

Stress indicators, immunity, and performance of quail fed on the basil leaf meal waste during the growth period

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ABSTRACT

This study aimed to evaluate the effects of adding basil (*Ocimum basilicum* L.) leaf meal waste to feed on stress indicators, immune responses, and performance of growing quails (*Coturnix coturnix japonica*). Two hundred female quails 3-week-old were allocated in a completely randomized design with four treatments: 0% (P0), 1.5% (P1), 3% (P2), and 4.5% (P3), each with five replicates of 10 birds. The Observed variables included oxygen saturation, heterophil/lymphocyte ratio, hepatic superoxide dismutase (SOD) activity and malondialdehyde (MDA) level, leukocyte profiles, and clearance ability against *Salmonella pullorum*. Growth performance was measured through feed intake, body weight gain, and feed conversion ratio. Results showed that 3% basil leaf meal waste improved oxygen saturation and SOD activity, reduced H/L ratio and MDA level, and produced the highest bacterial killing ability. The best growth performance and most efficient feed conversion were also observed in P2. It can be concluded that 3% inclusion effectively reduces oxidative stress, enhances immunity, and improves quail performance.

Keywords: Basil leaf meal waste, Immunity, Oxidative stress, Performance, Quail

INTRODUCTION

Quail (*Coturnix coturnix japonica*) are a poultry species that are widely farmed because of their high egg production. Quail egg production in one year ranges from 200 to 300 eggs (Amo *et al.*, 2013). Quail productivity is influenced by internal (genetic) and external (environmental)

factors. Environmental factors include feed nutrition, maintenance management, and microclimate (Utama *et al.*, 2021). Microclimate factors consist of wind speed, sunlight, humidity, and ambient temperature (Fajri and Ngatiman, 2017).

The ambient temperature in Indonesia ranges from 23 to 36°C (BMKG, 2023). In the

morning, it ranges from 23-28°C; at noon, 28-36°C; and in the afternoon, 26-30°C. The comfort zone for quail is 20-24°C (Ulupi *et al.*, 2016). On average, from noon to evening, the environmental temperature is above the comfort zone for quail, resulting in *heat stress*. Heat stress is a condition in which livestock are unable to maintain a balance between heat production and heat dissipation (Wasti *et al.*, 2020). High environmental temperatures disrupt physiological responses, so livestock kept above the comfort zone will experience physiological changes (Tamzil, 2014). During heat stress, the body produces excess heat. To mitigate these effects, livestock attempt to reduce feed intake (Xie *et al.*, 2017). With reduced feed intake, the nutrients required for growth are diminished. Prolonged heat stress leads to oxidative stress.

Oxidative stress is a condition of imbalance between the production of free radicals and reactive oxygen species (ROS) and the body's antioxidant defense system (Maheshwari *et al.*, 2017). Free radicals are unstable and reactive compounds because they have unpaired electrons in their outer orbitals (Ikrima *et al.*, 2020). During oxidative stress, glucocorticoid hormones increase, which can inhibit lymphocyte formation. El Shoukary *et al.* (2015) stated that a decrease in lymphocyte count indicates reduced immunity. The adverse effects of oxidative stress, as described above, can be overcome by adding antioxidants (Chi *et al.*, 2020).

Antioxidants are classified into endogenous (within the body) and exogenous (outside the body) antioxidants. Natural antioxidants can be obtained from plants, such as basil. Basil is a soft-stemmed plant that is commonly found in Indonesia and other tropical countries. Almost all parts of the basil plant can be used, from the roots, stems, leaves, flowers, and seeds (Hadiopentyanti and Wahyuni, 2008).

Basil is almost the same as kemangi. Various sources indicate that both belong to the same genus, *Ocimum*, but are different species. The species of basil is *Ocimum basilicum* L., and the species of kemangi is *Ocimum africanum* L. (Cronquist, 1981). Both have almost the same characteristics. Basil plants are often used as a

supplement in animal feed, mainly poultry feed. The addition of basil leaf powder to quail feed during the growth period at levels of 0%, 0.5%, 1%, and 1.5% has been tested. The results show that adding 1.5% resulted in the highest conversion rate (Aly *et al.*, 2024).

