This is an open-access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)

P-ISSN: 2304-3075; E-ISSN: 2305-4360

International Journal of Veterinary Science

www.ijvets.com; editor@ijvets.com

Research Article https://doi.org/10.47278/journal.ijvs/2025.156

# The Study of Bitter Leaf Extract *(Vernonia Amygdalina)* as an Alternative Feed Additive on Growth Performance, Carcass Quality, Intestine Morphology, and Microbial Counts of Broiler Chickens

Samadi [1,2,*], Sitti Wajizah [1,2], Sugito [2,3], Ilham [1,2], Taufiq Hidayat [4] and Indra Wahyudi [1,2]

[1]Department of Animal Science, Faculty of Agriculture, Universitas Syiah Kuala, Banda Aceh, 23111, Indonesia
[2]Research Center for Innovation and Feed Technology, Universitas Syiah Kuala, Banda Aceh, 23111, Indonesia
[3]Faculty of Veterinary, Universitas Syiah Kuala, Banda Aceh, 23111, Indonesia
[4]Department of Animal and Poultry Science, College of Agriculture and Bioresources, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK, S7N 5A8, Canada
***Corresponding author:** samadi177@usk.ac.id

**Article History:** 25-213 Received: 31-Jul-25 Revised: 23-Oct-25 Accepted: 12-Nov-25 Online First: 12-Dec-25

## ABSTRACT

The restriction on the use of antibiotics in animal feed has sparked renewed interest in phytogenic additives derived from plants and herbs. These bioactive plant compounds present a natural alternative to enhance animal health and productivity, whilst minimizing the risk of antimicrobial resistance. This study aims to evaluate bitter leaf extract (*Vernonia amygdalina*) as an alternative feed additive for broiler chickens. A total of 100 mixed-sex broiler chicks were randomly allocated to 20 cages (1×1m) under four treatments, with five replications. Each group received *V. amygdalina* extract in drinking water at concentrations of 0, 250, 500, and 750mg/L. The chickens were fed iso-protein and iso-energy diets (23% CP, 3200kcal ME/kg for days 1–14; 20% CP, 3200kcal ME/kg for days 15–28). Feed intake and water consumption were recorded daily, and carcass traits, intestinal morphology, and microbial counts were evaluated at the end of the experiment. The study showed that supplementation of *V. amygdalina* extract in drinking water for 28 days had no significant effect (P>0.05) on body weight, feed intake, feed conversion ratio, or water consumption. However, there was a noticeable trend towards increased weight and carcass percentage, with the 250mg/L treatment yielding the highest results (1357.20g; 74.78%) compared to the control group. Moreover, no adverse effects were observed on the morphology of the duodenal villi or on microbial counts (P>0.05). In conclusion, *V. amygdalina* extract can serve as a safe alternative feed additive, potentially replacing antibiotics in broiler chickens without negatively impacting growth performance, gastrointestinal health, or carcass quality.

**Keywords:** Broiler, Carcass Quality, Feed Additive, Intestine Morphology, Performance, *Vernonia amygdalina.*

## INTRODUCTION

Due to restrictions on the use of antibiotics as feed additives in animal nutrition, phytogenic feeds, such as aromatic plants and herbs, have been used as alternatives. Phytogenic compounds are plant-derived bioactive molecules that play essential roles in plant defense systems. Plant-derived feed additives have been intensively researched as antibiotic alternatives for animal health and productivity, due to their various biological functions and lower likelihood of generating resistance in finishing products (Wang et al. 2024; Aminullah et al. 2025). Multiple studies have examined the potential benefits of using plant extracts as alternative feed additives for broiler chickens. The use of non-antibiotic additives as pre-probiotics, phytochemicals, organic acids, and enzymes has been well documented and proven to enhance growth performance, improve gut health, modulate immune responses, and exhibit antimicrobial and antioxidant properties (Murugesan et al. 2015; Pirgozliev et al. 2019; Samadi et al. 2019; 2020; 2022; Imran et al. 2021; Wahyudi et al. 2021; Oni and Oke 2025).

Phytogenic feed additives generally improve animal growth and production performance by enhancing feed palatability, increasing feed intake, or improving the secretion and activity of endogenous enzymes and nutrient utilization (Wang et al. 2024). These benefits could make

**Cite This Article as:** Samadi, Wajizah S, Sugito, Ilham, Hidayat T and Wahyudi I, 2026. The study of bitter leaf extract *(Vernonia Amygdalina)* as an alternative feed additive on growth performance, carcass quality, intestine morphology, and microbial counts of broiler chickens. International Journal of Veterinary Science 15(2): 390-398. https://doi.org/10.47278/journal.ijvs/2025.156

plant-derived feed additives a viable option for sustainable poultry production. However, their effects might vary depending on the amount and the origin of phytochemicals involved. A study also suggests that the bioavailability and the biological effects of phytochemicals can be influenced by their interactions with other food components; these interactions can either enhance or impair their bioactivity (Phan et al. 2018). Bitter leaves *(Vernonia amygdalina)*, also called African leaf, are a small tree, around 2-5m tall, of the Asteraceae with an abundant bitter taste. Bitter leaf extract has been extensively studied, mainly for its health benefits and uses (Unigwe et al. 2025; Ikpamezie et al. 2025). Overall, bitter leaf extract has demonstrated beneficial antioxidant, antibacterial, hepatoprotective, and corrosion-inhibitory effects in several tests. Research found a substantial number of flavonoids in the extract, indicating excellent antioxidant effect (Imaga and Bamigbetan 2013). Raimi et al. (2020) discovered that an aqueous leaf extract of bitter leaf can reduce liver damage. Similarly, Uchendu (2018) investigated the hepatoprotective effects of an aqueous extract of bitter leaf on acetaminophen-induced liver injury in albino Wistar rats. Mandey et al. (2022) reported that bitter leaves contain high levels of linoleic and palmitic fatty acids and abundant amounts of the amino acids phenylalanine, serine, isoleucine, glycine, and arginine.

A recent study by Kismiati et al. (2023) on Japanese quail showed that supplementation of 20mL/L bitter leaf to drinking water had a significant effect on carcass weight, cholesterol, and meat Water Holding Capacity (WHC) without affecting carcass and non-carcass percentages, moisture, protein, fat, and meat color qualities. However, a dissimilar result was obtained by Mandey et al. (2022), who administered up to 30mL/L to broiler drinking water, with no effect on feed intake or carcass percentage. The differences between studies need to be examined to obtain more consistent results. In addition, research on *V. amygdalina* remains limited. Therefore, this study aims to determine the effect of bitter leaf extract as a feed additive at different concentrations on growth performance, carcass quality, intestinal morphology, and microbial counts in broiler chickens. The outcomes of this study can be used to provide recommendations to the feed industry, especially the feed additives industry, and to promote more sustainable farming practices that produce safe animal products for human consumers.

