Research Article

Pepper Elder Leaf Powder (PEP) Regulates Energy Homeostasis, Immune, and Stress Biomarkes in Heat-Stressed Indonesian Local Laying Quail

Andi Mushawwir[1]*, Heni Natalia Aritonang[2], Eli Sahara[3], St. Aisyah Sijid[4], Zeba F. Alam[5], Denny Rusmana[1], Ronnie Permana[1]

[1]Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, Padjadjaran University, Jl. Ir. Soekarno Km. 21 Jatinangor, Sumedang 45363, West Java, Indonesia; [2]Department of Animal Bioscience, Faculty of Food Security, Surabaya State University. Campus 3, Jl. Prof. Dr. Moestopo No. 4, Pacar Keling, Tambaksari District, Surabaya, East Java 60131, Indonesia; [3]Animal Science, Faculty of Agriculture, Sriwijaya University, Jalan Palembang-Prabumulih, KM 32, Inderalaya, Ogan Ilir Regency, 30662, South Sumatra, Indonesia; [4]Department of Biology, UIN Alauddin Makassar. Jl. H.M. Yasin Limpo No.36, Gowa 92118, Makassar, South Sulawesi, Indonesia; [5]Department of Biology, College of Science, De La Salle University, 2401 Taft Avenue, Manila 1004, Philippines

Abstract | Global climate change is a major environmental factor driving a decline in the productivity of laying quail by disrupting energy homeostasis, reducing immune responses, increasing oxidative stress, and lowering production performance, thereby threatening food security. Phytogenic ingredients rich in bioactive compounds, such as Pepper Elder Powder (PEP), may offer a nutritional strategy to enhance poultry physiological resistance and climate resilience. This study aimed to evaluate the effects of PEP supplementation on energy homeostasis, immune response, stress biomarkers, and production performance in laying quail reared under heat stress. A total of 400 laying quail aged 12 weeks were reared for eight weeks and allocated to four treatments: PEP-0 (control), PEP-75, PEP-150, and PEP-225 g/kg ration. Spectrophotometric techniques were used to analyse glucose, triglycerides, cholesterol, total protein, leukocyte count, heterophil/lymphocyte ratio (H/L), IgA, IgG, IL-6, IL-10, TNF-α, corticosterone, malondialdehyde (MDA), egg production, egg weight, and feed conversion ratio (FCR). Data were analysed using one-way ANOVA followed by Tukey's test at a significance level of 5%. The results showed that PEP supplementation did not affect blood glucose levels (P>0.05), but significantly increased triglyceride and cholesterol levels and decreased total protein (P<0.001). In addition, PEP increased leukocyte count, IgA, IgG, and IL-10, and decreased the H/L ratio, IL-6, TNF-α, corticosterone, and MDA (P<0.001). PEP supplementation also increased egg production and weight, and improved feed conversion efficiency compared to the control group (P<0.001). It was concluded that PEP supplementation, especially at a dose of 225 g/kg ration, improved metabolic homeostasis, enhanced immune status, suppressed stress responses, and improved production performance of laying quail reared under heat stress conditions.

Keywords | Global climate change, Physiology, *Peperomia pellucida*, Productivity, Quail, Food security.

Received | June 06, 2026; **Accepted** | August 01, 2026; **Published** | August 27, 2026
***Correspondence** |Andi Mushawwir, Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, University of Padjadjaran, **Email:** mushawwir@unpad.ac.id.
Citation | Mushawwir A, Aritonang HN, Sahara E, St. Aisyah Sijid, Alam Z F, Rusmana D, Permana R (2026). Pepper elder leaf powder (pep) regulates energy homeostasis, immune, and stress biomarkes in heat-stressed indonesian local laying quail Anim. Vet. Sci., 14(9):2066-2074.
DOI | https://dx.doi.org/10.17582/journal.aavs/2026/14.9.2066.2074
ISSN (Online) | 2307-8316

INTRODUCTION

Quail are a rapidly growing poultry commodity, valued for their relatively early sexual maturity, high egg productivity, high feed efficiency, and lower maintenance costs compared with laying hens. However, quail productivity is strongly influenced by environmental conditions, particularly global climate change, with temperature and humidity being key drivers (Campbell *et al*., 2022). In tropical regions such as Indonesia, ambient temperatures often exceed the livestock comfort zone (21-250C for quail), causing heat stress. This condition disrupts physiological homeostasis, reduces feed intake, alters energy metabolism, increases free radical formation (Olayiwola and Adedokun, 2025), suppresses the immune system (Chen *et al*., 2022), and ultimately reduces egg production and quality. Consequently, heat stress is a major limiting factor in modern livestock industry and threatens food security (Ribeiro *et al*., 2024; Olayiwola and Adedokun, 2025).

The physiological response to heat stress involves activation of endocrine signalling pathways, particularly in the hypothalamus, pituitary, and adrenal glands, followed by increased glucocorticoid secretion (El Sabry *et al*., 2022), with corticosterone the primary glucocorticoid in poultry. These hormonal increases trigger changes in carbohydrate, protein, and lipid metabolism to maintain energy balance but, in the long term, can lead to metabolic disorders and reduced production efficiency. Increased production of reactive oxygen species (ROS) during heat stress also significantly contributes to oxidative stress, characterised by increased lipid peroxidation, as indicated by malondialdehyde (MDA) levels, and decreased antioxidant capacity. This condition is also accompanied by an increased heterophil/lymphocyte (H/L) ratio, changes in cytokine profiles (Khan *et al*., 2023), and decreased humoral and cellular immune function, making poultry more susceptible to various health disorders (Ribeiro *et al*., 2024; Oke *et al*., 2026).