Basil plants have so far only been used as a source of basil seeds, which are used as an ingredient in beverages (Bumihherbal, 2017). Other parts, including the leaves, are considered waste. Basil leaves contain phytosterols, alkaloids, tannins, lignin, saponins, terpenoids, phenolics, flavonoids, and anthocyanins (Larasati and Apriliana, 2016; Rahman and Hossain, 2011). Based on phytochemical analysis and pharmacological testing, basil can be used as an anti-inflammatory, antimicrobial, antiviral, and antioxidant agent, as well as to boost the immune system (Khair-ul-Bariyah *et al.*, 2012). Information on the use of BLM (*basil leaf meal*) waste in quail feed is still limited. Therefore, the purpose of this study was to examine and analyze the administration of BLM waste to quails during the *grower* period on indicators of stress, immunity, and quail performance.

MATERIALS AND METHODS

Research Time and Location

The study was conducted from July to September 2025. The maintenance was carried out at Arkan Quail Farm, Ciampea, Bogor. The phytochemical testing of BLM waste was conducted at the IPB Bioactive Compounds Study Centre Laboratory, and proximate analysis was conducted at PT. Saraswati Indo Genetech Bogor Laboratory and white blood cell samples were analyzed at the IPB SKHB RSHP Research and Diagnostic Laboratory. SOD and MDA liver tests were conducted at the IPB Biotech Centre Laboratory, while immunity testing was conducted at the IPB SKHB Medical Microbiology Laboratory. All research procedures complied with the regulations of the IPB SKHB Animal Ethics Commission (No. 371/KEH/SKE/IX/2025).

Equipment and Materials

The quails were housed in 20 cages measuring 50 × 75 × 36 cm, with each cage containing 10 birds. Cage equipment includes feeders, water containers, incandescent lamps, and thermometers. Laboratory equipment is tailored to the testing procedures for each parameter. Materials used include 200 female quails in the *grower* phase (3–6 weeks old), BLM waste, and New Hope P100 commercial feed (crude protein 17%, metabolizable energy 2800 kcal/kg). Laboratory reagents and materials were prepared according to the procedures for the parameters being observed.

BLM Waste Production

Basil leaves were obtained from commercial farmers in Lamongan Regency. The preparation of basil leaf meal began with harvesting the plants at optimal maturity, drying them in the sun until completely dry, then sorting them to separate the leaves from the stems. The dry leaves were ground into meal using a machine.

Phytochemical and Proximate Analysis of BLM Waste

A quantitative analysis of the phytochemical content in BLM waste was conducted. Quantitative phytochemical analysis included phenols, flavonoids, and tannins, which were analysed according to the procedure described by Senguttuvan *et al.* (2014).

Nutritional content analysis included proximate analysis. The proximate analysis procedure was carried out based on AOAC (2005). This analysis parameters measured included for moisture content, ash content, crude protein, crude fat, and crude fibre.

Quail Maintenance

Quail maintenance included preparing the cages, measuring temperature, feeding twice a day, providing water *ad libitum*, and weighing the quails. Each cage contained 10 quails with different treatments. Temperature and humidity were recorded in the morning (06.00–07.00 AM), early afternoon (12.00–1.00 PM), and late

afternoon (4.00–5.00 PM). Feed with basil leaves was given starting at 3 weeks of age, and growth was measured up to 6 weeks. Blood samples were collected at 6 weeks of age after a 12-hour fast, placed in EDTA tubes, stored in a cooling box, and analysed.

Stress Indicators

Oxygen saturation was measured using a pulse oximeter (Jumadin *et al.*, 2023). The H/L ratio relates to the comparison between heterophils and lymphocytes (Maheshwari *et al.*, 2017). SOD analysis in the liver was performed using the Maskar *et al.* (2015) method with a spectrophotometer at 480 nm. MDA levels in the liver were calculated based on the linear regression curve of 1,1,3,3-tetramethoxypropane (TMP) at concentrations of 1, 5, 7, 13, and 18 μ (Ulhusna *et al.*, 2019).

Immunity Observation

The number of leukocytes were determined using a blood sample placed in a leukocyte diluent and an aspirator, then examined under a 400× microscope, and the results were multiplied by 50 (Maheshwari *et al.*, 2017). Leukocyte differentiation were determined in blood smear preparations to count heterophils, lymphocytes, monocytes, basophils, and eosinophils (Nurhayati *et al.*, 2019). A *clearance test* was performed to assess the immune system's ability to cope with exposure, using blood challenged with *Salmonella pullorum* bacteria (10⁸ cfu/mL) according to the method of Jackson *et al.* (1998).