## MATERIALS AND METHODS

### Animal, feeding, and data collection

The growth study of broiler chickens was conducted at a private farm in Banda Aceh. Carcass measurements were conducted at the Animal Nutrition and Feed Technology Laboratory, Science Department, The Faculty of Agriculture, Universitas Syiah Kuala. The analysis of the morphology and microflora of the small intestine was carried out at the Microbiology Laboratory of the Faculty of Veterinary, Universitas Syiah Kuala.

A total of 20 research cages measuring (1×1m) were prepared, and the floor was covered with wood-shaving litter. Each cage unit was equipped with drinking water and feed, with capacities adjusted to the age of the broilers. The cage floor, before being sprinkled with wood shavings, was first sprinkled with lime mixed with water. Prior to conducting the research, the cages and all research equipment were disinfected with Rodalon (Pyridam) at a dose of 15mL/10L of water to kill pathogenic microorganisms and insects. A total of 100 mixed-sex DOC were obtained from a commercial hatchery and randomly assigned to 20 research cages (1×1m), with 5 chickens per cage. The experiment comprised four drinking water treatments (P0, P1, P2 and P3), with each treatment involving 20 chickens divided into five cages (replicates). Each treatment group, consisting of five replications, was administered different concentrations of *V. amygdalina* extract in the drinking water, namely P0=0mg/L (control), P1=250mg/L, P2=500mg/L, and P3=750mg/L, provided ad libitum. The mean initial body weights of the chickens for each treatment (mean±SE) were 72.4±0.58g (P0), 71.5±0.47g (P1), 72.4±0.58g (P2), and 71.7±0.81g (P3), respectively.

To keep drinking water hygienic, the container was washed and refilled with fresh water daily. Feed was weighed every morning to determine the daily feed consumption. The cage temperature was controlled with a heating lamp and a fan. From the first to the seventh day, the cage temperature was maintained at 33°C; it was then decreased by 10°C each week until it reached 24°C, which was maintained until harvest on day 28. On the fourth day, all livestock were vaccinated with ND (Medivac La Sota-active) with a dose of 100mL per 100 DOCs through eye drops. On the 20$^{th}$ day, the chickens were vaccinated again with ND (Medivac La Sota-active) via injection of 0.5mL per chicken. To reduce stress, anti-stress agents and Vita Chick vitamins were added to the drinking water. The room conditions and cage ventilation were monitored continuously for 24 hours to ensure normal functioning throughout the research period.

Commercial feed was used in the research, which has been formulated to meat chickens' requirements according to recommendation of National Research Council (1994) with the content of protein and energy (23% protein; 3200kcal ME/kg feed at 1-14 days of age and 20% protein; 3200kcal ME/kg feed at 15-28 days of age). Feed intake and individual weight of broiler chickens were recorded at weekly intervals to measure Feed Intake (FI), Feed Conversion Ratio (FCR), and Body Weight Gain (BWG). Broiler mortality was recorded and weighted daily to account for growth and performance. At the end of the growth study, twenty broilers (one bird from each replicate) with the body weight close to the pen average were randomly selected to measure carcass, carcass, and carcass characteristics. Before selected broilers were slaughtered, feed was removed and animals were fasted overnight, with access only to water. All animals were slaughtered by cervical dislocation in an Islamic manner to obtain data for dressing percentage, carcass, and giblet characteristics. The intestinal tract was immediately removed. All carcass and giblet data were weighed and recorded for further analysis.

### Intestinal morphology

Measurement of small intestine morphology was performed using the method described by Wijayanti et al. (2017), in which intestine samples from each treatment group were cut into 1×1×1cm pieces. The small intestine samples were then immersed in a 10% Neutral Buffer

Formalin (NBF) solution. The samples were reduced in size, stored in tissue cassettes, and then refixed in 10% NBF. Following fixation, dehydration and clearing were performed using solutions of 70, 80, 90, and 96% alcohol, absolute alcohol, toluene, and paraffin, each for 2 hours. The organ samples were then embedded using an embedding set with liquid paraffin and cooled. The cooled blocks were then sectioned using a microtome with a thickness of ±4–5µm. Then, staining with Hematoxylin-Eosin (HE) was performed, followed by mounting in mounting medium. The preparations were then observed under a microscope at 50× magnification for each sample examined. Fig. 2 shows histopathological examination of the intestine of broiler chickens between controls and supplemented with *V. amygdalina* extracts in drinking water for 28 days.

### Microbial counts

The procedure for gut microbial analysis was as follows: gut contents were collected from a composite of five chickens per treatment and placed in sterile bottles. A total of 25g of intestinal contents was mixed with 225mL of Selenite Cysteine solution (for *Salmonella*) and Phosphate Buffer solution (for Lactic Acid Bacteria/LAB and *Escherichia coli*). This mixture was homogenized and incubated at 37°C for 2 hours. Following incubation, the homogenized sample was serially diluted to 106, and aliquots were inoculated in duplicate onto selective agar media: *Salmonella* on Salmonella-Shigella Agar (SSA), *E. coli* on Eosin-Methylene Blue Agar (EMBA), and LAB on de Man, Rogosa, and Sharpe Agar (MRSA). The inoculated plates were subsequently incubated under anaerobic conditions at 36°C for 24 to 48 hours. The number of bacterial colonies was counted using the Total Plate Count (TPC) method, and the average live bacterial population was determined based on the initial weight of the gut contents. The final results were expressed in logarithmic Colony-Forming Units (CFU/g). *E. coli* colonies on EMBA were identified by a metallic green color, while a pink or black color characterized Salmonella colonies on SSA.

### Statistical Analysis

All data obtained in this study were analyzed using SPSS statistical software (IBM SPSS Statistics 25.0). One-way ANOVA under a Completely Randomized Design (CRD) was employed to assess the effect of extracted *V. amygdalina* on broilers. The data were presented as mean±SEM. Differences among least squares means were declared significant at P<0.05, and trends were declared significant at P<0.010. Duncan's Multiple Range Test (DMRT) post hoc was employed to determine significant differences between experimental treatments (Steel and Torrie 1995).

## RESULTS

### Broiler performances

The effects of *V. amygdalina* extract supplementation in drinking water on broiler performance over a 28-day trial are presented in Table 1. Statistical analysis showed that there was no significant effect (P>0.05) of V. amygdalina extract on drinking water on any growth performance parameter at any dosage during the 28-day trial. Although statistical analysis in this study revealed that there was no significant effect among treatments (P>0.05), the numerical trend showed that P1 (1.37), P2 (1.40) and P3 (1.39) seem to had lower feed conversion ratio (FCR) compared to control (1.43), and also seem to had higher bodyweight gain on P1 (1683.2g) and P2 (1669.2g) compared to control (1655.15g). Furthermore, the numerical trend also revealed lower feed intake on P1 (2297.20g), P2 (2336.72g), and P3 (2254.12g) compared to control (2374.38g), and lower final water consumption on P1 (5711.20mL), P2 (5706.32mL), and P3 (5566.00mL) compared to control (5892.45mL). These trends suggest the potential impact on treatment, with further economic benefits. Therefore, further studies with a larger sample and different methods are needed to confirm this observation.