Various strategies have been developed to mitigate the negative impacts of heat stress, including improved housing systems, husbandry management, genetic selection, and nutritional approaches (Jimoh *et al*., 2022). Among these strategies, natural plant-based feed additives (phytogenic feed additives) are attracting growing attention for their bioactive compounds, which act as antioxidants, anti-inflammatories (Du *et al*., 2022), and immunomodulators (Hu *et al*., 2019). They also increase metabolic efficiency without leaving residues in livestock products (Paredes *et al*., 2024). Several studies have shown that phytogenic supplementation can improve production performance, increase antioxidant status, reduce stress biomarkers, and enhance the immune response in heat-stressed poultry (Jimoh *et al*., 2022; Olayiwola and Adedokun, 2025).

Pepper Elder (*Peperomia pellucida*) is a plant with potential as a natural feed additive. It contains bioactive compounds, including flavonoids, polyphenols, alkaloids, tannins, saponins, and other phenolic compounds, which exhibit strong antioxidant and anti-inflammatory activities (Krismiyanto *et al*., 2025), also someotjer herbs (Paredes *et al*., 2024). In vitro pharmacological studies have shown that *P. pellucida* extract can scavenge free radicals, inhibit the formation of inflammatory mediators, increase the activity of endogenous antioxidant enzymes, and protect tissues from oxidative damage. These biological activities indicate that a 2.5 mg methanol extract of *P. pellucida* and some herbs has the potential to act as a phytogenic agent to increase the physiological resistance of poultry to heat stress by improving metabolic balance, immune response, and oxidative status (Jimoh *et al*., 2022; Paredes-Lopez *et al*., 2025). However, in vivo trials at 150 mg/kg in the ration have not shown satisfactory results.

However, the use of Pepper Elder Powder (PEP) as a feed additive in laying quail, particularly under heat stress, remains very limited, leaving a lack of robust scientific information. Most previous studies have evaluated only one or two parameter groups (0 and 15 g/kg), such as production performance, antioxidant status, or immune indicators, in isolation (Ribeiro *et al*., 2024; Oke *et al*., 2026). This approach fails to fully characterise physiological responses, as adaptation to heat stress results from complex interactions among energy homeostasis, the immune system, oxidative stress, and production performance. Therefore, research integrating multiple physiological indicators is needed to more comprehensively understand the mechanisms underlying responses to phytogenic supplementation.

This study is strategically designed to assess how pepper elder powder supplementation affects energy balance, immune function, stress indicators, and production performance in laying quails exposed to heat stress. This study aims to provide scientific insights into the potential of *P. pellucida* as a natural feed additive to improve the physiological resilience and productivity of laying quails under tropical conditions, supporting the development of more effective and sustainable phytogenic-based nutritional strategies and food security.

MATERIALS AND METHODS

Animal sample, treatments and housing

Four hundred 12-week-old Indonesian local quail were randomly assigned to four experimental groups, each with five replicates, yielding 20 quail samples per experimental unit. Commercial battery cages for quail farming were used in this research and were constructed from standardised iron wire.

Pepper Elder Leaf Powder (PEP) was tested in four experimental groups: without PEP (PEP-0), supplemented with 75 g/kg of the basal ration (PEP-75), and supplemented with 150 g (PEP-150) and 225 g (PEP-225). The basal ration consisted of a mixture of fish meal, soybean meal, corn meal, fine bran, coconut meal, bone meal, and coconut oil. The nutritional content of the basal ration was 18.6% crude protein and 2,950 kcal of metabolisable energy.

The enclosure is located in the lowlands, with a relatively hot and humid annual climate. The cage microclimate was recorded daily throughout the study using a digital thermometer that stores temperature and humidity data. Daily temperature and humidity fluctuations during the study are shown in Figure 1.

PEP preparation

Pepper elder plant samples were collected from various locations in West Java, Indonesia. Leaves were separated from stems, sun-dried for one hour, then oven-dried for 48 hours at 40°C, and finally ground to a fine powder through a 50-mesh sieve. The powder was oven-dried for 2 hours at 40°C. A 100–250 mg portion was placed in a centrifuge tube, to which 1–5 mL of 80% methanol, a 20:80 methanol-water mix, or a methanol–water mixture with 0.1% formic acid was added. An internal standard could be added at this point to account for extraction and response variability. The mixture was vortexed for 1–2 minutes, sonicated for 20–30 minutes at low temperature, incubated at 4°C for 30–60 minutes, then centrifuged at around 10,000–15,000 × g for 10–15 minutes. Separation was performed using a C18 UHPLC column (100 × 2.1 mm, 1.7–2.6 µm). Detection typically utilised electrospray ionisation (ESI) in positive and negative modes. Several compounds, including pellitorine, sarmentine, piperine, and vitexin-2-O-rhamnoside, were quantified at 9.52, 5.35, 0.73, and 6.87 mg/g, respectively.

Blood sampling and sample analysis

Quail samples were fasted for 6 hours prior to blood collection. A 3 mL syringe and EDTA tube were used to collect blood samples from the jugular vein. Alcohol wipes were applied to the puncture site before and after blood collection.. All serum parameters related to immunity and stress were analysed by enzyme-linked immunosorbent assay (ELISA) using a commercial kit, following the manufacturer's protocol from Mybiosource (USA), except for leukocytes and the H/L ratio, which were analysed using a haematology analyser. All parameters related to energy homeostasis were quantified using a UV-Vis spectrophotometer, following the analysis guidelines provided with the Biolabo kit.