Performance Observation

Feed intake was calculated from the difference between the feed provided and the feed remaining per day (g/bird/day). Weight gain was calculated from the difference between the final and initial weights. Feed conversion was calculated from weekly feed consumption divided by weekly weight gain. Mortality was calculated as the percentage of dead quails from the initial total.

Data Analysis

This study used a completely randomized design (CRD). Data were analyzed using SAS Studio software. If there was a significant effect ($P < 0.05$), a follow-up test was performed using Duncan's test. Leukocyte data and differentiation, heterophil/lymphocyte ratio (H/L), and feed conversion were analyzed descriptively.

RESULTS AND DISCUSSION

Environmental Conditions During Rearing

The morning temperature ranged from 23.1 to 27.8°C, the afternoon temperature from 31.3 to 34.6°C, and the evening temperature from 31.7 to 34.0°C. According to Ulupi *et al.* (2016), the comfort zone for quails is 20–24°C, so only part of the morning temperature was within the comfort zone, while the afternoon and evening temperatures exceeded this limit. Morning humidity is 84–98%, afternoon humidity is 70–83%, and evening humidity is 56–85%, while the ideal humidity for quails is 60–70% (Raharjo *et al.*, 2018). High temperatures cause heat stress in quails. Observations during the study showed that quails experienced panting. This condition indicates that quails experience additional heat stress, which in turn causes oxidative stress. That

stress can be overcome by using exogenous antioxidants. One exogenous antioxidant is BLM waste.

Results of Phytochemical and Proximate Analysis of BLM Waste

The results of the phytochemical and proximate analyses of BLM waste are presented in Table 1 and 2.

Phenols are secondary metabolites belonging to the polyphenol group and play an important role as natural antioxidants (Tungmunnithum *et al.*, 2018). The main functions of phenolic compounds include antioxidant, antimicrobial, and anti-inflammatory activities (Kaur and Kapoor, 2002). Flavonoids are polyphenol derivatives with highly reactive hydroxyl groups that act as antioxidants (Dai and Mumper, 2010). As antioxidants, flavonoids can donate hydrogen atoms and stabilise free radicals, thereby slowing the oxidation process (Arifin and Ibrahim, 2018). Tannins are polyphenolic compounds composed of benzene rings and hydroxyl groups (Noer *et al.*, 2018). Tannins function to maintain the balance of pro-oxidant molecules through redox reactions (Molino *et al.*, 2023). Based on the above description, BLM waste has the potential to act

Table 1. Results of phytochemical analysis of BLM waste

Phytochemical Test	Quantitative %
Phenol	5.38
Flavonoids	0.64
Tannin	0.05

Results of tests conducted at the Biofarmaka Study Center Laboratory, IPB (2025)

Table 2. Results of proximate analysis of BLM waste

Nutrients	Percentage (%)
Crude protein	14.50
Crude fiber	13.80
Extract ether	3.61
Water	11.30
Ash	12.92

Test results at the PT. Saraswanti Indo Genetech Laboratory (2025).

Table 3. Stress indicators in quails with the addition of BLM waste in feed

Parameters	Treatments			
	P0	P1	P2	P3
Oxygen Saturation %	87.30±7.42	90.30±5.64	92.90±4.59	89.50±2.24
H/L	0.52±0.29	0.48±0.27	0.32±0.18	0.45±0.38
Liver SOD (units ml ⁻¹)	13.37±0.43 ^c	14.05±0.37 ^b	15.95±0.37 ^a	13.56±0.28 ^{bc}
Liver MDA (nmol mg ⁻¹)	1.74±0.09 ^a	1.31±0.08 ^b	1.15±0.05 ^c	1.54±0.84 ^a

Different superscripts on the same row indicate significant differences ($p < 0.05$); P0: without BLM waste (control); P1: 1.5% BLM waste; P2: 3% BLM waste; P3: 4.5% BLM waste 5%; P3: feed + moringa leaf flour 7.5%.

Table 4. Immunity of quails with the addition of BLM waste in feed

Parameters	Treatments			
	P0	P1	P2	P3
White blood cells (10 ³ mm ⁻³)	13.13±4.88	13.93±2.83	14.15±10.11	23.10±7.72
Leukocyte differentiation (%)				
Lymphocytes	50.40±21.12	63.00±15.00	54.60±24.82	49.00±20.65
Heterophil	37.80±23.08	27.20±11.98	37.20±23.06	43.00±18.76
Monocytes	5.00±2.54	3.20±2.86	3.60±2.40	3.60±2.40
Eosinophils	6.80±4.09	6.60±3.20	4.60±3.13	4.40±4.82
Basophils	ND	ND	ND	ND
Mortality of <i>S. pullorum</i> (%)	99.15±0.13 ^c	99.85±0.04 ^b	99.99±0.01 ^a	99.94±0.04 ^{ab}

Different superscripts on the same line indicate significant differences ($p < 0.05$); P0: without BLM waste (control); P1: 1.5% BLM waste; P2: 3% BLM waste; P3: 4.5% BLM waste ND: Not detected

as an antioxidant, thereby improving physiological performance.

