Open access
Vol. 23 No. 1: 74-81, April 2025
e-ISSN: 2622-3279, p-ISSN: 2657-0068
DOI: http://dx.doi.org/10.29244/jintp.23.1.74-81

Jurnal Ilmu Nutrisi dan Teknologi Pakan
http://journal.ipb.ac.id/index.php/jurnalintp

Performance and Health Profile of IPB-D1 Chickens Supplemented With Cassava (*Manihot Esculenta* Crantz) Leaf Extract via Drinking Water

S A H Ramadhani[1], M Ridla[2-3*], R Mutia[2], Nahrowi[2-3], W Hermana[2]

Corresponding email:
hmridla@apps.ipb.ac.id
ayuramadhani@apps.ipb.ac.id,

[1)] Study Program of Animal Nutrition and Feed Science, Faculty of Animal Science, IPB University, Kampus IPB Dramaga, Bogor 16680, Indonesia

[2)] Department of Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Kampus IPB Dramaga, Bogor 16680, Indonesia

[3)] Center for Tropical Animal Studies (CENTRAS), IPB University, Kampus IPB Baranangsiang, Jl. Raya Pajajaran, Bogor 16153, Indonesia

ABSTRACT

This study evaluated the effects of cassava (*Manihot esculenta* Crantz) leaf extract (CLE) on drinking water on the performance and health profile of IPB-D1 chickens. Cassava leaf is known to contain bioactive compounds, including flavonoids, tannins, and saponins which have antioxidant and antibacterial properties. These compounds can provide positive effects, such as improving feed efficiency, enhancing growth performance, and enhancing the health status of chickens by strengthening the immune system, and reducing oxidative stress. Cassava leaf extract acts as a phytogenic additive that can be added to livestock drinking water. This study used 180 mixed-sex IPB-D1 chickens aged 2-8 weeks were randomly assigned to three treatments: CLE0 (control), CLE1 (17 ml l^{-1} CLE), and CLE2 (50 ml l^{-1} CLE). Parameters observed included growth performance, organ morphometry, hematological profiles, and ileal histology. Data were analyzed using one-way ANOVA and Tukey's post hoc test ($p<0.05$). Cassava leaf extract supplementation did not adversely affect growth or feed intake. Final body weight, feed conversion ratio (FCR), and internal organs were not significantly different among the groups. A reduction in leukocyte counts was observed, particularly in the CLE2 group, with a relative increase in heterophils, though values remained within physiological limits. Histological analysis revealed an increase in villus surface area in the CLE2 group, followed by CLE1, compared to the control (CLE0), suggesting enhanced intestinal morphology and nutrient absorption potential. Cassava leaf extract can be given in the drinking water of IPB-D1 chickens without disrupting their performance and health profile. These findings indicate that cassava leaf extract can potentially improve gut structure without compromising performance, supporting its application as a safe and beneficial phytogenic additive in poultry diets.

Key words: cassava leaf extract, haematology, IPB-D1 chickens, performance, phytogenic additive

ABSTRAK

Penelitian ini mengevaluasi pengaruh pemberian ekstrak daun singkong (*Manihot esculenta Crantz)* (CLE) dalam air minum terhadap performa dan profil kesehatan ayam IPB-D1. Daun singkong diketahui mengandung senyawa bioaktif seperti flavonoid, tanin, dan saponin yang memiliki sifat antioksidan dan antibakteri. Senyawa-senyawa ini dapat memberikan efek positif, seperti meningkatkan efisiensi pakan, menunjang pertumbuhan, serta meningkatkan status kesehatan ayam dengan memperkuat sistem imun dan mengurangi stres oksidatif. Ekstrak daun singkong berfungsi sebagai aditif fitogenik yang dapat ditambahkan ke dalam air minum ternak. Penelitian ini menggunakan 180 ekor ayam IPB-D1 jantan dan betina berumur 2–8 minggu yang secara acak dibagi menjadi tiga perlakuan: CLE0 (kontrol), CLE1 (17 ml l^{-1} CLE), dan CLE2 (50 ml l^{-1} CLE). Parameter yang diamati meliputi performa pertumbuhan, morfometri organ, profil hematologi, dan histologi ileum. Data dianalisis menggunakan ANOVA satu arah dan uji lanjut Tukey ($p<0,05$). Pemberian ekstrak daun singkong tidak berdampak negatif terhadap pertumbuhan maupun konsumsi pakan. Bobot akhir, rasio konversi pakan (FCR), dan organ internal tidak menunjukkan perbedaan yang signifikan antar kelompok. Penurunan jumlah leukosit diamati, terutama pada kelompok CLE2, disertai peningkatan relatif heterofil, meskipun masih dalam batas fisiologis. Analisis histologis menunjukkan peningkatan luas permukaan vili pada kelompok CLE2, diikuti oleh CLE1, dibandingkan dengan kontrol (CLE0), yang mengindikasikan peningkatan morfologi usus dan potensi penyerapan nutrien. Ekstrak daun singkong dapat diberikan dalam air minum ayam IPB-D1 tanpa mengganggu performa maupun profil kesehatannya. Temuan ini menunjukkan bahwa ekstrak daun singkong berpotensi meningkatkan struktur usus tanpa menurunkan performa, sehingga mendukung penggunaannya sebagai aditif fitogenik yang aman dan bermanfaat dalam pakan unggas.