### Carcass and giblet characteristics

The result of *V. amygdalina* extract supplementation in drinking water on broiler carcass characteristics over a 28-day trial is shown in Table 2. Based on statistical analysis, there was no significant effect (P>0.05) of different *V. amygdalina* dosages on any carcass characteristics during the 28-day animal trial, indicating that the addition of *V. amygdalina* to drinking water had no adverse effect on the muscle tissue of the animals. Although the statistical analysis showed no significant effect (P>0.05), several numerical trends emerged among the treatment groups. The addition of *V. amygdalina* extract to drinking water tends to increase carcass weight and percentage, with P1 (1357.2g; 74.78%) being the highest among the treatments compared to the control (1282.4g; 72.23%). Ilham et al. (2023) also reported no effect (P>0.05) on the percentage and weight of carcass, breast, thigh, and back, while affecting wing weight and wing percentage (P<0.05) when using 0.25, 0.50, and 1.00g/L of Jamblang leaves (*Syzygium cumini L*) extract on drinking water for up to 30 days. A similar trend was also observed in breast weight and percentage, where groups receiving *V. amygdalina* extract (P1–P3) consistently showed higher values than the control, with P3 (502.67g; 38.59%) being the highest among treatments compared to the control (464.96g; 36.37%). Drumstick weight and percentage also showed a similar trend, where P1 (364.51g; 26.88%) was higher than the control (353.66g; 27.66%). Furthermore,

**Table 1:** The effect of *V. amygdalina* extract administration in drinking water on the performance of commercial broiler chickens was evaluated for a 28-day trial (n=5)

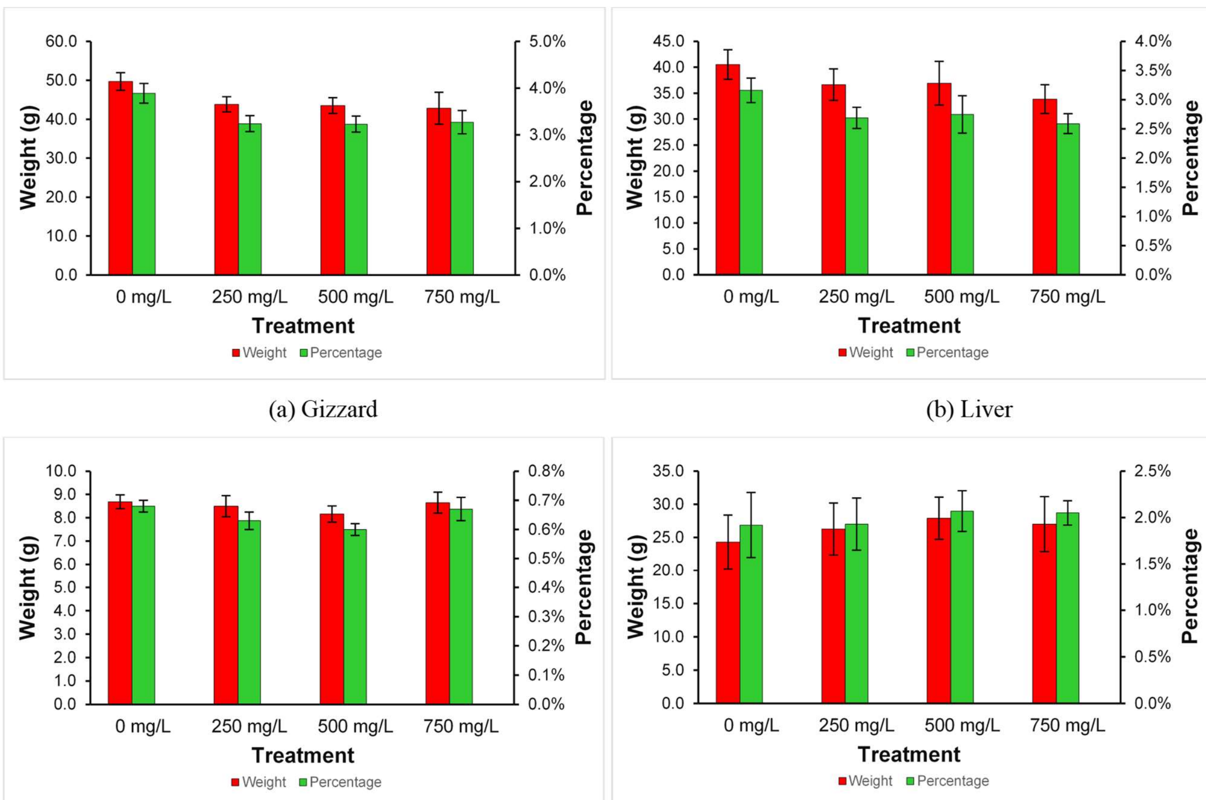
| Growth Performance | Treatment | | | | SEM | *P*–value |
|---|---|---|---|---|---|---|
| | P0 | P1 | P2 | P3 | | |
| Body Weight Gain (g) | 1655.15 | 1683.20 | 1669.20 | 1630.80 | 12.40 | 0.520 |
| Final Feed Intake (g) | 2374.38 | 2297.20 | 2336.72 | 2254.12 | 19.87 | 0.163 |
| Final Water Consumption (mL) | 5892.45 | 5711.20 | 5706.32 | 5566.00 | 50.60 | 0.150 |
| Feed Conversion Ratio | 1.43 | 1.37 | 1.40 | 1.39 | 0.01 | 0.234 |

P0= drinking water (control, no extract), P1= drinking water + 250mg/L *V. amygdalina* extract, P2= drinking water + 500mg/L *V. amygdalina* extract, P3= drinking water + 750mg/L *V. amygdalina* extract.

