

Effect of liquid extract of *Pandanus amaryllifolius* leaves on broiler performance, digestive tract size and immune response under high stocking density

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ABSTRACT

This study aimed to examine the effects of administration of a liquid extract of pandan leaves (*Pandanus amaryllifolius*) added to commercial rations on the performance, size of digestive tract and immune response of broiler chickens. The materials used in this study were 280 broiler chickens aged 8-35 days with an average initial weight of 118.0 ± 0.65 grams (density of 16 chickens/m²) and Pandan Leaf Liquid Extract (PLLE). The study was conducted using a Completely Randomized Design (CRD) with PLLE administration levels (control, 30, 60, 90, and 120 ml/kg) with four replications. The parameters observed were the performance, digestive tract size, and lymphoid organs. Data were analyzed using analysis of variance with a 5% accuracy level and Duncan's test at a 5% level. The results showed that the administration of PLLE at a level 30-120 ml/kg significantly improved ($P < 0.05$) feed intake, final body weight, feed conversion ratio, live weight, carcass weight, carcass percentage, and relative weight of lymphoid organs. Using PLLE, we were able to maintain digestive tract size in the form of duodenum and jejunum relative weight, duodenum and jejunum relative length, and duodenum weight/length ratio. Administration of PLLE at a dosage of 120 ml/kg is optimal for enhancing the performance, size of digestive tract and immune response of broiler chickens raised under high-density conditions.

Introduction

The demand for broiler chicken meat has been rising, but farming land availability remains limited. Although high stocking densities address this issue, they negatively affect broiler productivity. The variable microclimate of Indonesia makes broilers prone to oxidative stress under high-density conditions. Maximum broiler chicken production requires high-density maintenance, which should not exceed 30 kg per m² for good welfare (Meluzzi and Sirri, 2009). High density causes oxidative stress, producing excess free radicals that damage cells and cause death (Mahfudz *et al.*, 2015). Oxidative stress reduces antioxidant levels and hampers digestive organ growth, leading to poor nutrient absorption and suboptimal body weight. Oxidative stress inhibits villus growth and nutrient absorption, thereby affecting growth and size of digestive tract and immune reducing broiler performance and disease resistance. Performance indicators include body weight, feed consumption, and conversion, whereas lymphoid organ weight indicates the immune response (Shihah *et al.*, 2021). High density decreases the weight of the bursa of Fabricius, thymus, and spleen. One way to reduce the negative effects of oxidative stress, which is safe for both broilers and consumers, is to administer natural antioxidants. Antioxidants can counteract oxidative stress (Sugiharto, 2022; Yang *et al.*, 2016). Natural antioxidants can be obtained from pandan leaves. Pandan leaves contain phenolic substances, flavonoids, and polyphenols as antioxidants and saponins as antibacterial agents (Liaotrakoon *et al.*, 2021). These compounds optimize digestive tract development, reduce pathogenic bacteria, and improve nutrient absorption and productivity (Adedokun and Olojede 2019; Shilov *et al.*, 2020). Limited research exists on the effects of liquid Pandan leaf extract on digestion in broiler chickens. The digestive tract optimizes nutrient absorption and indicates broiler health through the lymphoid organ response. This study investigated the impact of pandan leaf extract on broiler performance, digestive tract size, and lymphoid organ weight at a high density.

Materials and methods

The experiments were approved by the Animal Ethical Committee of the Faculty of Animal and Agricultural Sciences (approval number 58-10/A-12/KEP-FPP). The materials were 280 broiler chickens aged 8-35 days with an initial weight of 118.0 ± 0.65 g, reared in an open house at 16 chickens/m². Average Temperature and humidity during rearing were 28.4°C and 77%, lighting according SOP management guide. The materials used included pandan leaves, commercial rations, and distilled water. Using a completely randomized design with five treatments and four replications, pandan leaf extract was administered at T0: Commercial feed +0 ml/kg, T1: +30 ml/kg, T2: +60 ml/kg, T3: +90 ml/kg, and T4: +120 ml/kg. Commercial Feed contains 22% crude protein and 2,900 kcal/kg of metabolizable energy. Vaccination includes ND IB vaccine as eye drops at 4 days of age, and gumboro vaccine in drinking water at 13 days of age.