Kata kunci: aditif fitogenik, ayam IPB-D1, ekstrak daun singkong, hematologi, performa

INTRODUCTION

Native chicken meat is highly valued by consumers for its distinctive flavor and superior taste (Hasriani *et al.* 2019). Furthermore, native chickens are often reared under relatively natural conditions with minimal reliance on pharmaceutical interventions or vaccinations, owing to their high adaptability and robust immune systems (Mubarak *et al.* 2018; Hasriani *et al.* 2019). Despite these advantages, a key limitation of native chickens is their relatively slow growth rate, which has prompted ongoing innovation in breeding programs. One such innovation is the development of the IPB-D1 chicken, a crossbred line derived from local Indonesian genetic resources namely Kampung, Pelung, and Sentul chickens and commercial broiler strains (Cobb) (Habib *et al.* 2020; Ridla *et al.* 2024). This hybrid line exhibits accelerated growth, efficient utilization of local feed resources, improved disease resistance, high adaptability, and retains the desirable meat quality associated with native chickens (Habib *et al.* 2020; Khairati *et al.* 2024).

A major challenge in intensive poultry production is maintaining optimal performance and health under confined management systems. Historically, antibiotic growth promoters (AGPs) have been used to improve productivity and feed efficiency. However, the long-term use of antibiotics raises significant health concerns, including the presence of drug residues in animal products and the development of antimicrobial resistance (Sapsuha *et al.* 2021). These issues pose risks to both animal and public health (El-Fateh *et al.* 2024). Consequently, there has been a growing interest in the development of safer, environmentally friendly alternatives such as natural feed additives (Sapsuha *et al.* 2023; Makanjuola *et al.* 2014) and probiotics (Ridla *et al.* 2024). One promising natural additive is cassava (*Manihot esculenta* Crantz) leaf, which is rich in bioactive compounds including flavonoids, tannins, and saponins that possess antioxidant and antimicrobial properties (Fachriyah *et al.* 2023). These compounds have the potential to enhance feed efficiency, promote growth performance, and support immune function by mitigating oxidative stress (Pasaribu 2019).

Cassava leaf extract (CLE) has been investigated as a phytogenic additive and offers a practical, cost-effective method of delivery in poultry systems. However, studies evaluating the supplementation of CLE through drinking water in chickens remain limited. This study aims to assess the effects of cassava leaf extract supplementation via drinking water on the performance and health profile of IPB-D1 chickens. Given that IPB-D1 chickens are designed for rearing systems with minimal antibiotic intervention, the use of cassava leaf extract as a natural additive aligns well with their intended management approach. The objective is to explore whether CLE can enhance performance and health or provide additional benefits in a breed already known for its strong disease resistance. Furthermore, the study seeks to identify any potential adverse effects associated with CLE use. The findings are expected to offer valuable insights into the applicability of cassava leaf extract as a natural additive in poultry production and support its potential integration into commercial poultry management practices.

METHODS

Materials

This study involved 180 mixed-sex IPB-D1 chickens aged 2 to 8 weeks, which were provided with drinking water supplemented with cassava (*Manihot esculenta* Crantz) leaf extract (CLE). The chickens were reared in a semi-closed housing system with rice husk used as litter material. The experimental ration was formulated based on the control diet composition reported by Sakinah *et al.* (2024a) for 2-week-old IPB-D1 chickens. The formulation and nutrient composition of the diet are detailed in Table 1.

Table 1 Feed formulation and nutrient composition

Feed Ingredient	%
Yellow corn	59.00
Rice bran	6.55
Corn gluten meal	6.50
Soybean meal	16.50
Meat bone meal	6.00
Crude palm oil	3.00
$CaCO_3$	0.80
NaCl	0.20
Premix	0.50
L-lysine	0.45
DL-methionine	0.40
Tryptophan	0.10
Total	100
Nutrient composition	
Dry matter, %	85.21
Ash, %	4.79
Crude protein, %	20.77
Crude fat, %	1.69
Crude fiber, %	2.42
Nitrogen-free extract, %	55.54
Gross energy (kcal g^{-1})	3810
Lysine, %	1.21
Methionine, %	0.75
Ca, %	0.83
P available, %	0.41
Na, %	0.18
Cl, %	0.19

Noted: $CaCO_3$= Calcium carbonate, NaCl= Natrium Chloride, Ca= Calcium, P= Phosphorus, Na= Natrium, Cl= Chloride.