**Table 2:** The effect of *V. amygdalina* extract administration in drinking water on carcass characteristics of commercial broiler chickens for a 28-day trial (n=5)

| Carcass Characteristics | Treatment | | | | SEM | *P*-value |
|---|---|---|---|---|---|---|
| | P0 | P1 | P2 | P3 | | |
| Carcass | | | | | | |
| Weight (g) | 1282.43 | 1357.22 | 1347.75 | 1302.20 | 15.04 | 0.242 |
| Percentage (%) | 72.23 | 74.78 | 76.05 | 74.92 | 0.85 | 0.472 |
| Breast | | | | | | |
| Weight (g) | 464.96 | 510.05 | 498.10 | 502.67 | 9.09 | 0.325 |
| Percentage (%) | 36.37 | 37.50 | 36.96 | 38.59 | 0.52 | 0.518 |
| Wings | | | | | | |
| Weight (g) | 132.52 | 129.62 | 131.59 | 124.85 | 1.67 | 0.395 |
| Percentage (%) | 10.37 | 9.57 | 9.77 | 9.59 | 0.16 | 0.244 |
| Drumpstick | | | | | | |
| Weight (g) | 353.66 | 364.51 | 350.48 | 347.46 | 4.52 | 0.602 |
| Percentage (%) | 27.66 | 26.88 | 26.00 | 26.67 | 0.34 | 0.394 |
| Back | | | | | | |
| Weight (g) | 321.12 | 314.81 | 289.57 | 301.96 | 7.11 | 0.433 |
| Percentage (%) | 25.08 | 23.23 | 21.51 | 23.24 | 0.61 | 0.244 |

P0= drinking water (control, no extract), P1= drinking water + 250mg/L *V. amygdalina* extract, P2= drinking water + 500mg/L *V. amygdalina* extract, P3= drinking water + 750mg/L *V. amygdalina* extract.




(a) Gizzard (b) Liver

(c) Heart (b) Abdominal Fat

**Fig. 1:** The Effect of *V. amygdalina* Extract Administration in Drinking Water on Giblet Characteristics of Commercial Broiler Chickens for a 28-day trial.

the trends also revealed that the weight and percentage of wings and back of all treatments (P1–P3) were lower than the control group (132.52g; 10.37%) and (321.12g; 25.08%), subsequently, indicating a shift in muscle distribution mainly from wings and back to breast rather than overall mass loss.

The effect of administering *V. amygdalina* on giblet characteristics is presented in Fig. 1. Research findings indicate that administration of *V. amygdalina* extract at up to 750mg/L in drinking water has not significantly reduced abdominal fat (P>0.05). Unigwe et al. (2025) also reported no significant difference in abdominal fat percentage (P>0.05), and even a tendency toward an increase in abdominal fat in finishing broiler chickens fed *V. amygdalina* powder at 2.5–7.5g/bird. In addition, no significant differences were observed in the percentage of heart organs (P>0.05), with a range of 0.60–0.68%, which remains within the normal range (0.42–0.75%). These results are consistent with those of Badaruddin et al. (2022), who administered *V. amygdalina* powder at 2–6%

in broiler chicken feed and reported a heart rate range of 0.58–0.66%. For the liver, there was no significant change in its percentage (P>0.05), ranging from 2.59–3.16%, although slightly above the normal range (1.70–2.80%). This is similar to the results of Badaruddin et al. (2022), which were 3.40 to 3.85%. Meanwhile, the gizzard also showed no significant changes (P>0.05), with a range of 3.23–3.89%. This figure was higher than the results of Badaruddin et al. (2022), which ranged from 2.44 to 2.64%, and both were higher than the normal range for the gizzard (1.9–2.3%).

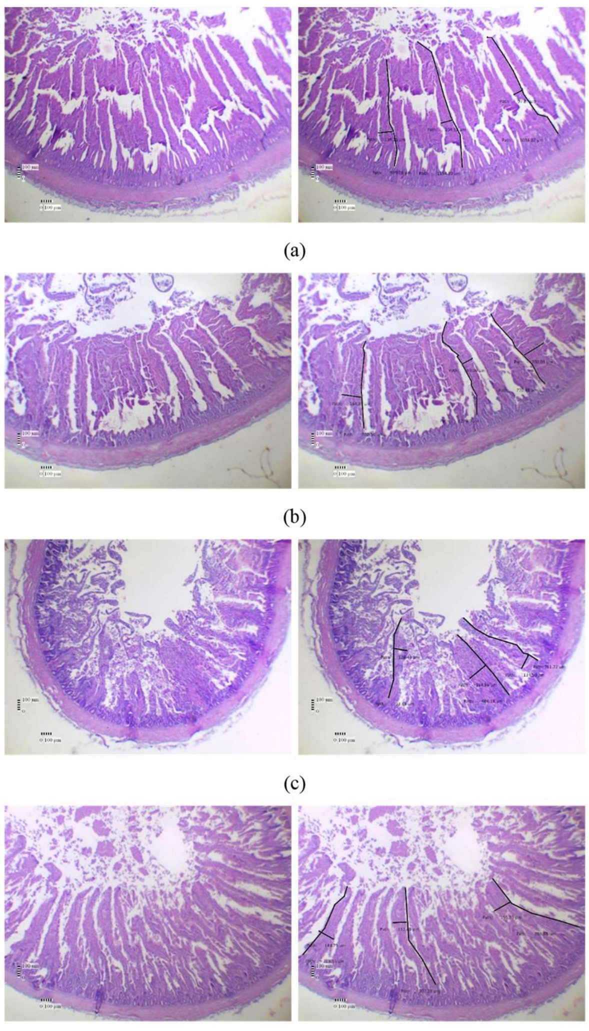



**Fig. 2:** Histopathological examination of the intestine of broiler chickens supplemented with *V. amygdalina* extracts in drinking water for a 28-day trial (a) control, (b) drinking water + 250mg/L *V. amygdalina* extract, (c) drinking water + 500mg/L *V. amygdalina* extract, (d) drinking water + 750mg/L *V. amygdalina* extract.

### Intestine morphology

The average height and width of the duodenal villi in broiler chickens are presented in Table 3. According to the data, the differences in these measurements among the treatments were not statistically significant (P>0.05). Despite the lack of significance, it is noteworthy that the highest numerical values for duodenal villi were recorded in treatment P0 (the control group, which did not receive any extract), with an average villi height of 965.14µm. In contrast, the lowest values were observed in treatment P1 (797.24µm), associated with the lowest dosage of *V. amygdalina* extract (250mg/L). Overall, the average base width of the duodenal villi was greater than that of the chickens that did not receive the extract, with P1 showing the highest numerical value. The observed trend of increased villus height at higher concentrations of the extract suggests that moderate to high doses of *V. amygdalina* may promote improved duodenal morphology compared to lower doses. A histological comparison of villus tissue growth in the duodenum is illustrated in Fig. 2.

**Table 3:** The effect of *V. amygdalina* extract administration in drinking water on the duodenum of commercial broiler chickens for a 28-day trial (n=5)

| Parameter | Treatment | | | | SEM | *P*–value |
|---|---|---|---|---|---|---|
| | P0 | P1 | P2 | P3 | | |
| Duodenal villus height (µm) | 965.14 | 797.24 | 897.45 | 911.82 | 32.49 | 0.354 |
| Base width of the duodenal villi (µm) | 138.94 | 168.21 | 147.31 | 148.62 | 5.41 | 0.305 |

P0= drinking water (control, no extract), P1= drinking water + 250mg/L *V. amygdalina* extract, P2= drinking water + 500mg/L *V. amygdalina* extract, P3= drinking water + 750mg/L *V. amygdalina* extract.