Data analysis

All research parameter data were analyzed with one-way ANOVA in a completely randomized design (CRD), followed by Tukey's Honestly Significant Difference Test at a 5% significance level to compare treatment means. Results are shown as mean ± standard deviation. Statistical analyses were conducted using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA).

A SHAP analysis was conducted using a Random Forest Regression model that included all parameters related to energy homeostasis, immune response, and stress biomarkers as predictor variables. The SHAP library in Python 3.11 was used to compute SHAP values, which quantify the influence of each parameter on the model's predictions, specifically FCR and HDP. The results were presented in a SHAP summary plot, with variables ranked by their average absolute SHAP value, facilitating the identification of the most impactful parameters.

RESULTS AND DISCUSSION

Energy homeostasis

The effect of PEP administration on energy homeostasis in quail during the egg-laying phase under heat stress is shown in Table 1.

Based on Table 1, PEP supplementation had different effects on energy homeostasis parameters in laying quail reared under heat stress. Glucose concentrations were relatively stable across all treatment groups and showed no significant differences ($P=0.604$), with values ranging from 204.00 ± 3.54 to 207.00 ± 3.54 mg/dL. In contrast, triglyceride levels increased significantly with increasing PEP doses ($P<0.001$), from 186.00 ± 1.58 mg/dL in the control group (PEP-0) to 240.00 ± 1.58 mg/dL in the PEP-225 group. A similar pattern was also observed in serum cholesterol levels, which increased gradually from 184.00 ± 2.55 mg/dL in PEP-0 to 213.00 ± 2.55 mg/dL in PEP-225 ($P<0.001$). In contrast, total protein concentration decreased significantly with increasing PEP dose, from 27.30 ± 0.70 g/dL in the control group to 22.86 ± 0.75 g/dL in the PEP-225 group ($P<0.001$). In general, the results of this study indicate that PEP supplementation did not affect blood glucose levels, but significantly altered the lipid metabolism profile and total protein concentration in laying quails experiencing heat stress.

The results showed that PEP supplementation significantly affected most energy homeostasis parameters, except blood

Links Researchers

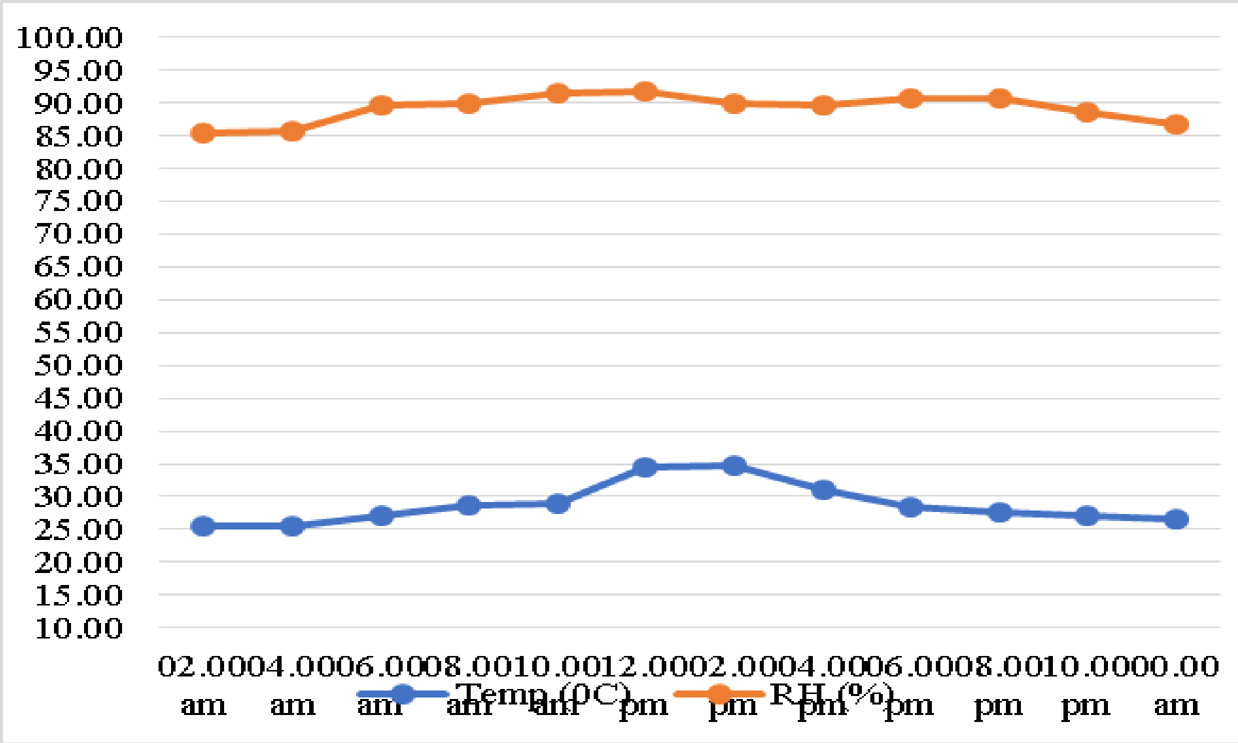


Figure 1: Daily temperature and humidity fluctuations during the study.

Table 1: Effects of PEP supplementation on energy homeostasis of heat-stressed laying quail.