Cassava Leaf Extract (CLE) Preparation

Fresh cassava leaves were thoroughly washed and weighed to a total of 500 g. The leaves were then homogenized using a blender with the addition of 1 L of clean water, resulting in a 1:2 (w/v) leaf-to-water ratio (Syafitri *et al.* 2015). The homogenate was filtered through a cloth to separate the liquid extract from the fibrous residue. The resulting filtrate was further clarified using filter paper with a pore size of 20–25 µm, yielding a light greenish-yellow cassava leaf extract (CLE). This extract was subsequently administered via drinking water at treatment-specific concentrations. Two dosage levels were used to explore dose-dependent effects: a low concentration (CLE1 = 17 mL CLE per liter of drinking water) and a high concentration (CLE2 = 50 mL CLE per liter of drinking water). The phytochemical profiles of both the cassava leaf extract and the treated drinking water are presented in Table 2.

Chicken Performance and Organs

The experimental period lasted from 2 to 8 weeks of age. Feed was provided twice daily at 07:00 WIB and 17:00 WIB, while drinking water was supplied ad libitum. Daily feed intake and water consumption were recorded, and body weight was measured weekly throughout the study. Internal organ evaluation was conducted using one bird per replicate, totaling 18 birds. Each selected chicken was weighed to determine slaughter body weight, then slaughtered and defeathered using a mechanical plucker. Dissection was performed to isolate and weigh internal organs. The parameters assessed included slaughter weight, relative organ weights, and relative intestinal lengths.

Blood Sampling and Profile Measurement

Blood sampling was performed on 8-week-old chickens by selecting one bird per replicate, totaling 18 birds. Approximately 1 mL of blood was collected from the brachial vein using sterile syringes. The puncture site was disinfected with 70% alcohol prior to collection. Blood samples were transferred into EDTA-coated tubes and immediately stored in an ice box containing ice gel to preserve plasma quality until hematological analysis could be conducted (Purnomo *et al.* 2015). The complete hematological analysis included measurements of red blood cell (RBC) count, white blood cell (WBC) count, hematocrit (HCT), hemoglobin (Hb) concentration, heterophil-to-lymphocyte (H/L) ratio, and leukocyte differential counts.

Table 2 Secondary metabolite content of CLE and treated drinking water

Composition	CLE	CLE0	CLE1	CLE2
Total phenol (mg GAE mL^{-1})	3.16	0	0.05	0.16
Flavonoid (mg RE mL^{-1})	88.37	0	1.47	4.42
Total antioxidant (mg L^{-1})	17.89	0	0.29	0.89
Hydrogen cyanide (ppm)	76.88	0	1.28	3.84

CLE = 100% cassava leaf extract; CLE0 = 0 ml CLE per liter of drinking water; CLE1 = 17 ml CLE per liter of drinking water; CLE2 = 50 ml CLE per liter of drinking water. The secondary metabolite content was analyzed in the Feed Science Technology lab, Faculty of Animal Science, IPB University in 2024.

Histological Analysis

Ileal segments approximately 3 cm in length were collected for histological analysis. The tissues were fixed in 10% buffered neutral formalin (BNF) for 48 hours and subsequently processed for hematoxylin and eosin (H&E) staining. Tissue dehydration was carried out using a graded ethanol series (each step lasting 10 seconds), followed by clearing with xylene and embedding in paraffin. Thin sections were prepared using a microtome, stained with H&E, and examined under a light microscope. Histological images were captured and analyzed using digital imaging software (Harimurti & Rahayu 2009).

Experimental Design and Data Analysis

A completely randomized design (CRD) was employed with three treatment groups:

CLE0: Control, 0 mL CLE per liter of drinking water
CLE1: 17 mL CLE per liter of drinking water
CLE2: 50 mL CLE per liter of drinking water

Each treatment was replicated six times. Data were analyzed using one-way ANOVA with IBM SPSS Statistics version 25. The data will be tested further using Tukey's post hoc test. Significance was declared at $p< 0.05$.

RESULTS AND DISCUSSION

Performance of IPB-D1 Chickens

The performance parameters observed in IPB-D1 chickens throughout the study included cassava leaf extract (CLE) intake, feed intake, water consumption, body weight gain, feed conversion ratio (FCR), and mortality rates (Tables 3 and 4).

Table 3 Total consumption of secondary metabolites by chickens during the rearing period

Composition	CLE0	CLE1	CLE2
Total Phenol (mg GAE $bird^{-1}$)	0	222.53 ± 7.68	662.06 ± 71.76
Flavonoid (mg RE $bird^{-1}$)	0	6,214.51 ± 493.64	18,490.18 ± 2004.21
Antioxidant (mg $bird^{-1}$)	0	1.26 ± 0.10	3.74 ± 0.41
HCN (mg $bird^{-1}$)	0	5.41 ± 0.43	16.09 ± 1.74

CLE0 = 0 ml CLE per liter of drinking water; CLE1 = 17 ml CLE per liter of drinking water; CLE2 = 50 ml CLE per liter of drinking water.