### Microbial counts

The effect of administering ethanol extract of *V. amygdalina* leaves at various concentrations through drinking water on 28-day-old broiler chickens is presented in Table 4. *E. coli* and *Salmonella* bacteria are pathogenic bacteria that have a detrimental effect on the growth and health of poultry, causing diseases such as colibacillosis and salmonellosis, which can lead to reduced production, mortality, and economic losses. On the other hand, phytochemicals, which are derived from plants, have been explored as alternatives to antibiotics in animal feed due to their antimicrobial, antiviral, and antifungal activities. These compounds, commonly found in herbs, spices, and their extracts (including essential oils), can disrupt the cell membranes of pathogens, inhibit protein synthesis, and disrupt bacterial metabolic pathways (Aminullah et al. 2025).

**Table 4:** The effect of *V. amygdalina* extract administration in drinking water on the bacterial population of commercial broiler chickens for a 28-day trial (n=1)

| Parameter | Treatment | | | |
|---|---|---|---|---|
| | P0 | P1 | P2 | P3 |
| Lactic acid bacteria (CFU/g) | $6.5\times10^6$ | $8.5\times10^6$ | $31\times10^6$ | $40\times10^6$ |
| *E. coli* (CFU/g) | $21\times10^3$ | - | - | - |
| *Salmonella* (CFU/g) | $292\times10^3$ | $208\times10^3$ | $115\times10^3$ | $48\times10^3$ |

P0= drinking water (control, no extract), P1= drinking water + 250mg/L *V. amygdalina* extract, P2= drinking water + 500mg/L *V. amygdalina* extract, P3= drinking water + 750mg/L *V. amygdalina* extract.

## DISCUSSION

### Broiler performances

The effectiveness of plant-sourced phytogenic additive compounds may vary depending on many factors, including diet, maturity, gut microbial composition, health status, feeding management, and rearing conditions (age, genetics, environment) that can lead to the inconsistency of results obtained with phytogenic feed additives (Yang et al. 2015; Abdelli et al. 2021). The current study was in accordance with the study conducted by Wahyudi et al. (2021) using 1, 1.5, and 2% of liquid probiotic containing *L. casei* and *S. cerevisiae* on broiler's drinking waters fed with commercial feed for up to 28 days showed no significant different (P>0.05) on body weight, feed intake, feed conversion ratio and water consumption. A recent study by Ilham et al. (2023) using 0.25, 0.50, and 1 g/L of Jamblang leaves extract in drinking water for up to 30 days showed no significant effect (P>0.05) on performance. However, current result was different from Mandey et al. (2022) using *V. amygdalina* juice on broiler's drinking water at 10, 20 and 30mL/L for up to 28 days fed with 70% commercial diet, 27% corn, 3% coconut oil showed significant increase (P<0.05) on final weight, weight gain and FCR but has no effect on total feed intake (P>0.05). Another study using Moringa (*Moringa oleifera*) and Sambiloto (*Andrographis paniculata*) leaf extracts in drinking water significantly improved broiler chicken performance, with significant (P<0.05) differences in feed consumption, body weight gain, and feed conversion (Astuti and Irawati, 2022).

**Carcass and giblet characteristics**

Adding a specific amount of plant-sourced phytogenic additive to a commercial broiler blend could significantly improve carcass energy retention and efficiency. However, it may not always correlate strongly with growth performance (Pirgozliev et al. 2015). Wahyudi et al. (2021) using up to 2% of liquid probiotic containing *L. casei* and *S. cerevisiae* on broiler's drinking waters for 28 days reported that there was no effect (P>0.05) on the weight and percentage of carcass, breast and back while significant effect (P<0.05) on drumstick percentage due to the probiotic's ability to deposit nutrients in edible portion of carcass. The observed trend of a relative reduction in wings and back, along with an increase in breast, although not significant (P>0.05), may have practical implications for broiler chicken production, especially in economically profitable parts such as the breast. However, further investigations are required to optimize levels, duration and response consistency.

Phytogenic feed additives, including essential oils, herbs, and spices, have been shown to improve growth performance, gut health, and meat quality while potentially reducing abdominal fat accumulation (Anwar et al. 2020; Pasaribu et al. 2024). The differing results in this study indicate that administering *V. amygdalina* is not always effective in reducing abdominal fat in chickens, due to factors such as composition, dose, extraction method, inclusion phase, and individual chicken responses (Aminullah et al. 2025). Abnormal changes in the weight of internal organs in chickens, such as the heart, liver, and gizzard, whether they enlarge or shrink, often indicate the presence of disease, nutritional issues, and genetic and environmental factors (Auza et al. 2021). Phytochemical compounds in feed can affect internal organs in chickens, including the digestive system, which may influence nutrient absorption and overall growth, as well as the liver and other organs involved in metabolism and detoxification (Abdel-Wareth et al. 2024). The heart muscle is highly susceptible to toxins and anti-nutrients. Heart enlargement can occur due to the accumulation of toxins in the heart muscle. If the blood contains toxins and anti-nutritional substances, it will trigger excessive contraction, leading to heart swelling. The size of the heart is also greatly influenced by the type, age, size, and activity of the animal, as well as the adaptability of the livestock (Nilawati et al. 2023).

The liver has a detoxification function carried out by liver enzymes, which convert potentially harmful substances into physiologically inactive substances. An enlarged liver indicates increased activity in neutralizing toxins (Auza et al. 2021). The study results showed that the administration of *V. amygdalina* extract up to 750mg/L in drinking water did not cause a significant change in liver organ percentage (P>0.05), ranging from 2.59-3.16%, slightly higher than the normal range of 1.70-2.80% (Auza et al. 2021). These results are consistent with the report by Badaruddin et al. (2022), which found that administering 2–6% *V. amygdalina* powder did not significantly affect liver percentage (P>0.05), which ranged from 3.40–3.85%. The study confirms previous research that the addition of African leaves, whether in the form of extracts or tannin- and saponin-containing powder, remains within safe threshold limits and does not cause toxic effects or affect liver detoxification activity (Nilawati et al. 2023).

The study results showed that the administration of *V. amygdalina* extract up to 750mg/L in drinking water did not cause significant changes in gizzard organ percentage (P>0.05), ranging from 3.23-3.89%, higher than the results of Badaruddin et al. (2022), who administered 2–6% *V. amygdalina* powder, which ranged from 2.44–2.64%. Both studies above showed gizzard percentages higher than the normal range of 1.9–2.3%. The gizzard is an organ with thick muscle layers that plays a crucial role in the mechanical digestion of feed. Gizzard size is influenced by the size of the livestock and the type of feed consumed. High-fiber feed or coarsely ground feed encourages the gizzard muscles to work more actively, thereby increasing the size and weight of the gizzard (Aguzey et al. 2018).

