was conducted in February 2025 at the Animal Production Laboratory and the Quality Testing and Analysis Laboratory, State Agricultural Polytechnic of Payakumbuh for a duration of 3 months.

Materials and tools used

- 1. The materials used in the fermentation of castor leaves and ginger are: castor leaves, ginger, EM4, molasses, and well water.
 - The materials used in maintenance are: broiler DOC, commercial feed, corn, fish meal, soybean meal, palm kernel cake, bran, oil and minerals.
- 2. The tools used in the fermentation of castor leaves and ginger are: knife, digital scale, blender, measuring cup, strainer, 20-liter capacity gallon, and bucket.
 - The tools used in maintenance are: 20 units of cages sized 0,6 m x 1 m, feed containers, drinking places, trays, analytical scale, 10 kg capacity feed scale, light bulbs, pitting, plugs, water liters, partitions, and thermometer.

Procedure for making fermentation of castor leaves and ginger

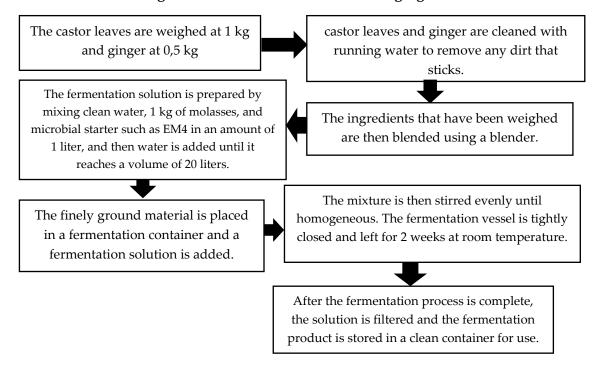


Figure 1. Stages of the fermentation process of castor leaves and ginger

Fermentation application of Castor leaves and ginger

The research was conducted on 100 broiler chickens from 1 day to 5 weeks of age. The feed used in the maintenance was a commercial feed and a basal feed. The commercial feed was given until the chickens were 1 week old, starting from the 2nd week a mixture of commercial and basal feeds was used to prevent stress from the new feed. The treatment involving the provision of fermented Castor leaves and ginger started from the 2nd week to the 5th week, administered through drinking water. The commercial feed contains a protein level of 21-22% and a metabolizable energy of 3000 Kcal. The basal feed was also formulated with a protein level of 22% and metabolizable energy of 3000 Kcal. The basal feed can be seen in Table 1.

Table 1. Ingredients and basic feed formulation for broilers during rearing

| Feed ingredients | Feed formulation (%) |
|------------------|----------------------|
| | |

| Corn | 53 |
|-------------------------|--------|
| Soybean meal | 34 |
| Palm kernel cake | 1 |
| Fish meal | 7 |
| Bran | 2 |
| Coconut oil | 2 |
| Mineral | 0 |
| Total | 100 |
| Nutritional content | |
| Crude protein (%) | 21,92 |
| Rough fiber (%) | 5,57 |
| Fat (%) | 2,49 |
| Ca (%) | 0,56 |
| P (%) | 0,48 |
| Metabolic energy (Kcal) | 3099,8 |

Description: Based on the Quality Testing Analysis and the Agricultural Polytechnic Analysis of Payakumbuh (2025) as well as the ration calculation based on the needs of broilers. The basal ration formulation is the same for all treatments.

Table 2. The content of phytochemical results of the fermentation of Castor leaves (*Jatropha curcas Lin*) and ginger (*Zingiber officinale Var*).

| Content | Before fermentation | After fermentation | | | |
|--------------------------|---------------------|--------------------|--|--|--|
| Flavonoid (ppm) | 330,49 | 99,64 | | | |
| Total phenol (ppm) | 1619,82 | 3093,66 | | | |
| Antioxidant activity (%) | 64,19 | <i>7</i> 5,11 | | | |

Description: Based on the Quality Testing Laboratory Analysis and the State Agricultural Polytechnic of Payakumbuh (2025).