Table 4 Average performance of IPB-D1 chickens aged 2–8 weeks

Variable	CLE0	CLE1	CLE2
Total feed intake, g bird^{-1}	2467.50 ± 113.53	2411.95 ± 55.56	2384.55 ± 158.84
Daily feed intake, g bird^{-1}day^{-1}	60.18 ± 2.77	58.83 ± 1.36	58.16 ± 3.87
Total water intake, ml bird^{-1}	4404.33 ± 540.12	4219.67 ± 335.19	4184.95 ± 453.62
Daily water intake, ml bird^{-1} day^{-1}	107.42 ± 13.17	102.92 ± 8.18	102.07 ± 11.06
Initial body weight, g bird^{-1}	146.00 ± 5.11	146.90 ± 5.97	148.20 ± 6.93
Final body weight, g bird^{-1}	887.03 ± 37.67	857.90 ± 43.07	874.60 ± 37.75
Weight gain, g bird^{-1}	740.37 ± 39.72	711.00 ± 42.07	726.40 ± 38.45
FCR (Feed Conversion Ratio)	3.34 ± 0.22	3.40 ± 0.25	3.29 ± 0.24
Mortality, %	0	0	0

CLE0 = 0 ml CLE per liter of drinking water; CLE1 = 17 ml CLE per liter of drinking water; CLE2 = 50 ml CLE per liter of drinking water.

The total intake of secondary metabolite compounds was estimated by multiplying the concentration of each compound present in the CLE by the total volume of drinking water consumed.

The administration of drinking water supplemented with cassava leaf extract (CLE) from 2 to 8 weeks of age did not result in significant differences in performance parameters among IPB-D1 chickens. The comparable feed intake across treatments may be attributed to consistent factors such as uniform nutrient composition, feed form, and palatability (Khairati *et al.* 2024). Similarly, the absence of significant differences in water consumption indicates that CLE supplementation, even at concentrations up to 50 mL/L, did not adversely affect the palatability of the drinking water.

Feed intake is a key indicator of digestive efficiency (Lestari *et al.* 2021). The similar average body weight gain (BWG) observed across all treatment groups suggests that nutrient digestion and assimilation capacities were not impaired. Notably, the average BWG recorded in this study exceeded values reported by Habib *et al.* (2020), who documented BWG ranging from 605 to 713.2 g per bird for IPB-D1 chickens reared in closed housing systems during the same age period.

Feed conversion ratio (FCR) values ranged from 3.29 to 3.40, demonstrating improved feed efficiency compared to values reported by Rajulani *et al.* (2022) for KUB chickens (FCR = 4.11–4.73; CP = 19.22%–19.66 %; EM = 3427.53–3440.82 kcal kg^{-1}) and by Sakinah *et al.* (2024a) for IPB-D1 chickens (FCR = 4.06–4.38; CP = 23.17%–23.41%; EM = 3004.95–3102.91 kcal kg^{-1}). This improvement suggests a potential role of bioactive compounds present in CLE, particularly at the higher dose (CLE2), in promoting gut health and enhancing nutrient absorption, thereby contributing to more efficient feed utilization (Paul *et al.* 2007). However, the lack of statistically significant improvement in performance parameters may indicate that the CLE dosage used was suboptimal, potentially too low to elicit a measurable biological response.

Furthermore, no mortality was recorded during the experimental period, indicating that CLE supplementation did not negatively affect the health or survival of IPB-D1 chickens.

Vital and Immune Organs

Vital organs are central to metabolic and detoxification processes, while immune organs are key components of the body's natural defense mechanisms. The relative weights of vital and immune organs are presented in Table 5.

No significant differences were observed in the relative weights of the liver, heart, kidneys, thymus, spleen, and bursa of Fabricius among the treatment groups. This suggests that supplementation with cassava leaf extract in drinking water did not impair the function of either vital or immune organs in response to bioactive or potentially toxic compounds.

The liver, as a central metabolic organ, plays a critical role in nutrient metabolism and detoxification processes (Piran *et al.* 2024). The concentration of hydrogen cyanide (HCN) in the supplemented drinking water ranged from 1.281 to 3.844 ppm. The reported tolerance threshold for HCN in poultry ranges between 0.5 and 3 mg kg^{-1} body weight (Cheeke & Shull 1985). At two weeks of age, chickens weighing approximately 0.146–0.148 kg are estimated to tolerate HCN doses up to 0.438–0.444 mg. Given that the average daily water intake for chickens at this age is around 47 mL, the estimated daily HCN intake was approximately 0.180 mg.

Table 5 Percentage weight of vital and immune organs of 8-week-old IPB-D1 chickens

Variable	CLE0	CLE1	CLE2
Liver, %	2.13±0.19	2.12±0.06	2.15±0.08
Heart, %	0.53±0.04	0.48±0.05	0.46±0.06
Kidney, %	0.52±0.17	0.41±0.10	0.41±0.06
Thymus, %	0.42±0.20	0.34±0.09	0.41±0.18
Spleen, %	0.27±0.05	0.26±0.03	0.22±0.03
Bursa of Fabricius, %	0.20±0.08	0.13±0.06	0.19±0.09

CLE0 = 0 ml CLE per liter of drinking water; CLE1 = 17 ml CLE per liter of drinking water; CLE2 = 50 ml CLE per liter of drinking water.