**Intestine morphology**

The lack of a noticeable effect from the administration of *V. amygdalina* extract in this study is likely due to the low dose used, which was insufficient to stimulate villus cell growth or protect the digestive tract of chickens. Several studies have indicated that the height and width of the base of duodenal villi are among the primary morphological indicators for evaluating the absorption capacity of the small intestine, particularly the duodenum, as wider and taller villi provide a larger surface area for nutrient absorption (Shazali et al. 2019). The effects observed are not due to stimulation of villus growth in the digestive tract, but rather to stimulation of enzyme secretion involved in the digestive process. Tokofai et al. (2023) reported that feeding *V. amygdalina* leaf powder to broiler chickens did not show any effect on growth performance, digestive enzyme activity, or nutrient absorption function. However, the inclusion of *V. amygdalina* leaf powder at 3g/kg in the diet resulted in a significant increase in amylase and trypsin concentrations (P<0.05), both of which are digestive enzymes.

In the case of increased width of the duodenal villi following the administration of *V. amygdalina* extract, it is suspected that this effect is due to the activity of bioactive compounds found in the extract. According to Patathananone et al. (2023), the increase in villus size in the duodenum is likely due to the bioactive compounds present in *V. amygdalina*, such as flavonoids, saponins, and tannins. These compounds possess antioxidant, anti-inflammatory, and antimicrobial properties. Their effects can help reduce oxidative stress and microinflammation in the intestinal mucosa, thereby maintaining integrity and promoting the regeneration of villous tissue. This finding is consistent with previous research by Ugbogu et al. (2021), which indicated that *V. amygdalina* administration can support gastrointestinal health and enhance digestive tract absorption function in chickens. This support occurs through both direct effects on mucosal tissue and modulation of the intestinal microbiota.

**Microbial counts**

The results of bacterial population testing in the intestines of broiler chickens after 28 days of rearing showed that *the V. amygdalina* ethanol extract was bactericidal against *E. coli*, as indicated by undetectable levels of *E. coli* at the lowest concentration (250mg/L). Imarenezor et al. (2021) also reported that ethanol and water extracts could inhibit the growth of *E. coli* at a concentration of 20mg/40mL, with ethanol extracts generally showing greater efficacy. This inhibition is likely due to the presence of various bioactive compounds in the leaves, such as flavonoids, terpenoids, saponins, alkaloids, anthraquinones, and phenols. The study also showed that ethanol extracts from African leaves can inhibit the growth of *Salmonella* at concentrations up to 750mg/L in drinking water. However, the effect is not as significant as that on *E. coli*. In their study using several *Salmonella* isolates, Ahmad et al. (2024) reported that *the V. amygdalina* ethanol extract had Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of 50 and 100mg/L, respectively. The use of *V. amygdalina* extract in mice infected with *Salmonella typhimurium* at a dose of 50mg/kg body weight alleviated liver damage caused by the bacterial infection.

In comparison, a dose of 200mg/kg body weight improved liver and kidney damage in Wistar rats (Amushie and Ebuehi 2022). The mechanism of microbial inhibition primarily involves disruption of cell membranes and cellular processes. Major bioactive compounds, such as flavonoids, saponins, and tannins, contribute to these antimicrobial effects by disrupting cell structure, inhibiting enzyme activity, and disrupting energy metabolism (Tura et al. 2024; Urban et al. 2025).

Conversely, in this study, the administration of *V. amygdalina* extract increased the population of lactic acid bacteria (LAB) with increasing concentrations up to 750 mg/L in drinking water, although the effect was relatively small. These results are similar to those reported by Ukwah and Ezeonu (2014) in broiler chickens, where the administration of 2% *V. amygdalina* powder significantly increased the presence of *Lactobacillus* and *Enterococcus* up to the fourth week of rearing, while *Salmonella* and *E. coli* continued to decrease. *V. amygdalina* extract can influence LAB by acting as a prebiotic, selectively stimulating their growth and/or activity, and potentially increasing vitamin production, such as folate, in the intestinal environment. However, its specific effects may vary depending on the extract concentration and the type of lactic acid bacteria, as it selectively stimulates *Lactobacillus* while potentially reducing the population of other bacteria such as Bacteroides (Ezeonu and Ukwah 2009). The antagonistic properties of LAB against pathogenic bacteria in the avian digestive tract may occur through competitive mechanisms for nutrients and attachment sites in the intestine, thereby preventing pathogens from colonizing and causing disease. LAB also produces various antimicrobial compounds, such as lactic acid, bacteriocins, and hydrogen peroxide, which directly inhibit or kill pathogenic bacteria, thereby influencing the host's immune response and potentially enhancing chickens' resistance to infections (Wyszyńska and Godlewska 2021).

**Conclusion**

Based on the results of the study, it was concluded that extracted *V. amygdalina* can be used as an antibiotic alternative in broiler chickens, indicating that its administration in drinking water had no detrimental effects on performance, carcass, and giblet characteristics. Intestinal morphology and microbial counts in broiler chickens showed no adverse effects with the administration of *V. amygdalina* as an animal feed additive. It is recommended that future studies concentrate on optimizing dosage levels, identifying active compounds responsible for its bioactivity, and evaluating its long-term effects on immune response and meat quality under commercial production conditions.

## DECLARATIONS

**Funding:** This research was financially supported by Universitas Syiah Kuala through the Professor Research Grant 2023, as stipulated in the Assignment Agreement No. 52/UN11.2.1/PT.01.03/PNBP/2023.

**Acknowledgement:** The authors would like to thank the Institution of Research and Community Service Universitas Syiah Kuala for supporting this study under the Professorship scheme in 2023.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Data Availability:** All data generated are included in the article.

**Ethics Statement:** The Committee on Veterinary Ethics approved this study for the Utilization of Animals at the Faculty of Veterinary, Universitas Syiah Kuala (Approval No. 268/KEPH/VII/2023).

**Author's Contribution:** SAM conceptualized and designed the study, supervised the experimental work, and prepared the initial draft of the manuscript. ILH conducted the experiments and collected the data. IW performed the statistical analysis and compiled the tables and figures. SW, SGT, and TH contributed to manuscript drafting, data review, and critical revisions. All authors read and approved the final version of the manuscript.

**Generative AI Statement:** The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

**Publisher's Note:** All claims stated in this article are exclusively those of the authors and do not necessarily represent those of their affiliated organizations or those of the publisher, the editors, and the reviewers. Any product that may be evaluated/assessed in this article or claimed by its manufacturer is not guaranteed or endorsed by the publisher/editors.