Research method

This research uses a Completely Randomized Design (CRD) with 5 treatments and 4 replications. The treatments tested are as follows:

- A: No addition of fermented Castor leaves and ginger.
- B: Addition of 0,5% fermented Castor leaves and ginger in 1 liter of water.
- C: Addition of 1% fermented Castor leaves and ginger in 1 liter of water.
- D: Addition of 1,5% fermented Castor leaves and ginger in 1 liter of water.
- E: Addition of 2% fermented Castor leaves and ginger in 1 liter of water.

Data collection of lymphoid organs was conducted when the chickens were 35 days old, by taking samples from 1 chicken per cage unit, resulting in a total of 20 chickens for 20 cage unit. Thus, the total number of chickens is 20 for 20 cage units. Lymphoid organs were obtained through surgery on chickens to take the lymphoid organs, namely the spleen, bursa of fabricius, and thymus using a scalpel and tweezers. The lymphoid organs were weighed using an analytical balance with a precision of 0,01 grams. The data obtained from the final weight, the weight of the lymphoid organs consists of the spleen, thymus, and bursa fabricius.

- 1. Percentage of the spleen
 - Calculated using the method of [8], which involves weighing the spleen organ that has been cleaned of any attached blood. Weighing the spleen organ (g) is divided by the live weight (g) x 100%.
- 2. Percentage of the fabricius bursa

Calculated using the method of [9] by weighing the cleaned bursa fabricius organ. Weighing the bursa fabricius organ (g) divided by the live weight (g) \times 100%.

3. Thymus Percentage

Calculated according to the method described by [9], by weighing the cleaned thymus organ. The thymus weight (g) was divided by the live body weight (g) and multiplied by 100% to obtain the percentage.

4. Final Body Weight

Determined based on the method of [10], by weighing the body weight of broilers at 35 days of age after being fasted and prior to slaughter.

The obtained data were subjected to analysis of variance (ANOVA) to determine the effect of the treatments. Subsequently, Duncan's Multiple Range Test (DMRT) was applied to identify significant differences between treatment groups [11].

Results And Discussion

The results of the study with the addition of fermented castor leaves (*Jatropha curcas, Lin*) and ginger (*Zingiber officinale* var.) in drinking water on the percentage of lymphoid organ weight and final weight can be seen in Table 3.

Table 3. Effect of Fermented Jatropha curcas Leaf and Zingiber officinale Supplementation in Drinking Water on Final Body Weight, Bursa of Fabricius Percentage, Spleen, and Thymus

| Treatment | Final weight | Bursa of | Thymus (%) | Spleen (%) |
|-----------|-------------------|-------------------|----------------------|------------------|
| | (kg) | fabricius (%) | | |
| A | $1,839 \pm 0,108$ | 0.19 ± 0.065 | $0,23 \pm 0,022$ | $0,15 \pm 0,079$ |
| В | $1,933 \pm 0,045$ | $0,20 \pm 0,059$ | $0,25 \pm 0,020$ | 0.11 ± 0.036 |
| C | $1,895 \pm 0,030$ | $0,20 \pm 0,048$ | $0,24 \pm 0,010$ | 0.11 ± 0.022 |
| D | $1,884 \pm 0,096$ | $0,21 \pm 0,0049$ | $0,29 \pm 0,030$ | $0,10 \pm 0,018$ |
| E | $1,871 \pm 0,039$ | $0,22 \pm 0,0314$ | $0,\!27 \pm 0,\!069$ | $0,11 \pm 0,021$ |

Final weight

The final body weight of broilers across all treatment groups showed relatively uniform values, ranging from 1.839 ± 0.108 kg to 1.933 ± 0.045 kg. Differences among the treatment groups did not follow a distinct pattern and were not statistically significant. The supplementation of fermented *Jatropha curcas* leaves and *Zingiber officinale* in drinking water produced final body weights that remained within the normal growth range for broilers harvested at 35 days of age.

Statistical analysis indicated that the differences in final body weight between groups were not significant (P>0.05), suggesting that the variations observed were more influenced by individual biological differences rather than by the treatment effects [12]. These findings imply that the inclusion of fermented *Jatropha* and ginger has not yet demonstrated a statistically significant impact on broiler final body weight.