Making liquid extract of pandan leaves

The liquid extract of the pandan leaves was prepared using a maceration technique, washed and dried, chopped into small pieces of ± 1 cm, placed in a blender, and mixed with distilled water at a ratio of 1:3; that is, every 100 g of pandan leaves was mixed with 300 ml of distilled water, placed in a beaker glass to be left for one night, filtered, placed in a plastic bottle container, and stored in a refrigerator.

The liquid extract of pandan leaves was administered to chickens aged 8-35 days. The liquid extract of pandan leaves was mixed evenly into the commercial feed of broiler chickens by stirring. Feed and drinks were provided ad libitum

Data collection

Data collection was conducted on the 36th day using the following parameters.

Feed consumption (g/bird) = feed given - remaining feed (g)

Body weight was determined by measuring the final weight at 35 days of age.

Feed conversion= Feed Consumption (g) : Body weight gain (g)

Live weight= Final weight of chickens that had been fasted before being slaughtered

Carcass weight= Weight of chicken after being slaughtered - (weight of blood, feathers, head, legs and digestive tract)

Carcass percentage= "Carcass weight (g)" / "Live weight (g)" × 100%.

Organ relative weight= Digestive organ weight (g)/Live weight (g) × 100%

Organ relative length= Length of digestive organ (cm)/Digestive organ weight(g)

Ratio of weight and length of organs= Digestive organ weight (g)/Digestive organ length (cm)

Lymphoid relative weight

% Organ Weight = (Organ Weight)/Live Weight × 100%

Data analysis

Data were analyzed using analysis of variance at a 5% significance level. Data showing a significant effect ($P < 0.05$) were further tested by Duncan's test, and the data obtained were analyzed using SPSS software.

Results

Performance

Broiler performance is presented in Table 1. Based on the results, the performance, including feed intake, did not significantly differ, while the final result showed that treatment significantly (improve or decrease) ($P < 0.05$) on body weight and feed conversion ratio. Treatment T4 had the highest final body weight and the lowest feed conversion ratio.

Live weight, carcass weight and carcass percentage

The result showed The treatment had a significant effect on live weight, carcass weight, and carcass age ($P < 0.05$). Administration of 30–120 ml/kg PLLE improves broiler live weight. T3 had the highest carcass weight, whereas T3 and T4 had the highest carcass percentage. The carcass percentages in this study ranged from 74.88 to 78.34%.

Digestive tract size

As shown in Table 3. the administration of the liquid extract of pandan leaves at different levels was able to provide a significant effect by maintaining better ($P < 0.05$) the absolute weight of the duodenum, jejunum, ileum, and large intestine and the relative weights of the duodenum, jejunum, ileum, and large intestine, whereas the absolute weight of the ceca and the relative weight of the ceca were not significantly affected

($P > 0.05$).

The administration of pandan leaf liquid extract (PLLE) at different levels can significantly affect the form of better maintenance of the digestive organs ($P < 0.05$) in terms of the absolute length of the duodenum, ceca, large intestine, and relative length of the duodenum, jejunum, and ileum. Administration of the liquid extract of pandan leaves did not have a significant effect on the absolute length of the jejunum and ileum and the relative length of the ceca and large intestine ($P > 0.05$).

Based on Table 3. it can be seen that the administration of PLLE at different levels can provide a significant effect in the form of better maintenance ($P < 0.05$) on the weight and length ratio of the duodenum, jejunum, and ileum, but has not been able to provide a significant effect ($P > 0.05$) on the weight and length ratio of the ceca and large intestine.

Lymphoid organs

The relative weights of lymphoid organs are presented in Table 4. The results showed that all treatments had a significant effect ($P < 0.05$) on the relative weights of the bursa fabricius, thymus, and spleen. The highest relative weights of lymphoid organs (bursa fabricius, thymus, and spleen) were observed in the treatment without PLLE (T0).