The protein content of BLM waste is sufficient for use as a feed supplement. Protein is needed to maintain basic life functions and support muscle and bone growth, as well as egg production (Kantja *et al.*, 2022). The crude fibre content of BLM waste is relatively high, which can be a limiting factor for quails (Kamel *et al.*, 2020). This condition occurs because poultry, including quails, lack cellulase enzymes that aid in fibre digestion (Prawitasari *et al.*, 2012).

BLM waste contains nutrients, especially protein, which can improve feed quality and support physiological processes and growth. However, its high crude fibre content can inhibit digestion and nutrient absorption, so its amount and proportion in the ration must be carefully regulated to avoid negative impacts on health and productivity.

Stress Indicators

Stress indicator observations in this study included oxygen saturation, H/L, SOD levels, and liver MDA levels. The results of these observations are presented in Table 3.

Chakravarty *et al.* (2022) stated that oxygen saturation measures the percentage of oxygen in the blood. The addition of BLM waste increased oxygen saturation compared to P0, with the highest value in P2 and a decrease in P3. According to Abu *et al.* (2024), a value of <90% indicates hypoxemia. The increase in P2 indicates that 3% BLM waste can improve oxygen circulation. In contrast, the decrease in P3 indicates an adaptive response to excessive antioxidant doses, as flavonoids and phenolics at high doses can act as pro-oxidants (Andrés *et al.*, 2023).

The H/L ratio is the ratio of heterophils to

Table 5. Quail performance during the growth period with the addition of BLM waste in feed

Parameters	Treatments			
	P0	P1	P2	P3
Feed intake (g b ¹ d ⁻¹)	15,49±0,09 ^a	15,22±0,17 ^b	15,06±0,12 ^b	15,19±0,11 ^b
Body weight gain (g b ⁻¹)	85,61±5,61	90,50±5,81	93,08±1,85	92,00±5,98
Feed conversion	3,81±0,21	3,53±0,21	3,39±0,06	3,48±0,21
Mortality %	0	0	0	0

Different superscripts on the same row indicate significant differences ($p < 0.05$); g: grams; b: birds; d: days; P0: without BLM waste (control); P1: 1.5% BLM waste; P2: 3% BLM waste; P3: 4.5% BLM waste.

lymphocytes and is used to assess stress in quails (Nengsih and Mustika, 2020). The lowest value was found in P2 and increased in P3. The normal H/L value in quails is 0.45–0.5 (Thrall *et al.*, 2012). A low H/L value indicates low stress, as oxidative stress increases glucocorticoid hormones, which in turn increase heterophils and decrease lymphocytes (Wasti *et al.*, 2020). The low H/L in P2 was due to the bioactive compounds in basil leaves, which increased with dose and contributed to heat tolerance, antioxidant status, and immune function (Guo *et al.*, 2023).

SOD is an endogenous antioxidant that catalyzes the dismutation of free radicals through redox reactions (Younus, 2018). The administration of 3% BLM waste produced the highest liver SOD, indicating a high antioxidant capacity against oxidative stress. This increase in liver SOD levels was due to the flavonoid content. According to Van de Wier *et al.* (2017), flavonoids have antioxidant properties that stabilise free radicals, reduce their reactivity, and enhance endogenous antioxidant activity.

MDA is a product of lipid peroxidation and is used to assess cell damage induced by oxidative stress (Jumadin *et al.*, 2022). The administration of BLM waste reduced liver MDA, especially in P2 (3%), indicating low oxidative stress and correlating with increased SOD. This condition is because high antioxidant status generally reduces MDA (Fadhila *et al.*, 2023).

Treatment P2 produced the best physiological conditions of the four stress indicators observed. Although the highest BLM

waste level was found in P3, optimal results were obtained in P2. This outcome was because the increase in BLM waste level was followed by an increase in tannin concentration in the feed. Tannins can bind to proteins and minerals, forming complex compounds that are difficult for the body to absorb (Naumann *et al.*, 2017).