## REFERENCES


Abdelli N, Solà-Oriol D and Pérez JF, 2021. Phytogenic feed additives in poultry: Achievements, prospective and challenges. Animals 11(12): 3471 https://doi.org/10.3390/ANI11123471

Abdel-Wareth AAA, Williams AN, Salahuddin M, Gadekar S and Lohakare J, 2024. Algae as an alternative source of protein in poultry diets for sustainable production and disease resistance: present status and future considerations. Frontiers in Veterinary Science 11: 1382163. https://doi.org/10.3389/FVETS.2024.1382163

Aguzey HA, Gao Z, Wu H and Guilan C, 2018. Influence of feed form and particle size on gizzard, intestinal morphology and microbiota composition of broiler chicken. Poultry Fisheries and Wildlife Sciences 6(2). https://doi.org/10.4172/2375-446X.1000196

Ahmad M, Usman A and Usman HD, 2024. Antibacterial Activity of Vernonia Amygdalina (Bitter Leaf) Extracts against Clinical isolates of salmonella species. UMYU Journal of Microbiology Research (UJMR) 9: 308–314. https://doi.org/10.47430/UJMR.2493.037

Aminullah N, Mostamand A, Zahir A, Mahaq O and Azizi MN, 2025. Phytogenic feed additives as alternatives to antibiotics in poultry production: A review. Veterinary World 18: 141–154. https://doi.org/10.14202/VETWORLD.2025.141-154

Amushie I and Ebuehi OAT, 2022. Therapeutic effect of *Vernonia amygdalina* aqueous leaf extract on *Salmonella typhimurium*-infected female wistar rats. International Journal of Pharmaceutical and Bio Medical Science 2: 211–222. https://doi.org/10.47191/IJPBMS/V2-I7-04

Anwar P, Jiyanto J, Hadi N and Santi MA, 2020. Pengaruh Tepung Daun Kipait (Tithonia diversifolia) dalam Pakan terhadap Performa, Persentase Karkas, Lemak Abdominal, dan Giblet Broiler. Jurnal Agripet 20: 111–117. https://doi.org/10.17969/AGRIPET.V20I2.16172

Astuti P and Irawati DA, 2022. Broiler chicken performance given Moringa (*Moringa oliefera* Lam) and Sambiloto (*Andrographis paniculata*) leaf extract in drinking water. Jurnal Ilmiah Peternakan Terpadu 10: 92–100. https://doi.org/10.23960/JIPT.V10I1.P92-100

Auza FA, Purwanti S, Syamsu JA and Natsir A, 2021. The relative weight of internal organs and digestive tract in native chickens age 12 weeks that are given various levels of BSF larvae meal (*Hermetia illucens* L) in the ration. IOP Conference Series: Earth and Environmental Science 788: 012064. https://doi.org/10.1088/1755-1315/788/1/012064

Badaruddin R, Auza FA, Saili T, Pagala MA and Munadi LO, 2022. Percentage of internal organs of broiler chickens given Vernonia amygdalina flour feed additives. IOP Conference Series: Earth and Environmental Science 1107: 012069. https://doi.org/10.1088/1755-1315/1107/1/012069

Ezeonu I and Ukwah B, 2009. In vivo studies on the prebiotic effects of *Vernonia amygdalina* aqueous leaf extract on human intestinal microflora. Bio-research 7: 396–401. https://doi.org/10.4314/br.v7i1.45451

Ikpamezie LC, Aladi NO, Ogbuewu IP, Kadurumba OE, Anyanwu VC and Okeudo NJ, 2025. Growth dynamics of ISA Brown pullets on dietary bitter leaf (Vernonia amygdalina) meal intervention. International Journal of Agriculture and Rural Development 28: 7369–7372. https://orcid.org/0000-0003-2420-7121

Ilham I, Wahyudi I, Hidayat T, Allaily A, Wajizah S and Samadi S, 2023. Effect of jamblang extract (Syzygium cumini L) on performance, carcass and giblet characteristics of broilers. E3S Web of Conferences 373: 01008. https://doi.org/10.1051/e3sconf/202337301008

Imaga NOA and Bamigbetan DO, 2013. In vivo biochemical assessment of aqueous extracts of Vernonia amygdalina (Bitter leaf). International Journal of Nutrition and Metabolism 5: 22–27. https://doi.org/10.5897/IJNAM12.001

Imarenezor EPK, Abhadionmhen OA, Briska J, Shinggu PP and Danya S, 2021. Antimicrobial properties of Vernonia amygdalina on Escherichia coli and Proteus species isolated from urine samples: Potential antimicrobial alternative for urinary tract infection. International Journal of Biological and Pharmaceutical Sciences Archive 2: 127–134. https://doi.org/10.53771/IJBPSA.2021.2.1.0074

Imran, Wajizah S, and Samadi, 2021. Influence of liquid probiotic inclusion as feed additives on lipid profiles and meat cholesterol content of commercial broiler chickens. IOP Conference Series: Earth and Environmental Science 667: 012075. https://doi.org/10.1088/1755-1315/667/1/012075

Kismiati S, Sarjana TA, Mahfudz LD and Prayitno DS, 2023. African leaf (*Vernonia amygdalina*) extracts improve Japanese quail (Coturnix coturnix japonica) carcass traits. Veterinary World 16: 773–778. https://doi.org/10.14202/VETWORLD.2023.773-778

Mandey JS, Sompie M and Wolayan FR, 2022. Growth assessment of broiler chickens given Bitter leaves (*Vernonia amygdalina*) as phyto-additive, potentially antimicrobial agents of lipids and amino acids. Proceedings of the 9th International Seminar on Tropical Animal Production (ISTAP 2021) 18: 21–25. https://doi.org/10.2991/ABSR.K.220207.005

Murugesan GR, Syed B, Haldar S and Pender C, 2015. Phytogenic feed additives as an alternative to antibiotic growth promoters in broiler chickens. Frontiers in Veterinary Sciences 2: 21. https://doi.org/10.3389/fvets.2015.00021

National Research Council (NRC), 1994. Nutrient Requirements of Poultry, 9th ed. National Academy Press, Washington DC.

Nilawati Fati N, Yulia E, Malvin T and Ramaiyulis, 2023. Physiological organ profile of broilers added fermented extract of Bitter leaves (*Vernonia amydalina*) in drinking water. Eksakta : Berkala Ilmiah Bidang MIPA 24: 295–304. https://doi.org/10.24036/eksakta/vol24-iss02/286

Oni AI and Oke OE, 2025. Gut health modulation through phytogenics in poultry: mechanisms, benefits, and applications. Frontiers in Veterinary Science 12: 1616734. https://doi.org/10.3389/fvets.2025.1616734

Pasaribu T, Sinurat AP, Silalahi M and Lase JA, 2024. Phytogenic cocktails fed in different feeding regimes as alternatives to antibiotics for improving performance, intestinal microbial, and carcass characteristics of slow growth chickens. Veterinary World 17: 1423–1429. https://doi.org/10.14202/VETWORLD.2024.1423-1429

Patathananone S, Pothiwan M, Uapipatanakul B and Kunu W, 2023. Inhibitory effects of *Vernonia amygdalina* leaf extracts on free radical scavenging, tyrosinase, and amylase activities. Preventive Nutrition and Food Science 28: 302–311. https://doi.org/10.3746/PNF.2023.28.3.302

Phan MAT, Paterson J, Bucknall M and Arcot J, 2018.