This value falls below the established toxicity threshold, indicating that the observed HCN concentrations remained within safe limits and did not compromise hepatic function in IPB-D1 chickens.

Rutin is the predominant flavonoid found in cassava leaves (Fachriyah *et al.* 2023). Upon ingestion, rutin is enzymatically hydrolyzed into quercetin, an active antioxidant. Quercetin has been shown to mitigate oxidative stress and enhance endogenous antioxidant defense mechanisms (Rahman *et al.* 2022; Zahra *et al.* 2024). These antioxidant properties may contribute to maintaining immune organ integrity, potentially supporting the functional capacity of lymphoid organs such as the bursa of Fabricius, which plays a key role in B-cell maturation (Satrian *et al.* 2022). Furthermore, cassava leaf extract demonstrates strong antioxidant activity, with an IC_{50} value of 17.88 ppm, classifying it as a highly potent antioxidant (Sukandiarsyah *et al.* 2023).

Gastrointestinal Organ Characteristics

The administration of drinking water supplemented with cassava leaf extract during the rearing period (weeks 2-8) did not affect the relative weight and length of gastrointestinal organs in IPB-D1 chickens. The percentage weight and relative length of gastrointestinal tract organs are presented in Table 6.

The relative weight of the proventriculus in this study ranged from 0.46% to 0.47%, which is consistent with the findings of Khairati *et al.* (2024), who reported values between 0.47% and 0.54% in 9-week-old IPB-D1 chickens. In contrast, the relative gizzard weights observed in this study (2.27%-2.45%) were slightly lower than those reported by the same authors (2.80%–3.33%). These differences may be attributed to variations in dietary composition and the inclusion of functional bioactive compounds in the drinking water (Rosanti *et al.* 2021).

The absence of significant differences in gallbladder weight among treatment groups suggests that supplementation with cassava leaf extract did not disrupt hepatic metabolic activity, thereby preserving bile storage capacity. This observation aligns with the findings of Putra *et al.* (2017), who noted that bile acid production is closely linked to hepatic workload. Overall, the data indicate that both hepatic and biliary systems were functioning within physiological parameters, with no evidence of hypertrophy or pathological changes. The relative weight of the pancreas ranged from 0.20% to 0.26%, which is comparable to values reported by Ridla *et al.* (2024) and Khairati *et al.* (2024), who observed pancreatic weights ranging from 0.19% to 0.25% in IPB-D1 chickens.

No significant differences were found in the relative weight or length of the small intestine segments (duodenum, jejunum, and ileum) across treatment groups receiving cassava leaf extract. This may be due to the suboptimal dosage administered; while supplementation up to 50 mL per liter of drinking water may stimulate small intestinal development, the effect may not yet be sufficient to yield statistically significant differences. Similarly, the relative weight and length of the cecum and colon were unaffected by the treatments, indicating that cassava leaf extract did not compromise cecal functions related to secondary fermentation and nutrient recovery, nor did it impair colonic functions involved in water and mineral absorption.

Hematological Profile

The hematological profile is a critical indicator of an animal's physiological and health status. In the present study, the administration of cassava leaf extract via drinking water during the 2- to 8-week rearing period significantly influenced ($p < 0.05$) the total leukocyte count and heterophil percentage in IPB-D1 chickens. However, no significant effects were observed on erythrocyte count, hemoglobin concentration, hematocrit, lymphocyte percentage, heterophil-to-lymphocyte (H/L) ratio, eosinophil, monocyte, or basophil levels.

Table 6 Percentage weight and relative length of digestive tract organs in 8-week-old IPB-D1 chickens

Variable	CLE0	CLE1	CLE2
	% body weight		
Proventriculus	0.46 ± 0.09	0.46 ± 0.09	0.47 ± 0.06
Gizzard	2.45 ± 0.71	2.27 ± 0.46	2.39 ± 0.34
Gall bladder	0.07 ± 0.02	0.07 ± 0.02	0.06 ± 0.03
Pancreas	0.26 ± 0.10	0.20 ± 0.02	0.22 ± 0.04
Duodenum	0.58 ± 0.09	0.64 ± 0.14	0.61 ± 0.12
Jejunum	0.97 ± 0.14	1.03 ± 0.08	0.99 ± 0.12
Ileum	0.64 ± 0.15	0.70 ± 0.05	0.75 ± 0.09
Cecum	0.46 ± 0.13	0.44 ± 0.05	0.52 ± 0.14
Colon	0.19 ± 0.05	0.16 ± 0.03	0.14 ± 0.03
	Cm 100 g^{-1} body weight		
Duodenum	2.78 ± 0.49	2.85 ± 0.30	2.80 ± 0.26
Jejunum	6.24 ± 0.71	6.10 ± 0.36	6.15 ± 0.49
Ileum	5.10 ± 0.83	5.22 ± 0.30	5.32 ± 0.52
Cecum	1.20 ± 0.22	1.16 ± 0.15	1.22 ± 0.12
Colon	0.84 ± 0.22	0.83 ± 0.12	0.69 ± 0.19

CLE0 = 0 ml CLE per liter of drinking water; CLE1 = 17 ml CLE per liter of drinking water; CLE2 = 50 ml CLE per liter of drinking water.