Parameter	PEP-0	PEP-75	PEP-150	PEP-225	P-value
Glucose (mg/dL)	205.00 ± 3.54^{a}	204.00 ± 3.54^{a}	205.00 ± 3.54^{a}	207.00 ± 3.54^{a}	0.604
Triglycerides (mg/dL)	186.00 ± 1.58^{a}	200.00 ± 1.58^{b}	215.00 ± 1.58^{c}	240.00 ± 1.58^{d}	<0.001
Cholesterol (mg/dL)	184.00 ± 2.55^{a}	192.00 ± 2.55^{b}	204.00 ± 2.55^{c}	213.00 ± 2.55^{d}	<0.001
Total protein (mg/dL)	27.30 ± 0.70^{c}	24.92 ± 0.55^{b}	25.37 ± 0.77^{b}	22.86 ± 0.75^{a}	<0.001

Different notations on the same line show significant differences.

glucose levels. The stability of glucose concentrations across all treatment groups indicates that PEP supplementation did not disrupt glucose regulatory mechanisms, even though the quails were reared under heat stress. In poultry, blood glucose levels are maintained within a relatively constant range through homeostatic mechanisms involving hepatic gluconeogenesis, glycogenolysis, and hormonal regulation by glucagon and corticosterone (Yilmaz *et al.*, 2023). Therefore, the absence of changes in glucose levels indicates that basic energy requirements remain met despite alterations in other metabolic pathways (Ribeiro *et al.*, 2024).

Conversely, the increased triglyceride and cholesterol levels in the PEP-supplemented group indicate changes in lipid metabolism leading to increased availability of yolk precursors. In laying hens, triglycerides and cholesterol are the main components of very low-density lipoprotein (VLDL) (Olayiwola and Adedokun, 2025), and vitellogenin is synthesised in the liver and then transported to the ovaries to support follicle development and yolk formation (Olayiwola and Adedokun, 2025). Therefore, the increase in these two parameters in this study reflects increased reproductive metabolic activity rather than impaired lipid metabolism (Lay *et al.*, 2023). This is consistent with the increased egg production and weight observed in the group with higher PEP supplementation (Oke *et al.*, 2024; Barros *et al.*, 2024).

These changes in lipid metabolism are thought to be related to bioactive compounds in *P. pellucida*, particularly flavonoids, polyphenols, and other phenolic compounds that increase metabolic efficiency through antioxidant activity and reduced oxidative stress. Reducing the formation of reactive oxygen species (ROS) allows more metabolic energy to be allocated to the synthesis of biomolecules that support reproductive function rather than maintaining adaptive responses to heat stress. Furthermore, phytogenic compounds have been reported to improve liver function, the primary organ involved in lipid metabolism, thereby increasing lipoprotein synthesis, which is necessary for egg formation (Aritonang *et al.*, 2024; Li *et al.*, 2024).

This study also observed a decrease in total protein levels with higher PEP doses. This reduction likely indicates increased use of amino acids to synthesise egg proteins and other molecules critical for reproduction. In laying hens, many plasma proteins, especially vitellogenin and yolk precursors, are actively transferred to the ovaries during peak production (Li *et al*., 2024). Thus, a decline in total protein alongside increased egg production suggests improved efficiency in directing proteins to reproductive tissues, rather than a protein deficiency (Barros *et al*., 2024).

Immune responses and stress biomarkers

The immune response and stress biomarker levels of laying-phase quail given PEP and maintained under heat stress conditions are shown in Table 2.

Based on Table 2, PEP supplementation significantly affected all immune response parameters and stress biomarkers in laying quails reared under heat stress conditions (P<0.05). The number of leukocytes increased gradually with increasing PEP dose, from 26.87 ± 0.62 × 10^3/mm^3 in the control group (PEP-0) to 30.15 ± 0.56 × 10^3/mm^3 in the PEP-225 group (P < 0.001). In contrast, the heterophil/lymphocyte (H/L) ratio decreased significantly from 0.77 ± 0.04 in PEP-0 to 0.54 ± 0.04 in PEP-225 (P<0.001). Humoral immunity parameters also showed significant increases, as indicated by higher concentrations of IgA, IgG, and IL-10, with the highest values consistently observed in the PEP-225 group (P<0.001). Conversely, the concentrations of the pro-inflammatory cytokines IL-6 and TNF-α decreased significantly with increasing PEP dose. Furthermore, stress biomarkers also decreased significantly. Serum corticosterone concentration decreased from 3.44 ± 0.37 ng/mL in the control group to 0.87 ± 0.07 ng/mL in the PEP-225 group, while malondialdehyde (MDA) levels decreased from 3.67 ± 0.24 to 0.65 ± 0.08 nmol/mL (P<0.001). Overall, these results indicate that PEP supplementation significantly improves immune status and reduces biomarkers of physiological and oxidative stress in laying quail under heat stress.

Research results show that PEP supplementation significantly improves immune status and reduces stress biomarkers in laying quail under heat stress. Increased leukocyte counts, IgA, IgG, and IL-10 levels, accompanied by decreases in the H/L ratio, IL-6, TNF-α, corticosterone, and MDA, indicate that PEP can improve immune system balance and reduce the physiological impacts of heat stress (Du *et al*., 2022; Basiouni *et al*., 2023). Based on current research findings, the response pattern indicates that the bioactive compounds in PEP act synergistically as immunomodulators and antioxidants, thereby enhancing birds' ability to adapt to unfavourable environments (Hu *et al*., 2019; Li *et al*., 2024).