Discussion

The provision of Pandan leaf liquid extract in rations did not significantly affect broiler chicken feed consumption ($P > 0.05$). This aligns with the results of Antara *et al.* (2025), who found that pandan leaf water extract (3-9%) in drinking water had no significant effect on feed intake. Similarly, *Pandanus amaryllifolius* Roxb. (0.5-2%) showed no significant effect on broiler feed consumption (Widodo *et al.*, 2022). Feed consumption by broilers is influenced by genetics, energy and protein levels, palatability, and physiological conditions. The extract (up to 120 ml/kg) did not affect feed energy and protein content. While *Pandanus amaryllifolius* contains 2 acetyl-1-pyrroline for relaxation (Bhat *et al.*, 2021; Wang *et al.*, 2024), feed intake in broiler predominantly affecting by their voracious appetite and driven by excesses target deposition hence less affected by addition of PPLE (Azis *et al.*, 2011; Liu *et al.*, 2019)

Administering Pandan leaf liquid extract led to an increase in the body weight of broiler chickens in treatments T2, T3, and T4, but no significant difference was observed in treatments T1 and T0. Final body weight serves as an indicator of production performance, with a high final body weight reflecting good production performance (Alghirani *et al.*, 2021). Factors such as feed quality, feeding method, environmental temperature, and chicks/DOC quality influence the final body weight of broiler chickens (Tallentire *et al.*, 2016; Morgan *et al.*, 2023). The essential oil content of pandan leaves is believed to affect the final body weight of broiler chickens. These essential oils enhance feed digestibility, thereby increasing body weight. They aid digestive enzyme function by stimu-

Table 1. Performance of Broiler Chickens Given PLLE in Rations.

Parameters	T0	T1	T2	T3	T4	P- value
Feed Intake (g/bird/day)	2281.15±27.61	2278.21±13.17	2272.16±42.52	2321.49±54.81	2294.51±44.44	0.44
Final Body Weight (g/bird)	1415.75±15.59 ^d	1445.25±25.20 ^{cd}	1464.25±12.87 ^{bc}	1486.50±19.12 ^b	1520.75±26.32 ^a	<0.01
Feed Conversion Ratio	1.41±0.01 ^c	1.38±0.02 ^{bc}	1.35±0.02 ^b	1.36±0.03 ^b	1.31±0.03 ^a	<0.01

Different superscripts values on the same row indicate significant differences

Table 2. Live Weight, Carcass Weight and Carcass Percentage of Broiler Chickens Given PLLE in the ration.

Parameters	T0	T1	T2	T3	T4	P- value
Live Weight (g/bird)	1288.50±29.51 ^b	1359.50±10.21 ^a	1375.00±34.36 ^a	1366.50±45.23 ^a	1411.00±45.75 ^a	<0.01
Carcass Weight (g)	965±36.45 ^c	1040.50±26.26 ^b	1048.25±25.81 ^{ab}	1059.75±34.39 ^{ab}	1104.25±49.07 ^a	<0.01
Carcass Percentage (%)	74.88±1.73 ^b	76.53±1.64 ^{ab}	76.26±1.99 ^{ab}	77.56±0.64 ^a	78.34±1.14 ^a	0.05

Different superscripts values on the same row indicate significant differences

Table 3. Size of the Digestive Tract of Broiler Chickens Given PLLE in the ration.