Immunity

Immunity observations in this study included leukocyte count, leukocyte differentiation, and the mortality rate of *S. pullorum* bacteria, as presented in Table 4.

Leukocytes are the main components of the immune system that play roles in phagocytosis and antibody production (Marshall *et al.*, 2018). The mean leukocyte count in this study ranged from 13.13 to 23.10 × 10³/mm³, which is still within the normal range. The normal leukocyte count in quails ranges from 13 to 25 × 10³/mm³ (Campbell, 2022).

Leukocyte differentiation includes lymphocytes, heterophils, monocytes, eosinophils, and basophils. The average lymphocyte count in this study was within normal limits, ranging from 49% to 63%. Mahmoud *et al.* (2013) reported that normal lymphocytes in quails range from 46.25–73.75%. Lymphocytes play a role in adaptive immune responses through B lymphocytes (antibody production) and T lymphocytes (antigen recognition, interferon production, communication with B lymphocytes) (Resmawati *et al.*, 2016). The average heterophil count in this study was within normal limits, ranging from 27.20% to 43.00%. Normal

heterophils in quails range from 25–50% (Campbell, 2022). Heterophils play a role in quickly detecting and killing pathogens through phagocytosis (Harahap *et al.*, 2023). The average monocyte count in this study was within normal limits, ranging from 3.20% to 5.00%. Normal monocytes in quails range from 1.14–6.75% (Mahmoud *et al.*, 2013). Monocytes function as macrophages, destroying dead cells and pathogens and regulating inflammatory responses (Han *et al.*, 2023). The average eosinophil count in this study was within normal limits, ranging from 4.40% to 6.80%. According to Campbell (2022), normal eosinophils in quails are 0–15%. Lokapirnasari and Yulianto (2014) state that eosinophils neutralise inflammation and indicate that quails do not experience allergies or infections. Basophils were not detected in this study. Normal basophil levels in quails are 0–2% (Campbell, 2022). Basophils play a role in defending the body against allergens. Moreira *et al.* (2013) stated, basophils release histamine upon contact with allergens, thereby activating other immune cells.

The immunity of quails was tested with a challenge test using the bacterium *S. pullorum*. P2 and P3 showed the highest ability to kill bacteria, with P2 reaching 99.99%, a significant difference from P0. This condition is due to natural antimicrobial compounds in basil such as eugenol, methyl chavicol, and linalool (Hussain *et al.*, 2008). In P3, a slight decrease was observed due to the saturation effect, in which excessive antioxidant administration suppressed the synthesis of endogenous enzymes (Andrés *et al.*, 2023). Leukocyte concentration and differentiation play a crucial role in maintaining body resistance. The best results in the *clearance* test against *S. pullorum* bacteria were obtained with the addition of 3% and 4.5% BLM waste in the feed.

Quail Performance

Performance observations of quails in this study included feed consumption, initial body weight, final body weight, body weight gain, feed conversion, and mortality, as presented in

Table 5.

Statistical results indicate a significant effect on quail feed consumption. Factors influencing consumption are feed energy content and environmental temperature (Anggitasari *et al.*, 2016). P0 had the highest consumption and P2 the lowest.

Statistical analysis shows that there is no significant difference in quail weight gain between treatments. Although not significant, the highest BLM waste addition was in P3, while the best body weight gain results were obtained in P2. This result indicates that high doses of phenolic compounds can cause pro-oxidant effects, inhibit digestive enzyme activity, and reduce metabolic efficiency (Vlaicu *et al.*, 2023).

Feed conversion is an indicator of feed utilisation efficiency (Yi *et al.*, 2018). P2 (3%) conversion showed the highest efficiency, indicating optimal feed utilisation despite heat stress. Although the highest BLM waste addition was in P3, the best performance was obtained in P2. An increase in BLM waste levels is accompanied by an increase in tannin content, which can bind, precipitate, and inhibit protein synthesis (Sunani and Hendriani, 2023). As a result, the protein that should be metabolised for growth cannot be utilised optimally.

CONCLUSION

The administration of 3% BLM waste to quails during the growth period increased oxygen saturation, SOD, and the ability to kill *S. pullorum* bacteria. It reduced the H/L ratio, MDA, and feed conversion. Thus, administering BLM waste can reduce oxidative stress, improve immunity, and enhance performance. The addition of BLM waste in layer chicken feed during the laying period should be studied to produce eggs as a functional food.

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CONFLICTS OF INTEREST

There is no conflict of interest in this research.

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