An increase in leukocyte counts and in IgA and IgG concentrations indicates improved innate and humoral immune responses. These increases indicate that PEP supplementation can enhance immune competence without triggering an excessive inflammatory response. These results are related to the flavonoid, polyphenol and phenolic compound content of *P. pellucida,* which is known to increase lymphocyte activity, stimulate immunoglobulin synthesis, and improve immune system function (Krismiyanto *et al*., 2025).

The results of this study also showed an increase in IL-10, followed by decreases in IL-6 and TNF-α, indicating that PEP helps maintain the balance between anti-inflammatory and pro-inflammatory responses. IL-10 is an anti-inflammatory cytokine that suppresses the production of pro-inflammatory cytokines, whereas IL-6 and TNF-α are key mediators of inflammation that generally increase during heat stress (Jimoh *et al*., 2022; Mahasneh *et al*., 2024). The decrease in these two cytokines indicates reduced activation of the inflammatory process triggered by

Table 2: Effects of pepper elder powder (pep) supplementation on immune responses and stress biomarkers in heat-stressed laying quail.

Parameters	PEP-0	PEP-75	PEP-150	PEP-225	P-value
Immune responses :					
Leukocyte (×10^3/mm^3)	26.87 ± 0.62^a	27.71 ± 0.44^b	28.70 ± 0.28^c	30.15 ± 0.56^d	<0.001
H/L ratio	0.77 ± 0.04^d	0.68 ± 0.04^c	0.65 ± 0.02^b	0.54 ± 0.04^a	<0.001
IgA (μg/mL)	535.43 ± 3.88^c	541.41 ± 2.50^b	543.92 ± 4.03^b	551.22 ± 1.68^a	<0.001
IgG (mg/mL)	2.85 ± 0.29^a	3.08 ± 0.44^b	3.85 ± 0.69^c	4.08 ± 0.17^d	0.001
IL-6 (pg/mL)	0.80 ± 0.04^d	0.71 ± 0.04^c	0.68 ± 0.02^b	0.57 ± 0.04^a	<0.001
IL-10 (pg/mL)	475.14 ± 3.88^a	481.12 ± 2.50^b	483.63 ± 4.03^b	488.93 ± 3.18^c	<0.001
TNF-α (pg/mL)	23.75 ± 0.47^d	19.37 ± 1.31^c	12.50 ± 1.51^b	9.38 ± 1.08^a	<0.001
Stress biomarkers :					
Corticosterone (ng/mL)	3.44 ± 0.37^d	2.31 ± 0.37^c	1.89 ± 0.35^b	0.87 ± 0.07^a	<0.001
MDA (nmol/mL)	3.67 ± 0.24^d	2.49 ± 0.32^c	1.81 ± 0.36^b	0.65 ± 0.08^a	<0.001

Different notations on the same line show significant differences.

oxidative stress, thereby minimising tissue damage (Chen *et al.*, 2022; Oke *et al.*, 2024).

In addition to improving immune status, PEP supplementation also reduced the H/L ratio, corticosterone, and MDA. The H/L ratio is a sensitive haematological indicator of stress in poultry, corticosterone is a key stress-response hormone, and MDA is a marker of lipid peroxidation. The reduction in these three parameters indicates that PEP can suppress stress-response activation and reduce oxidative damage at the cellular level (Abdullahi *et al.*, 2025; Oke *et al.*, 2026). This activity is thought to originate from the ability of *P. pellucida*'s antioxidant compounds to scavenge free radicals, enhance the endogenous antioxidant system, and protect cell membranes from lipid peroxidation (Li *et al.*, 2024; Ribeiro *et al.*, 2024).

The results of this study indicate that PEP supplementation can improve immune competence while reducing physiological and oxidative stress in laying quail experiencing heat stress and global climate change. These improvements in immune status and reductions in stress biomarkers are suspected to be the main mechanisms supporting the increased productivity of laying quail in the group receiving PEP supplementation.

Perfomance

The performance profile of laying-phase quail given PEP in the ration, maintained under heat-stress conditions, is shown in Table 3.

According to Table 3, PEP supplementation significantly influenced all production performance parameters of laying quail under heat stress (P<0.01). Egg production (%HDP) rose markedly with higher PEP doses, from 71.00 ± 1.91% in the control group (PEP-0) to 80.10 ± 1.54% in the PEP-225 group (P<0.001). Although the PEP-75 and PEP-150 groups exhibited similar egg production, both surpassed the control. Egg weight also increased notably with PEP, from 8.34 ± 0.37 g in PEP-0 to 9.82 ± 0.14 g in PEP-225 (P<0.001). Additionally, the feed conversion ratio (FCR) decreased significantly, reflecting better feed efficiency. The lowest FCR was in the PEP-225 group (3.87 ± 0.05), while the control group had the highest (4.08 ± 0.08) (P<0.001). Overall, the findings indicate that PEP supplementation enhances laying quail productivity by boosting egg production and body weight and improving feed efficiency under heat stress amid global climate change.

The research results showed that PEP supplementation significantly increased egg production, egg weight, and feed conversion efficiency in laying quail reared under heat stress conditions. This increase in production performance is thought to result from improved physiological conditions, including enhanced immune status and reduced oxidative stress observed in this study. In poultry, global climate change or heat stress generally causes decreased feed consumption, metabolic disorders, and increased energy requirements to maintain homeostasis, resulting in decreased egg productivity (El Sabry *et al.*, 2022). Therefore, PEP's ability to improve physiological conditions is likely to contribute directly to increased productivity (Khan *et al.*, 2023; Ribeiro *et al.*, 2024).