Table 7 Blood profile of IPB-D1 chickens at 8 weeks of age

Variable	CLE0	CLE1	CLE2
Erythrocytes, 10^6 mm^{-3}	2.48 ± 0.37	2.21 ± 0.21	2.37 ± 0.36
Leukocytes, 10^3 mm^{-3}	11.21 ± 0.67^a	9.48 ± 1.05^b	9.19 ± 1.19^b
Hemoglobin, g dl^{-1}	8.07 ± 0.87	8.40 ± 0.89	8.23 ± 1.56
Hematocrit, %	24.33 ± 3.08	26.33 ± 2.25	27.00 ± 2.00
Heterophils, %	30.01 ± 4.04^b	33.21 ± 2.92^{ab}	34.98 ± 0.79^a
Lymphocytes, %	56.23 ± 4.28	56.91 ± 1.61	54.13 ± 2.09
H/L ratio	0.54 ± 0.11	0.56 ± 0.06	0.63 ± 0.07
Eosinophils, %	7.49 ± 2.53	6.65 ± 2.47	6.64 ± 2.23
Monocytes, %	5.40 ± 1.91	4.88 ± 1.35	4.58 ± 1.08
Basophils, %	0.87 ± 0.07	0.83 ± 0.07	0.81 ± 0.06

CLE0 = 0 ml CLE per liter of drinking water; CLE1 = 17 ml CLE per liter of drinking water; CLE2 = 50 ml CLE per liter of drinking water, H/L ratio= heterophil-to-lymphocyte. Means that have different superscripts in the same row indicate a significant difference ($p<0.05$)

These results suggest that while cassava leaf extract may modulate certain components of the immune response, it does not adversely affect overall hematological homeostasis. A detailed summary of the hematological parameters is provided in Table 7.

The mean leukocyte count ranged from 9.19 to 11.21 × 10^3 mm^{-3}, which is comparable to the values reported by Khairati *et al.* (2024) at 9.87 × 10^3 mm^{-3} and Ridla *et al.* (2024) at 10.76 × 10^3 mm^{-3}. Notably, the leukocyte count in IPB-D1 chickens supplemented with cassava leaf extract was lower than these reference values. This reduction may be attributed to the presence of bioactive compounds in cassava leaves such as saponins, tannins, and hydrocyanic acid (HCN) which, even at low concentrations, are known to exert mild immunosuppressive effects. These compounds are hypothesized to modulate hematopoietic activity in the bone marrow, the primary site of leukocyte production.
Despite the overall decrease in total leukocyte count, a significant increase in heterophil percentage was observed, suggesting that the innate immune response remains active in chickens receiving cassava leaf extract. Elevated heterophil levels likely represent an early immune response to phytochemical exposure, as suggested by Nasrullah *et al.* (2020). The prompt recruitment of heterophils may reduce the need for a robust adaptive immune response, including lymphocyte activation and antibody production.

The heterophil-to-lymphocyte (H/L) ratio among treatment groups ranged from 0.54 to 0.63. According to Siegel (1995), an H/L ratio of 0.2 indicates low stress, 0.5 indicates moderate stress, and 0.8 indicates high stress levels in poultry. Therefore, the H/L ratios observed in this study fall within the moderate stress range. Such stress levels may be associated with environmental factors such as heat stress or stocking density, as noted by Haerul *et al.* (2024).

Histopathology of the Small Intestine

The findings indicate that supplementation with cassava leaf extract (CLE) did not compromise intestinal health in IPB-D1 chickens. Histopathological analysis focused on the ileum segment of the small intestine in 8-week-old birds, as shown in Figure 1. The ileum is a critical region for nutrient absorption including amino acids, vitamins, and minerals making it a key site for assessing gut integrity and nutrient utilization.

The results demonstrated that the villus architecture in CLE-treated groups remained intact or was even enhanced, suggesting that CLE did not exert harmful effects on the intestinal mucosa. Observations of well-organized, elongated villi and narrower crypts are indicative of improved mucosal development and greater absorptive capacity. Since villus surface area is positively correlated with nutrient absorption efficiency, the preservation or enhancement of these morphological features may contribute to improved digestive and metabolic performance (Sakinah *et al.* 2024b; Kusuma *et al.* 2020).