Parameters	T0	T1	T2	T3	T4	P- value
Absolute Weight of Organ (g)						
Duodenum	5.51±0.33 ^d	8.75±0.44 ^c	9.07±0.19 ^{bc}	10.55±0.60 ^a	10.39±0.59 ^{ab}	<0.01
Jejunum	13.68±0.86 ^c	17.10±0.46 ^b	18.52±0.72 ^b	23.15±1.22 ^a	23.58±0.86 ^a	<0.01
Ileum	10.92±0.31 ^d	12.72±0.40 ^c	14.92±0.71 ^b	16.82±0.62 ^a	17.15±0.25 ^a	<0.01
Ceca	5.48±0.55	5.82±0.36	5.98±0.60	5.62±0.22	6.15±0.18	0.61
Large Intestine	1.18±0.10 ^d	1.58±0.05 ^c	1.82±0.06 ^{bc}	2.05±0.09 ^b	2.35±0.12 ^a	<0.01
Absolute Length of Organ (cm)						
Duodenum	27.50±0.50 ^c	30.25±0.48 ^b	32.00±1.08 ^{ab}	33.50±0.87 ^a	32.75±0.48 ^a	<0.01
Jejunum	52.75±17.59	74.50±1.85	83.50±1.50	85.50±0.96	84.50±0.29	0.18
Ileum	53.00±17.71	79.75±1.38	80.25±1.03	59.75±19.96	79.75±1.25	0.52
Ceca	16.50±0.50 ^c	18.00±0.58 ^{bc}	18.25±0.25 ^{bc}	19.25±0.48 ^{ab}	21.00±1.08 ^a	0.00
Large Intestine	6.12±0.52 ^d	7.75±0.25 ^{cd}	8.88±0.43 ^{bc}	10.00±0.71 ^{ab}	10.88±0.85 ^a	<0.01
Relative Weight of Organs (%)						
Duodenum	0.42±0.02 ^d	0.64±0.03 ^c	0.67±0.01 ^{bc}	0.77±0.04 ^a	0.73±0.03 ^{ab}	<0.01
Jejunum	1.06±0.06 ^c	1.26±0.03 ^b	1.34±0.04 ^b	1.69±0.06 ^a	1.68±0.07 ^a	<0.01
Ileum	0.85±0.02 ^c	0.94±0.03 ^c	1.08±0.04 ^b	1.23±0.04 ^a	1.22±0.02 ^a	<0.01
Ceca	0.42±0.05	0.43±0.03	0.44±0.04	0.42±0.02	0.44±0.01	0.99
Large Intestine	0.09±0.01 ^d	0.12±0.003 ^c	0.13±0.006 ^{bc}	0.15±0.004 ^{ab}	0.17±0.008 ^a	<0.01
Relative Length of Organs (cm/g)						
Duodenum	5.03±0.24 ^a	3.48±0.18 ^b	3.53±0.11 ^b	3.20±0.15 ^b	3.18±0.12 ^b	<0.01
Jejunum	5.12±0.39 ^a	4.36±0.13 ^{bc}	4.52±0.14 ^{ab}	3.72±0.17 ^{cd}	3.60±0.14 ^d	<0.01
Ileum	6.30±0.10 ^a	6.29±0.27 ^a	5.40±0.22 ^b	4.91±0.22 ^{bc}	4.65±0.07 ^c	<0.01
Ceca	3.09±0.28	3.12±0.17	3.16±0.36	3.44±0.21	3.43±0.24	0.77
Large Intestine	5.20±0.27	4.94±0.29	4.89±0.32	4.87±0.21	4.64±0.32	0.74
Organ Weight and Length Ratio (g/cm)						
Duodenum	0.20±0.01 ^b	0.29±0.02 ^a	0.28±0.01 ^a	0.32±0.02 ^a	0.32±0.01 ^a	<0.01
Jejunum	0.20±0.01 ^b	0.23±0.01 ^b	0.22±0.01 ^b	0.28±0.01 ^b	1.40±0.06 ^a	<0.01
Ileum	0.16±0.002 ^b	0.16±0.01 ^b	0.19±0.01 ^a	0.20±0.01 ^a	0.28±0.01 ^a	<0.01
Ceca	0.33±0.04	0.32±0.02	0.33±0.03	0.29±0.02	0.29±0.02	0.73
Large Intestine	0.19±0.01	0.20±0.01	0.21±0.02	0.21±0.01	0.22±0.02	0.75

Different superscripts values on the same row indicate significant differences

Table 4. Lymphoid Organs Relative Weight (%) of Broiler Chickens Given PLLE in the ration.