The increased egg production and weight in the PEP-supplemented group were also associated with improved lipid metabolism, as indicated by higher triglyceride and cholesterol levels (Table 1). These two components are the main precursors of very low-density lipoprotein (VLDL) and vitellogenin, both synthesised in the liver to support follicle development and yolk formation. Therefore, increased lipid metabolism, while remaining within the physiological range, can increase nutrient supply to the ovaries, thereby supporting more optimal egg formation (Yilmaz *et al.*, 2023; Barros *et al.*, 2024).

Besides boosting egg production, PEP supplementation improves Feed Conversion Ratio (FCR), indicating increased nutrient efficiency. This FCR improvement likely results from lower physiological stress, as evidenced by reduced corticosterone, MDA, and proinflammatory cytokines, thereby allowing more energy to support follicle development and egg formation rather than heat-stress adaptation. The flavonoids and polyphenols in P. pellucida are also known to promote digestive health, boost metabolic processes, and improve nutrient absorption, thereby supporting overall poultry productivity (Barros etal., 2024; Krismiyanto *et al.*, 2025).

Table 3: Effects of pepper elder powder (PEP) supplementation on productive performance of heat-stressed laying quail.

Parameter	PEP-0	PEP-75	PEP-150	PEP-225	P-value
Egg production (% HDP)	71.00 ± 1.91[a]	78.10 ± 2.07[b]	78.57 ± 1.01[b]	80.10 ± 1.54[c]	<0.001
Egg weight (g)	8.34 ± 0.37[a]	8.83 ± 0.26[b]	9.33 ± 0.43[c]	9.82 ± 0.14[c]	<0.001
Feed conversion ratio (FCR)	4.08 ± 0.08[c]	3.97 ± 0.06[b]	4.02 ± 0.03[bc]	3.87 ± 0.05[a]	<0.001

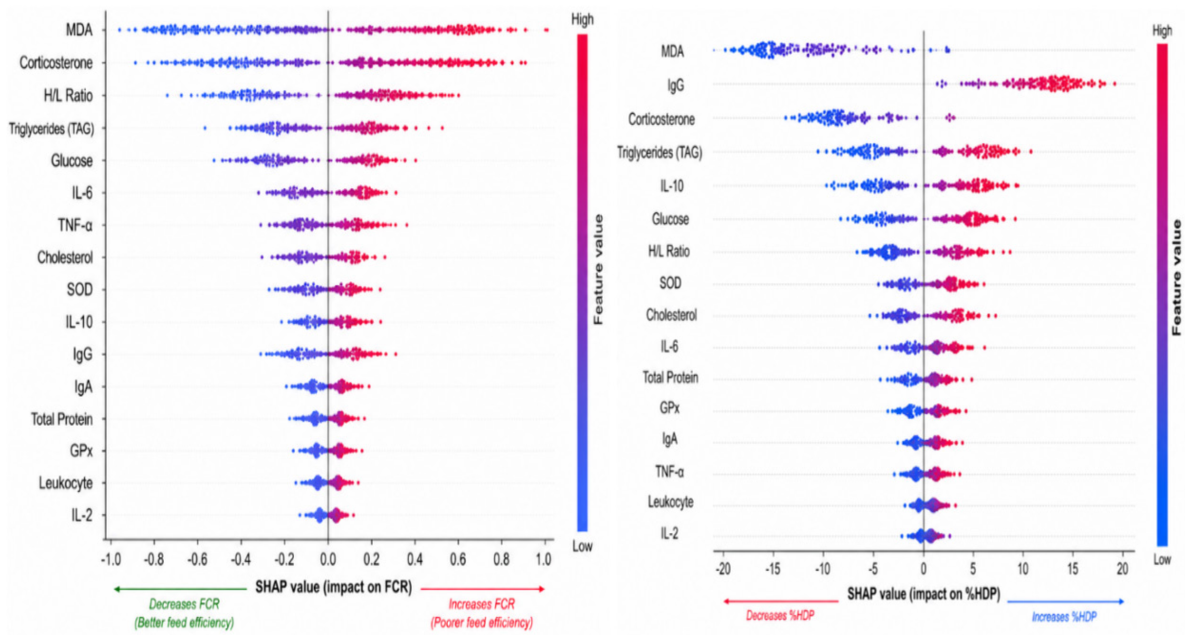
Different notations on the same line show significant differences.

Overall, the results of this study indicate that PEP supplementation can improve the production performance of laying quail by improving metabolic efficiency and physiological conditions during heat stress, thereby increasing feed efficiency and egg production (%HDP). The positive and negative contributions of each measured parameter to FCR and %HDP are shown using SHAP (Shapley additive explanations) analysis. Visualisation of SHAP analysis results for the predicted contributions of various parameters to FCR and %HDP is shown in Figure 2.

SHAP analysis revealed that biomarkers related to oxidative stress, physiological stress, and immune status differentially influenced Feed Conversion Ratio (FCR) predictions (Figure 2A). The most influential biomarkers for higher FCR were MDA, corticosterone, H/L ratio, IL-6, and TNF-α. Elevated levels of these markers generally corresponded to positive SHAP values, suggesting higher FCR or lower feed utilisation efficiency. In contrast, increased levels of IgG, IL-10, triglycerides (TAG), glucose, and SOD were linked to negative SHAP values, indicating improved feed efficiency. Other parameters, such as cholesterol, total protein, GPx, IgA, leukocyte count, and IL-2, had smaller effects on the model's predictions. Overall, these findings highlight that managing oxidative stress and boosting immune function are key to enhancing feed utilisation efficiency.