Nevertheless, the final body weights recorded in this study are comparable to those reported by [13], who observed average final weights ranging from 1981 to 2102.25 g/bird following the supplementation of *Piper betle* extract in drinking water at 8 weeks of age. Similarly, a study

by [14], reported lower final weights, ranging from 1380.75 g/bird to 1510.88 g/bird, with the inclusion of fermented fruit peel meal in the diet at 35 days of age.

This finding is consistent with the results of [15], who reported that low-dose supplementation of fermented herbal materials did not significantly affect broiler growth performance. According to [16], it was also noted that natural feed additives to performance responses are often gradual and require a longer application period to provide significant results.

Herbal plants such as *Jatropha* leaves and ginger contain bioactive compounds that are thought to support digestive tract performance and metabolic efficiency, but their success depends on the dose, formulation, and duration of administration [17]. Fermentation with microorganisms such as *Lactobacillus* or yeast has the potential to increase the bioavailability of these active compounds [18], but optimal adjustments are needed in their use to achieve significant results Scientifically, final body weight is a complex indicator influenced by various physiological processes, including energy metabolism and gut health. The lack of significant effects observed in this study highlights the need for further refinement in the dosage and frequency of fermented phytogenic supplementation using *Jatropha* and ginger. This aligns with the findings of [19], who suggested that herbal interventions often require long-term strategies to produce consistent effects on growth parameters.

Bursa of Fabricius

The bursa of Fabricius is a primary lymphoid organ that plays a crucial role in the maturation and differentiation of B cells and is highly sensitive to immunostimulant stimuli. The percentage of bursa of Fabricius weight per broiler at five weeks of age is shown in Table 3. The table indicates that supplementation with fermented *Jatropha curcas* leaves and *Zingiber officinale* resulted in bursa percentages ranging from $0.19 \pm 0.065\%$ to $0.22 \pm 0.0314\%$ across all treatment groups, with minimal fluctuation. These values fall within the normal physiological range and do not exhibit a dominant trend favoring any specific treatment group.

Statistical analysis revealed that differences in bursa weight percentage were not significant (P>0.05), indicating that the treatments did not exert a strong enough effect to differentiate lymphoid organ development among groups [20]. The observed variation is more likely attributable to natural individual differences than to responses elicited by the treatments.

Previous studies have reported that herbal plants can modulate poultry immune activity [21], with phytochemicals acting as natural immunostimulants that enhance immunoglobulin production [22], [23]. Although the relative bursa weights observed in the current study (0.19%–0.22%) are modest, they are higher than those reported by [9], who recorded bursa weights between 0.07% and 0.11%, and comparable to the findings of [24], who reported values ranging from 0.20% to 0.22%.

Such differences may be attributed to the types and dosages of natural feed additives used, as well as variations in treatment protocols. [25] reported bursa weight percentages ranging from $0.16 \pm 0.030\%$ to $0.196 \pm 0.027\%$ with the use of *Bawang Dayak*-based eco-enzymes, while [26] documented much higher values, ranging from $0.82 \pm 1.10\%$ to $0.95 \pm 1.15\%$. These findings highlight that the efficacy of feed additives in enhancing bursa weight is strongly influenced by their composition, properties, and the method of administration.

A healthy bursa of Fabricius weight at 3–5 weeks of age is generally within 0.2%–0.4% of body weight. The values obtained in this study (0.19%–0.22%) fall within this normal range, indicating no disruption to the broilers' immune function. According to research by [27], the optimal size of the bursa of fabricius indicates lymphocyte activity and healty. The shrinking size of the bursa fabricius can occur due to environmental stress, maintenance management, or feed toxins [28]. While excessive enlargement may indicate immune system activation due to pathogenic exposure, though it does not always reflect improved health. The stable bursa size despite fermented herbal supplementation in this study suggests that the additives did not suppress immune function and may be suitable as natural immunostimulants [29].