Parameters	T0	T1	T2	T3	T4	P- value
Bursa Fabricius	0.034±0.01 ^c	0.055±0.01 ^{bc}	0.067±0.04 ^{abc}	0.099±0.04 ^a	0.095±0.03 ^{ab}	0.02
Thymus	0.132±0.01 ^c	0.158±0.02 ^{bc}	0.190±0.06 ^{ab}	0.221±0.04 ^a	0.203±0.03 ^a	0.02
Spleen	0.083±0.002 ^c	0.099±0.013 ^b	0.122±0.015 ^a	0.124±0.015 ^a	0.127±0.010 ^a	<0.001

Different superscripts values on the same row indicate significant differences

lating the gallbladder to secrete bile and prompting the release of pancreatic juices, such as amylase, protease, and lipase, which digest carbohydrates, proteins, and fat. This process accelerates the feed and growth rates, leading to increased meat production (Kusmiyati *et al.*, 2022). Despite similar consumption across treatments, the absorbed nutrients varied, resulting in different weight gains and final weights. Additionally, the active flavonoid ingredients in pandans aid nutrient absorption during digestion, efficiently contributing to significant body weight gain. Flavonoids help maintain intestinal barrier integrity and modulate the gut microbiota, indirectly influencing nutrient absorption and overall health (Xiong *et al.*, 2023). This can potentially affect body weight by enhancing nutrient absorption. Flavonoids are acidic and promote the growth of *Lactobacillus*, which benefits from digestion (Singh *et al.*, 2025). *Lactobacillus* adapts to acidic conditions during poultry digestion and produces organic compounds that lower the intestinal pH, making the digestive tract more acidic and inhibiting pathogenic bacterial growth (Zulhelmi *et al.*, 2022).

The antibacterial compounds in PLLE prevent free radicals through their antioxidant activity, leading to higher broiler chicken growth and live weight. According to Hieu *et al.* (2022), cell damage inhibits chicken growth and can prevent antioxidant activity. The increased live weight results from enhanced feed nutrient absorption and inhibition of pathogenic bacteria due to antibacterial compounds in pandan leaf extract-mixed rations. Tang *et al.* (2025) found that pandan leaves contain terpenoids, which Huang *et al.* (2022) noted form strong polymer bonds with trans-membrane proteins, disrupting bacterial cell growth. Obianwuna *et al.* (2024) confirmed that antibacterial compounds increase broiler chicken weight by enhancing intestinal mucosal permeability and stopping pathogenic bacterial growth. Antibacterial activity also increases chicken immunity and optimizes body weight, as supported by Duarte-Mata and Salinas-Carmona (2023).

Administration of liquid Pandan leaf extract in rations affected feed conversion. Higher extract concentrations led to lower conversion values. Feed conversion determines feed use efficiency and productivity, with

low conversion indicating improved meat conversion ability in broiler chickens (Zampiga *et al.*, 2021). Feed conversion is influenced by genetics, temperature, nutrient content, digestive tract transit, ration form, and nutrient balance (Lentle *et al.*, 2006; Liu *et al.*, 2012). Increasing Pandan leaf extract did not affect feed consumption but increased body weight and decreased feed conversion. This effect was attributed to the extract, as the nutritional content remained constant across treatments. Pandan leaves contain antioxidants from flavonoids and essential oils, which improve blood flow and metabolism, and optimize ration utilization (Trinity *et al.*, 2016; Ognik *et al.*, 2016). Flavonoids maintain the digestive microorganism balance by lowering pH and increasing non-pathogenic bacteria (Baky *et al.*, 2021). Higher extract use decreases pathogenic bacteria while increasing lactic acid bacteria (LAB), creating optimal protein digestion conditions. LAB growth improves digestive health and nutrient absorption (Palupi *et al.*, 2023). *Lactobacillus* aids in digestion through enzyme production and improves feed nutrient breakdown and metabolism (Wulandari *et al.*, 2018; Wang *et al.*, 2021).