SHAP analysis showed that biomarkers of oxidative stress, immunity, and energy metabolism contributed differently to the prediction of egg production (%HDP) (Figure 2B). Among all parameters analysed, MDA had the largest contribution to the prediction model, followed by IgG, corticosterone, triglycerides (TAG), IL-10, and glucose. Higher MDA and corticosterone values tended to reduce predicted %HDP, whereas increased levels of IgG, IL-10, TAG, and glucose tended to increase egg production. Other parameters, such as the H/L ratio, SOD, cholesterol, IL-6, and total protein, showed moderate contributions, while GPx, IgA, TNF-α, leukocyte count, and IL-2 had relatively smaller effects. Overall, the SHAP analysis indicated that controlling oxidative stress, improving immune status, and improving energy metabolism were the main factors supporting the increase in %HDP.

SHAP analysis showed that stress biomarkers and immune responses were the most significant contributors to production efficiency in heat-stressed laying quail. In the FCR prediction model, MDA, corticosterone, and the H/L ratio had the highest SHAP values, indicating that increases in these three parameters were associated with poorer feed conversion efficiency. Conversely, increases in IgG, IgA, IL-10, and leukocyte count tended to reduce FCR, indicating more efficient nutrient utilisation. A similar pattern was observed in the egg production (%HDP) prediction model, where IgG was the dominant predictor, followed by MDA, corticosterone, triglycerides, and IL-10. These results indicate that improved immune status, accompanied by reduced physiological and oxidative stress, is the main factor supporting increased egg production. These findings align with previous research showing that heat stress diverts metabolic energy to maintain homeostasis, thereby reducing productivity (Saracila et al., 2021). Phytogenics rich in phenolic and flavonoid compounds can improve immune balance, reduce



A B

Figure 2: SHAP analysis for the contribution of biomarkers measured in research to FCR (A) and % HDP (B).

oxidative stress, and increase nutrient utilisation efficiency (Paredes-Lopez *et al*., 2025), as well as poultry production performance (Li *et al*., 2024; Oke *et al*., 2024; Krismiyanto *et al*., 2025). Thus, the SHAP analysis not only identified the most influential variables but also confirmed that improved immune status and stress management were the main mechanisms underlying improved performance in laying quail after PEP supplementation and its impact on food security.

CONCLUSION

Supplementing with Pepper Elder Powder (PEP) notably enhanced physiological balance and production in heat-stressed laying quail. PEP boosted lipid metabolism, as shown by higher triglyceride and cholesterol levels, while keeping glucose levels stable. It also strengthened immune health by raising leukocyte counts, IgA, IgG, and IL-10, and lowering the heterophil/lymphocyte (H/L) ratio, IL-6, TNF-α, corticosterone, and MDA—markers of stress and oxidative damage. These physiological benefits coincided with increased egg production, larger eggs, and better feed conversion.

Recent SHAP analysis confirms that reducing oxidative stress and boosting immune function are key factors in improving production performance with PEP supplementation. Overall, PEP, particularly at 225 g/kg, shows promise as a phytogenic feed additive to improve the resilience and productivity of laying quail facing heat stress.

ACKNOWLEDGEMENTS

The author expresses appreciation to the parties who supported this research, especially EDWISE Yogyakarta and Al Mustofa Sukabumi.

NOVELTY STATEMENT

This study is the first to comprehensively evaluate the effects of Pepper Elder Powder (Peperomia pellucida) on energy homeostasis, immune responses, stress biomarkers, and production performance in heat-stressed laying quail. Furthermore, this study introduces SHAP (Shapley additive explanations) analysis, which integrates multiple physiological responses, offering a more comprehensive approach to assessing phytogenic effectiveness in poultry. Visualisation of SHAP analysis results for the predicted contributions of various parameters to FCR and %HDP.

AUTHORS' CONTRIBUTION

All authors listed in this article have made equal contributions, from planning and implementation to writing.

Ethical approval

The procedures and implementation of this research have been carefully evaluated by the Animal Welfare and Ethics Assessment Authority, the Animal Experiment Ethics Agency, and the Ministry of Agriculture of the Republic of Indonesia, under certificate number 0502/KEP.08/KEP/10/2025.

Generate AI and AI assisted technology statement

The authors, in preparing the article, did not use AI applications or similar technology.

Conflict of interest

We have no conflict of interest regarding this article.

REFERENCES

Abdullahi HS, Zaruwa MZ, Ukamaka IA, Nweze CC (2025). Comparative evaluation of selected medicinal plant extracts on health biomarkers and antimicrobial activity in broiler chickens. Intern. J. Latest Tech. in Eng. Manag. Appl. Sci., 14(10): 107–112. https://doi.org/10.51583/IJLTEMAS.2025.1410000014

Aritonang H N, Mushawwir A, Adriani L, Puspitasari T (2024). Lipid regulation by early administration of irradiated chitosan and glutathione in heat-stressed broilers. IOP Conf. Series: Earth and Environ. Sci., 1292:012011. https://doi.org/10.1088/1755-1315/1292/1/012011

Barros HSS, Oliveira RF, Minafra CS, Gomide APC, Araújo Neto FR, Santos RC, Silva Júnior AJ, Fernandes RTV, Oliveira MC (2024). Functional oil supplementation improves productive performance, lipid metabolism, and antioxidant status of heat-stressed japanese quail. Poult. Sci., 103(12):104644. https://doi.org/10.1016/j.psj.2024.104041

Basiouni S, Schubert A, Eisenreich W (2023). Anti-inflammatory and antioxidative phytogenic substances for mitigating oxidative stress and inflammation in poultry. Vet. Sci., 10(1):55. 10 https://doi.org/10.3390/vetsci10010055

Campbell DLM, Hinch GN, Downing JA, Lee C (2022). Fear and stress responses of poultry housed in different production systems: implications for welfare and productivity. Poult. Sci., 101(9):102066.