These findings are particularly noteworthy given the increasing interest in phytogenic feed additives, such as cassava leaf extract, as alternatives to antibiotic growth promoters. The results are consistent with previous studies demonstrating that dietary strategies that maintain or enhance villus morphology are associated with better feed conversion efficiency and growth performance (Azhar *et al.* 2022). Furthermore, the antioxidant and antimicrobial activities of cassava leaf extract attributable to its content of polyphenols, flavonoids, and other bioactive compounds may help sustain intestinal homeostasis and protect epithelial tissues from oxidative or inflammatory damage. In conclusion, histological evidence supports the safe use of cassava leaf extract as a functional feed additive in poultry nutrition. Its inclusion may enhance performance outcomes while preserving intestinal health, contributing to the development of more sustainable and antibiotic-free poultry production systems.

Figure 1 Ileum histopathology of IPB-D1 chickens at 8 weeks of age

CONCLUSION

Cassava leaf extract (CLE) can be safely included in the drinking water of IPB-D1 chickens without negatively affecting growth performance or health profile indicators. Supplementation with 50 mL CLE per liter of drinking water reduces leukocyte levels and increases heterophil percentage in IPB-D1 chickens. There is potential for increased performance and health by administering cassava leaf extract up to 50 ml CLE. Further studies using graded levels of cassava leaf extract are recommended to establish the optimal dosage for maximizing physiological and productive benefits in chickens.

ACKNOWLEDGMENT

This research was funded by the Directorate General of Higher Education Research and Technology Ministry of Higher Education Science and Technology under the 2025 Research program implementation.

REFERENCES

Azhar M, Rahmawati, Sara U & Taufik M. 2022 Respons organ saluran pencernaan dan morfologi usus halus ayam lokal dengan in-ovo feeding menggunakan L-arginine. *Journal of Animal Science and Biotechnology*, 8(1): 1-10.

Cheeke PR & Shull LR. 1985. *Natural Toxicant in Feed and Poisonous Plants.* Westport Connecticut (US): AVI Publishing Company.

Fachriyah E, Haryanto IB, Kusrini D, Sarjono PR & Ngadiwiyana. 2023. Antioxidant activity of flavonoids from cassava leaf (*Manihot esculenta* Crantz). *Journal of Scientific and Applied Chemistry.* 26(1): 10-18.

Habib MFA, Murtini S, Cyrilla L, Arief II, Mutia R & Sumantri C. 2020. Performa pertumbuhan ayam IPB-D1 pada perlakuan pakan dan manajemen pemeliharaan yang berbeda. *Jurnal Agripet.* 20(2): 177-186.

Harimurti S & Rahayu ES. 2009 Morfologi usus ayam broiler yang disuplementasi dengan probiotik strain tunggal dan campuran. *Agricultural Technology Journal*. 29(3): 179-183.

Hasriani, Arifin & Pata AA. 2019. Faktor-faktor yang mempengaruhi permintaan ayam kampung di Kabupaten Maros. *Jurnal Agribis.* 1(1): 53-64.

Haerul M, Hermana W & Sumiati. 2024. Pemberian tepung bawang Dayak (*Eleutherine palmifolia*) dalam pakan terhadap profil hematologi darah puyuh. *Jurnal Ilmu Nutrisi dan Teknologi Pakan.* 22(2): 72-78.

Khairati N, Ridla M, Hermana W & Nahrowi. 2024. Impact of diets incorporating black soldier fly larvae meal and lactic acid bacteria-supplemented drinking water on the health profile and performance of IPB-D1 chickens. *Livestock Research for Rural Development.* 36(4).

Kusuma AY, Osfar S & Djunaidi IH. 2020 Pengaruh fermentasi campuran bungkil inti sawit dan onggok (FBISO) sebagai pengganti jagung dalam pakan terhadap karakteristik vili usus ayam pedaging. *Journal of Animal Science University of Padjadjaran*. 20(2): 126-137.

Lestari PK, Siska I & Anggrayni YL. 2021. Pengaruh substitusi tepung daun singkong (*Manihot utilissima*) dalam ransum terhadap bobot hidup, persentase karkas dan lemak abdominal broiler. *Jurnal Green Swarnadwipa.* 10(2): 242-251.

Makanjuola BA, Obi OO, Olorungbohunmi TO, Morakinyo OA, Oladele-Bukola MO & Boladuro BA. 2014. Effect of *Moringa oleifera* leaf meal as a substitute for antibiotics on the performance and blood parameters of broiler chickens. *Livestock Research for Rural Development*. 26(8).

El-Fateh M, Bilal M & Zhao X. 2024. Effect of antibiotic growth promoters (AGPs) on feed conversion ratio (FCR) of broiler chickens: A meta-analysis. *Poultry Science*. 103(12): 1-11.

Mubarak PR, Mahfudz LD & Sunarti D. 2018. Pengaruh pemberian probiotik pada level protein pakan berbeda terhadap perlemakan ayam kampung. *Jurnal Sain Peternakan Indonesia.* 13(4): 357-364.