Interactions between phytochemicals from fruits and vegetables: Effects on bioactivities and bioavailability. Critical Reviews in Food Science and Nutrition 58: 1310–1329. https://doi.org/10.1080/10408398.2016.1254595

Pirgozliev V, Beccaccia A, Rose SP and Bravo D, 2015. Partitioning of dietary energy of chickens fed maize- or wheat-based diets with and without a commercial blend of phytogenic feed additives. Journal of Animal Science 93: 1695–1702. https://doi.org/10.2527/jas.2014-8175

Pirgozliev V, Mansbridge SC, Rose SP, Lillehoj HS and Bravo D, 2019. Immune modulation, growth performance, and nutrient retention in broiler chickens fed a blend of phytogenic feed additives. Poultry Science 98: 3443–3449. https://doi.org/10.3382/PS/PEY472

Raimi CO, Oyelade WA, & Iyanda AO, 2020. Histopathological effect of aqueous bitter leaf extract (Vernonia amygdalina) on acetaminophen-induced liver damage in albino rat (Rattus norvegicus). Nigerian Journal of Animal Production 47: 24–31. https://doi.org/10.51791/njap.v47i4.95

Samadi, Delima M, and Herawati, 2019. Effect of various feed additives administration on performance and hematological parameters of local chickens (Gallus domesticus). IOP Conference Series: Earth and Environmental Science 260: 012065. https://doi.org/10.1088/1755-1315/260/1/012065

Samadi, Wajizah S, and Tarman A, 2020. Potency of several local phytogenic feed additives as antioxidant and antimicrobial sources for non-ruminant animals. IOP Conference Series: Earth and Environmental Science 425: 012029. https://doi.org/10.1088/1755-1315/425/1/012029

Samadi, Wajizah, S, Tarman A, Ilham, and Wahyudi I, 2022. Influence of Syzygium cumini extract as feed additives on performance and haematological parameters of commercial broiler chickens. IOP Conference Series: Earth and Environmental Science 951: 012079. https://doi.org/10.1088/1755-1315/951/1/012079

Shazali N, Loh TC, Foo HL and Samsudin AA, 2019. Gut microflora and intestinal morphology changes of broiler chickens fed reducing dietary protein supplemented with lysine, methionine, and threonine in tropical environment. Revista Brasileira de Zootecnia 48: e20170265. https://doi.org/10.1590/rbz4820170265

Steel PGD and Torrie JH, 1995. Prinsip dan Prosedur Statistika: suatu Pendekatan Geometrik, Terjemahan B. Sumantri. PT Gramedia, Jakarta.

Tokofai BM, Orounladji BM, Idoh K, Oke OE and Agbonon A, 2023. Effect of Vernonia amygdalina leaf meal on growth performance, intestinal mucosa activity, digestive enzymes, absorption capacity, and immunity in broiler chickens. Journal of Applied Animal Nutrition 11: 1–8. https://doi.org/https://doi.org/10.3920/JAAN2022.0006

Tura AM, Anbessa M, Tulu ED and Tilinti BZ, 2024. Exploring Vernonia amygdalina's leaf extracts for phytochemical screening and its anti-bacterial activities. International Journal of Food Properties 27: 960–974. https://doi.org/10.1080/10942912.2024.2377242

Uchendu I, 2018. Effect of aqueous extract of Bitter leaf (*Vernonia Amygdalina*) against acetaminophen-induced liver damage in rats. Bioequivalence & Bioavailability International Journal 2(1): 000122. https://doi.org/10.23880/BEBA-16000122

Ugbogu EA, Emmanuel O, Dike ED, Agi GO, Ugbogu OC, Ibe C and Iweala EJ, 2021. The phytochemistry, ethnobotanical, and pharmacological potentials of the medicinal plant-*Vernonia amygdalina* L. (bitter Leaf). Clinical Complementary Medicine and Pharmacology 1: 100006. https://doi.org/10.1016/J.CCMP.2021.100006

Ukwah B and Ezeonu I, 2014. In vivo studies on the prebiotic effects of *Vernonia amygdalina* leaf powder on broilers' intestinal microflora and pathogen shedding. Journal of Biological and Chemical Research 31: 464–473.

Unigwe RC, Emmanuel RC, Ukwueze CS, Egwu LU and Shobowale OM, 2025. Effects of bitter leaf (Vernonia amygdalina) powder on liver enzymes, lipid profiles and carcass traits of finisher broiler chickens. Nigerian Journal of Animal Science and Technology 8(1): 104–112. https://doi.org/10.63658/njast/2025.vol.8.1/02.14-112

Urban J, Kareem KY, Matuszewski A, Bień D, Ciborowska P, Lutostański K and Michalczuk M, 2025. Enhancing broiler chicken health and performance: the impact of phytobiotics on growth, gut microbiota, antioxidants, and immunity. Phytochemistry Reviews 24: 2131–2145. https://doi.org/10.1007/s11101-024-09994-0

Wahyudi I, Khairi F, Tarman A and Wajizah S, 2021. Effect of liquid probiotic administration on performances, carcass and giblet characteristics in commercial broiler chickens. IOP Conference Series: Earth and Environmental Science 667: 012026. https://doi.org/10.1088/1755-1315/667/1/012026

Wang J, Deng L, Chen M, Che Y, Li L, Zhu L, Chen G and Feng T, 2024. Phytogenic feed additives as natural antibiotic alternatives in animal health and production: A review of the literature of the last decade. Animal Nutrition 17: 244–264. https://doi.org/10.1016/J.ANINU.2024.01.012

Wijayanti KKD, Berata IK, Samsuri and Sudira IW, 2017. Histopatologi Usus Halus Tikus Putih Jantan yang Diberikan Deksametason dan Vitamin E. Buletin Veteriner Udayana 9: 47–53.

Wyszyńska AK and Godlewska R, 2021. Lactic Acid Bacteria – A promising tool for controlling chicken campylobacter infection. Frontiers in Microbiology 12: 703441 https://doi.org/10.3389/FMICB.2021.703441

Yang C, Chowdhury MAK, Hou Y and Gong J, 2015. Phytogenic compounds as alternatives to in-feed antibiotics: Potentials and challenges in application. Pathogens 4: 137. https://doi.org/10.3390/PATHOGENS4010137