Chen Y, Zhang H, Wang J, Li X, Zhao R, Sun Z (2022). Environmental stress modulates inflammatory responses and immune function in poultry. Front. Vet. Sci., 9:876214.

Du J, Wang H, Wang Y (2022). Antioxidative and anti-inflammatory effects of vitamin C on the liver of laying hens under chronic heat stress. Front. Vet. Sci., 9:963180. https://doi.org/10.3389/fvets.2022.1052553

El Sabry MI, Goonewardene LA, Zuidhof MJ, Ranade AS, Yang X (2022). Effect of housing systems on behaviour, welfare, and productivity of laying hens. Animal, 12(4): 487–501.

Hu R, He Y, Arowolo MAA, Wu S, He J (2019). Polyphenols as potential attenuators of heat stress in poultry production. Antioxidants, 8(3):67. https://doi.org/10.3390/

antiox8030067

Jimoh OA, Omede AA, Adejumo IO (2022). Performance, hematobiochemical indices and oxidative stress status of broiler chickens fed phytogenic supplements during heat stress. J. Animm. Sci. Techn., 64(5):970–985. https://doi.org/10.5187/jast.2022.e46

Khan S, Rahman M, Akter Y, Islam T, Hossain M (2023). Housing environment and welfare assessment in commercial poultry production systems. Poult. Sci., 102(7):102725.

Krismiyanto L, Mangisah I, Yunianto VD, Mulyono M, Maulana AI (2025). Effects of encapsulated peperomia pellucida extract on growth performance, immune status, antioxidant capacity, and protein digestibility of broilers under high stocking density. Adv. Vet. Res., 19(2):131–140.

Lay DC Jr, Fulton RM, Hester PY, Karcher DM, Kjaer JB, Mench JA, Mullens BA, Newberry RC, Nicol CJ, O'Sullivan NP, Porter RE (2023). Hen welfare in different housing systems and implications for productivity. World's Poult. Sci. J., 79(2):215–232.

Li Y, Wang K, Li C, Zhang H, Liu X (2024). Oxidative stress in poultry and the therapeutic role of herbal medicine in intestinal health and metabolic regulation. Antioxidants, 13(11):1375. https://doi.org/10.3390/antiox13111375

Mahasneh ZMH, Al-Dalain SYA, Al-Zghoul MB (2024). Effects of medicinal plants on alleviating heat stress in poultry: A rev. Anim., 14(23):3243-3248.

Oke OE, Abbas AO, Adewumi AA (2026). Heat stress in quail: impacts on health and productivity and mitigation strategies. Poult. Sci., 105(8):114021.

Oke OE, Akosile MA, Iyasere OS, Adebowale TO, Oso AO, Sogunle OM (2024). Oxidative stress in poultry production: mechanisms, consequences and nutritional intervention strategies. Poult. Sci., 103(9):104400. https://doi.org/10.1016/j.psj.2024.104003

Olayiwola SF, Adedokun AS. (2025). Heat stress in poultry: the role of nutritional supplements in gut health and productivity. Front. Vet. Sci., 12(10):57-53. https://doi.org/10.3389/fvets.2025.1691532

Paredes D, Huaynate R A R, Vásquez M R S, Perales-Camacho R A, Morales-Cauti S, Beteta-Blas X, Aldava-Pardave U (2024). Modulation of gut microbiota, morphometry, blood profiles, and performance of broiler chickens supplemented with ethanolic extracts of leaves of piper aduncum, morinda citrifolia, and artocarpus altilis. Front. Vet. Sci., 11(8):42-51. https://doi.org/10.3389/fvets.2024.1286152

Paredes-López DM, Robles-Huaynate RA, Camacho RAP, Santiago CVA, Gonzales JPD, Pardave UA (2025). Piper aduncum polyphenols and flavonoids enhance gut health and productive performance in broiler chickens. Front. Vet. Sci., 12(10):37-43. https://doi.org/10.3389/fvets.2025.1597948

Ribeiro AG, Silva RS, Silva DA, Nascimento JCS, Souza LFA, Silva EG, Ribeiro JES, Campos DB, Alves CVBV, Saraiva EP, Costa FGP, Guerra RR (2024). Heat stress in japanese quails (Coturnix japonica): Benefits of Phytase Supplementation. Animal, 14(24):3599. https://doi.org/10.3390/ani14243599

Saracila M, Panaite TD, Papuc CP, Criste RD (2021). Heat stress in broiler chickens and the effect of dietary polyphenols, with special reference to willow bark supplements—A review. Antioxidants, 10(5):686.

Yilmaz A, Kaya M, Karadas F (2023). Housing environment influences oxidative stress, immune function, and productive performance in laying birds. Animal, 13(4):682. https://doi.org/10.3390/antiox10050686