The antioxidant and antibacterial contents of pandan leaves in broiler chickens reduce free radicals and oxidative stress, thereby optimizing carcass production. According to Oke *et al.* (2024), antioxidants inhibit free radicals by converting them to harmless compounds. Treatments T1, T2, T3, and T4 produced greater live and carcass weights than T0, indicating sufficient antioxidant and antibacterial activity without excess, allowing normal cell function. Excessive antioxidant and antibacterial compounds can cause cell damage (Surai *et al.*, 2019). High cage density increases the temperature and ammonia levels, causing lung irritation and oxidative stress in broilers (Swelum *et al.*, 2021). This leads to an increased body temperature and decreased feed consumption. Blood vessel disorders in poultry can cause oxygen deficiency and oxidative stress (Lake *et al.*, 2020), whereas lung disorders disrupt heat release (Iftitah *et al.*, 2022; Wu *et al.*, 2023). Antioxidants prevent oxidative stress from pathogenic bacteria, increase nutrient absorption, and increase live and carcass weights. A higher live weight correlates with a higher carcass weight. Live weight and carcass weight affect the carcass percentage. Significant effects on live and carcass weights lead to significant effects on carcass percentage, as reported by Avrilliani *et al.* (2024). The liquid extract of pandan leaves from T1 (30 ml/kg ration) and T2 (60 ml/kg ration) showed no significant difference ($P > 0.05$) compared to T0, but carcass percentage increased ($P \leq 0.05$) in T3 (90 ml/kg ration) and T4 (120 ml/kg ration). This occurred because bioactive substances in pandan leaf extract at 30-60 ml/kg ration did not increase the broiler carcass percentage.

The use of liquid extract of pandan leaves at a 90 ml/kg ratio provided optimal improvement, marked by increased weight of the duodenum, jejunum, and ileum. The extract contained bioactive substances that act as antioxidants to combat oxidative stress. Antioxidants function as free radical scavengers that prevent cell damage, particularly in intestinal villi. Lumbanraja *et al.* (2024) noted that flavonoids in pandan leaves have natural antioxidant capacity. Oke *et al.* (2024) found that antioxidants neutralize free radicals, protecting against oxidative stress damage. Antioxidants prevent damage to intestinal villi cells, leading to optimal development and weight increase in the digestive organs (Sunu *et al.*, 2021; Xu *et al.*, 2025a). Active ingredients, such as flavonoids and tannins, increase the weight of the small intestine of broiler chickens. Alagawany *et al.* (2021) noted that herbal flavonoids protect the mucosa of the small intestine, thereby enabling optimal development. High temperature and humidity trigger oxidative stress through free radical attack on cell membranes. The active compounds in pandan leaf extracts, including alkaloids, tannins, flavonoids, and polyphenols, increase antioxidant activity by capturing free radicals and promoting intestinal villus cell development and optimal digestive organ weight.

The consumption of the active ingredients in pandan leaf extracts correlates with increased small intestine length in broiler chickens. Obianwuna *et al.* (2024) reported that plant antioxidants improve the development of the broiler chicken small intestine. Xu *et al.* (2025b) reported

that tannins positively affected the length of the small intestine of broiler chickens. The optimal increase in digestive tract length after pandan leaf extract administration is attributed to its antibacterial compounds that inhibit pathogenic bacteria. Djenar *et al.* (2020) identified tannins, saponins, polyphenols, and flavonoids as antibacterial components in pandan leaves. This inhibition leads to optimal development of the digestive tract length. Increased digestive organ length indicates greater intestinal surface area and enhanced feed nutrient absorption duration. Ravindran and Abdollahi (2021) noted that increased digestive tract length improved nutrient absorption. Pandan leaf extract antioxidants reduce oxidative stress in high-density broiler farming by optimizing intestinal villus growth and length. Thanebal *et al.* (2021) reported that the antioxidant activity of pandan leaves suppressed oxidative stress in the body. The increase in digestive tract length corresponds to villus length, optimizing nutrient absorption. Anjani *et al.* (2025) linked small intestine nutrient absorption to villi length growth.

The increasing consumption of active ingredients aligns with increased broiler duodenum weight and length ratio. El Sabry and Yalcin (2023) stated that the duodenum is the site of chemical absorption of digestive products, especially fat, iron, and vitamins. Flavonoids and tannins positively affect small intestine development. Farid *et al.* (2024) found that Black Garlic's Allicin improves intestinal microflora balance and enhances nutrient absorption. The duodenum, jejunum, and ileum walls were thickened due to increased villi numbers and improved feed nutrient absorption. Simon *et al.* (2019) reported that digestive absorption occurs on the surfaces of intestinal microvilli. The high villus count is attributed to the flavonoids of the pandan leaf extract, which inhibits pathogenic bacteria and optimizes villi development. Prakatur *et al.* (2019) confirmed flavonoids increased intestinal villi development. Broilers in high-temperature locations experience oxidative stress caused by natural antioxidants in the pandan leaf extracts. Mishra and Ja (2019) noted antioxidants repair oxidative stress and restore damaged intestinal membranes. Dewi *et al.* (2019) stated pandan leaf extract contains tannin, alkaloid, flavonoid, polyphenol and saponin compounds as antioxidants.