According to research by [30], the provision of natural feed additives in the form of herbal infusions also showed similar results, namely no significant effect on the bursa fabricius. However, there was an increase in positive trends that were not always reflected statistically. This phenomenon confirms that adaptive immune organs such as bursa have high sensitivity to various internal and external factors, including stress and environmental management. According to research by [27], the bursa response to immunostimulants substances varies greatly and the effects bioactive substances such as flavonoids or saponins do not always result in directly measurable morphological changes.

Supporting this, [31] showed that adaptive immune responses require adequate stimulation and time. From an immunological perspective, the enhancement of bursa function is not solely indicated by its size, but also by cellular activity within the organ. Therefore, the absence of significant morphometric changes does not rule out the possibility of immunomodulatory effects at the molecular level. Further studies using histological and gene expression approaches are necessary to assess the true potential of fermented herbal combinations in modulating the immune system in broilers [32].

Thymus

The thymus is one of the key organs of the immune system in broilers. It plays an essential role in the development and maturation of immune cells, particularly T lymphocytes (T cells), which are crucial for defending against viral, bacterial, and other pathogenic infections. According to [33], the thymus is susceptible to shrinkage due to stress or even atrophy in response to disease. The percentage of thymus weight per broiler at 5 weeks of age is presented in Table 3. The table shows that the addition of fermented *Jatropha curcas* leaves and *Zingiber officinale* resulted in thymus weight percentages ranging from $0.23 \pm 0.022 \%$ to $0.29 \pm 0,030 \%$ across all treatment groups, reflecting physiological stability of the broiler immune system. The observed fluctuations remain within the normal range, with no treatment group showing a notably dominant effect.

Statistical analysis indicated that the differences in thymus weight percentage were not significant (P>0.05), suggesting that the supplementation with fermented *Jatropha* and ginger did not cause a statistically measurable change in relative thymus size [34]. This may be attributed to the physiological adaptability of broilers, which remained within tolerable limits. The normal thymus size in broilers aged 3–5 weeks ranges from 0.2% to 0.4% of body weight; the observed values of 0.23%–0.29% fall within this physiological norm, indicating healthy

birds. A reduced thymus size may indicate stress, while an enlarged thymus may suggest immune hyperactivity. According to [35], thymus size can be influenced by factors such as herbal supplementation, fermentation products, stress levels, and ambient temperature.

Ginger contains bioactive compounds that are known to affect the production of interleukins and interferons—cytokines involved in T cell differentiation [36], [37]. Additionally, [38] reported that ginger's anti-inflammatory properties help reduce tissue swelling and inflammation, allowing the thymus to function more efficiently in T cell production. *Jatropha curcas* leaves have also been shown to enhance immune gene expression by modulating gut microflora [39], [40]. Supporting this, [41] observed that increased thymus weight correlated with heightened immune activity in broilers, suggesting that the combination of ginger and *Jatropha* could enhance T cell maturation and overall immune efficiency [35], [42].

In this study, the thymus percentage ranged from $0.23 \pm 0.022\%$ to $0.29 \pm 0.030\%$. In comparison, [8] reported relative thymus weights between 0.090% and 0.197%, while [29] noted that empirical ginger extract supplementation in drinking water resulted in thymus percentages ranging from 0.25% to 0.34%. The values observed in the current study are higher than those reported by [24], who found relative thymus weights between $0.16 \pm 0.02\%$ and $0.22 \pm 0.05\%$ in broilers supplemented with fermented ginger and African leaf extract. Furthermore, broilers supplemented with eco-enzymes in drinking water exhibited lower thymus weights, ranging from $0.16 \pm 0.062\%$ to $0.198 \pm 0.079\%$ [43].

These findings align with [44], who concluded that oral administration of fermented phytogenic substances in broilers does not necessarily result in significant changes in primary lymphoid organs. The effects of such natural compounds are often indirect and require a longer period to induce structural changes.

The thymus plays a central role in T cell maturation, which is vital for cell-mediated immune responses. According to [45], thymus size does not always correlate directly with immune activity; increased immune function may occur without noticeable changes in organ morphology. Gingerol, a key compound in ginger, and the controlled levels of potentially toxic compounds in *Jatropha* leaves may exert immunomodulatory effects, but require an optimized combination to produce significant physiological and statistical outcomes.