Nasrullah N, Isroli I & Sugiharto S. 2020. Pengaruh penambahan jamu dalam ration terhadap profil darah putih dalam darah ayam petelur. *Jurnal Sain Peternakan Indonesia.* 15(3): 315-319.

Pasaribu T. 2019. Peluang zat aktif bioaktif tanaman sebagai alternatif imbuhan pakan antibiotik pada ayam. *Jurnal Litbang Pertanian.* 38(2): 96-104.

Paul S K, Samanta G, Halder G & Biswas P. 2007. Effect of a combination of organic acid salts as antibiotic replacer on the performance and gut health of broiler chickens. *Livestock Research for Rural Development.* 19.

Piran VB, Novian DR & Ndaong NA. 2024. Uji fungsi hati dan performa tumbuh ayam broiler yang diberikan pakan kombinasi tepung kelor (*Moringa Oleifera* Lam.) dan jeruk nipis (*Citrus aurantifolia*). *Jurnal Veteriner Nusantara.* 7(3): 1-12.

Purnomo D, Sugiharto & Isroli. 2015. Total leukosit dan diferensial leukosit darah ayam broiler akibat penggunaan tepung onggok fermentasi *rhizopus oryzae* pada ransum. *Jurnal Ilmu-Ilmu Peternakan.* 25(3): 59-68.

Putra RP, Mahfudz LD & Suthama N. 2017. Pemberian tepung daun ubi jalar fermentasi terhadap kinerja hati ayam kampung super. *Agromedia: Berkala Ilmiah Ilmu-Ilmu Pertanian.* 35(1): 34-41.

Rahman H, Jannah R, Elisma, & Sani F. 2022. Uji preklinik: aktivitas penyembuhan luka bakar ekstrak air daun singkong (*Manihot esculenta*). *Jurnal Pharmascience.* 9(2): 280-286.

Rajulani C, Bahri S & Zainudin S. 2022. Performans ayam kampung unggul balitnak (KUB) yang diberi ampas tahu fermentasi menggunakan microbacter Alfaafa-11. *Gorontalo Journal Equatorial Animals.* 1(2): 81-86.

Ridla M, Sakinah RG, Hermana W & Nahrowi. 2024. Jackbean meal as a soybean meal substitute and lactic acid bacteria supplementation on the performance and health profile of IPB-D1 chickens. *Livestock Research for Rural Development.* 36(5).

Rosanti I, Siska I & Anggrayni YL. 2021. Pengaruh substitusi tepung daun singkong (*Manihot utilissima*) dalam ransum terhadap persentase organ dalam ayam broiler. *Jurnal Green Swarnadwipa.* 10(1): 171-175.

Sakinah RG, Hermana W & Ridla M. 2024a. Jack bean (*Canavalia ensiformis*) meal inclusion and lactic acid bacteria supplementation on performance of the IPB-D1 chickens. *Jurnal Ilmu Nutrisi dan Teknologi Pakan.* 22(2): 79-84.

Sakinah RG, Alifianty OF, Hermana W, Nahrowi & Ridla M. 2024b. Influence of the inclusion of soaked and peeled jack bean meals in the broiler ration on internal vital organs, digestive tract organs, and intestinal villi morphology. *Journal Peternakan*. 21(2): 172-179.

Sapsuha Y, Sjafani N, Fatmona S & Tjokrodiningrat S. 2023. Butterfly pea (*Clitoria ternatea*) extract as a potential feed additive for broiler chickens. *Livestock Research for Rural Development*. 35.

Sapsuha Y, Suprijatna E, Kismiati S & Sugiharto. 2021 Combination of probiotic and phytobiotic as an alternative for antibiotic growth promoter for broilers chickens - a review. *Livestock Research for Rural Development.* 33(4).

Satrian A, Siska I & Jiyanto. 2022. Pengaruh penggunaan air rebusan daun sirih terhadap bobot bursa Fabricius, tymus, dan limpa broiler. *Jurnal Green Swarnadwipa*. 11(2): 275-281.

Siegel HS. 1995. Stress, strain, and resistance. *British Poultry Science*. 36(1): 3-22.

Sukandiarsyah F, Purwaningsih I & Ratnawaty GJ. 2023. Aktivitas antioksidan ekstrak metanol dan n-Heksana rimpang temu ireng

(*Curcuma aeruginosa* Roxb.) metode DPPH. *Jurnal Mandala Pharmacon Indonesia.* 9(1): 62-70.

Syafitri YE, Yunianto VD & Suthama N. 2015. Pemberian ekstrak daun beluntas (*Pluchea indica Less*) dan klorin terhadap massa kalsium dan massa protein daging pada ayam broiler. *Animal Agriculture Journal.* 4(1): 155-164.

Zahra AJ, Susianti, Happy TA & Soleha TU. 2024 Peran flavonoid sebagai antiulser dan atioksidan pada ulkus duodenum. *Medula.* 14(12): 2290-2296.