The utilization of Pandan Leaf Liquid Extract (PLLE) effectively supports the growth and development of immune organs. This treatment starts by 8 days support. Song *et al.* (2021) indicate that the immune system and its functions do not fully develop until 13 days of age. The majority of immune organ development in broilers occurs after 14 days of age. PLLE at 90-120 ml/kg of ration ($P \leq 0.05$) increased bursa fabricius weight in broiler chickens. The low weight without PLLE was due to the more difficult antibody formation compared to treatment with PLLE. Pandan leaf extract helps overcome stress in broiler chickens and affects body resistance. The heat stress index (HSI) value was 160, with chickens remaining tolerant below this value (Tesakul *et al.*, 2025). Chicken health is related to cage comfort, with cooler cages providing better health. The relative weight of bursa fabricius ranged from 0.0340-0.0948%, with a normal size of 0.09% of live weight (Toghyani *et al.*, 2010). According to Shihah *et al.* (2021), bursa development varies with age. Body resistance is influenced by temperature, diseases, and feed consumption, which affect lymphoid organs. Environmental stress and high temperatures can cause bursa shrinkage in broilers (Oluwagbenga and Fraley 2023). The bursa is crucial for immune system development and B lymphocyte maturation (Latif *et al.*, 2014). PLLE contains flavonoids that function as antioxidants and activate the bursa follicles. Flavonoids protect cells, prevent inflammation, and act as antibiotics (Liu *et al.*, 2025; Zhou *et al.*, 2025). Bursa follicles produce lymphocytes that differentiate into B and plasma cells for antibody production (Szocs *et al.*, 2024).

The average relative thymus weight was 0.1319-0.2206% of the live weight (Table 4). A low thymus weight likely results from heat stress, which reduces lymphocyte and antibody production. The low weight also stems from lymphoid organs, which inhibit microbes from entering through the beak, nose, and eyes. The thymus is located along the ventral neck to the thyroid gland (Gündüz *et al.*, 2024). The relative thymus weight

in the control treatment (T0) was lower ($P \leq 0.05$) than T1-T4 treatments (Table 4). PLLE contains flavonoids, which act as antioxidants (Djenar *et al.*, 2020). Pandan leaf extract increased the chicken immune response by preventing thymus overwork. Flavonoids increase thymus-producing T cells and enhance antibody production. The thymus requires the bursa of fabricius' B cells to produce antibodies.

The spleen filters the blood from pathogens and abnormal cells, facilitating interactions between antigen-presenting cells (APCs) and lymphocytes. APCs regulate T- and B-cell responses to blood antigens (Farid *et al.*, 2019). Pandan leaf extract contains flavonoids with antioxidant and antibacterial effects, which reduce spleen function by inhibiting pathogens. These flavonoids eliminate radicals and pathogens from chicken bodies (Banjarnahor and Artanti, 2014; Cheng *et al.*, 2024). The control treatment (T0) and the 30% PLLE treatment (T1) exhibited lower relative spleen weights than the other treatments. The reduced spleen weight observed at T0 can be attributed to the absence of flavonoids, which are necessary for acidification of the digestive tract and maintenance of microbial balance.

Conclusion

Pandan leaf liquid extract (PLLE) at a concentration of 120 ml/kg improves broiler chicken performance, size of digestive tract and immune response at high density. This optimal level enhances digestive tract size, lymphoid organ weight, slaughter and carcass weights, and final body weight, and reduces feed conversion even though feed intake does not change. Pandan leaf extract can serve as a natural antioxidant source that is beneficial to poultry.

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Conflict of interest

The authors have no conflict of interest to declare.

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