The bioactive compounds in fermented herbal plants may stimulate the immune system via gut microbiota modulation and enhancement of mucosal immune responses, rather than directly altering organ size. This supports the growing perspective that the efficacy of natural immunostimulants should not be evaluated solely based on morphometric changes, but also on functional and molecular immune responses [46].

Spleen

The spleen is a vital organ involved in maintaining fluid balance, transporting white blood cells, and managing immune responses against infections and diseases. The relative spleen weight per broiler at 5 weeks of age is presented in Table 3. The data show that the inclusion of fermented *Jatropha curcas* leaves and *Zingiber officinale* in drinking water resulted in spleen weight percentages ranging from $0.10 \pm 0.018\%$ to $0.15 \pm 0.079\%$. All treatments yielded values

within the physiological norm for 35 day old broilers, with minimal variation observed among groups.

Statistical analysis revealed no significant differences (P>0.05) among the treatments, indicating that supplementation with fermented *Jatropha* and ginger had no significant effect on the development of the spleen as a secondary lymphoid organ [47]. These findings suggest that the non-specific immune response, as reflected by spleen weight, remained unaffected, implying that the broilers were not subjected to excessive stress or immunological stimulation during the supplementation period.

According to [48], while herbal formulations may enhance other performance parameters, their effects on spleen size are not always significant. [49] also noted that the spleen is more sensitive to chronic stress or systemic infections than to herbal treatments. A reduction in spleen size in some treatments may indicate the absence of an immune response to pathogens, implying that the chickens were in good health.

Other studies, such as those by [50], [51], have reported that stable spleen size can serve as an indicator of favorable environmental and management conditions. [52] added that optimal feeding and environmental management tend not to stimulate a significant increase in spleen size. Therefore, the absence of marked enlargement suggests a lack of physiological stress or infection, implying that fermented *Jatropha* and ginger may help reduce stress in broilers, thereby maintaining normal spleen morphology.

The present findings are in agreement with [24], who reported spleen weight ranges of $0.10 \pm 0.07\%$ to $0.15 \pm 0.07\%$ in broilers supplemented with fermented ginger and African leaf extract. Similarly, [43] found spleen weights ranging from $0.107 \pm 0.034\%$ to $0.154 \pm 0.102\%$ with the inclusion of dayak onion–based eco enzymes. [53] reported relative spleen weights of 0.11%–0.18% when noni juice was added to drinking water, and [54] found spleen percentages between 0.14%–0.16% with the addition of *Uncaria gambir* extract (0–0.3 g/kg feed).

In contrast, [55] reported higher spleen weights ranging from $0.26 \pm 0.01\%$ to $0.293 \pm 0.03\%$. However, [56] found similar outcomes to the current study, where supplementation with fermented herbal plants did not significantly affect relative spleen weights. This consistent spleen response may reflect systemic immune stability, particularly under well-managed rearing conditions.

The spleen plays a crucial role in blood filtration and immune responses to pathogens. According to [57], not all immunostimulant interventions lead to changes in spleen size, as its primary function is more related to phagocytic activity and antigen presentation. Compounds such as gingerol from ginger or secondary metabolites from *Jatropha* may exert systemic effects without significantly altering spleen mass.

Physiologically, the spleen functions as a reservoir for erythrocytes and a site for secondary lymphocyte production. The non-significant changes observed in this study may be interpreted as indicators of immune system stability, indicating no systemic inflammation or excessive immune response during the rearing period. This aligns with the findings of [45], who reported that the immunological effects of natural feed additives often occur internally and may not be reflected in the macroscopic morphology of lymphoid organs.

Conclusion

The supplementation of fermented *Jatropha curcas* leaves and *Zingiber officinale* in drinking water had no significant effect on final body weight or the relative weights of the bursa of Fabricius, thymus, and spleen. Inclusion levels ranging from 0.5% to 2% were well tolerated and did not adversely affect these lymphoid organ parameters or broiler growth performance.

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