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Research Article

Effects of a Multi-Component Feed Additive on Performance and Physiology of Heat-Stressed Laying Ducks Reared Without Water Play

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Abstract | Environmental aspects that are generally problematic for local poultry are feed and the physical environment. The feed provided is expected to increase the productivity of local ducks in West Java. Temperatures above the comfort zone for laying ducks (19-22^{0}C) induce oxidative stress, impairing optimal production. The "Three in One" strategy features a unified feed formulation augmented with feed additives that provides three concurrent advantages: reducing oxidative stress triggered by elevated environmental temperatures, enhancing the performance of local West Javanese ducks, and generating eggs with increased antioxidant levels. This study aims to determine the effects of providing active substances and micronutrients with potential as additives. Ninety laying phase Cihateup ducks were used as samples in vivo tests at various levels of feed. This study was designed as an experimental study using a t-test. Treatments consisted of two: P1 with feed additives (a combination of flavonoids, methionine, linoleate, and allicin, each at 0.5 mL/kg bw) and P2 without feed additives. Each treatment unit consisted of 45 ducks. Observations were made on blood biochemistry, production performance, ileum histopathology, HSP and 3β- HSD mRNA expression. The results of the study showed that the biochemical condition of the blood of ducks with the provision of feed additives showed a good profile ($P < 0.05$), namely uric acid 10.67 g/dL, urea 1.89 g/dL, albumin 2.75 g/dL, total protein 1.94 g/dL, glucose 244.18 g/dL, Triglycerides 101.89 g/dL, Reticulocyte count 6.15%, MDA 1.15 nm/M-1cm-1, the performance of ducks that received different feed additives was very significantly higher ($P<0.01$), namely an average HDP of 83.97%, an average egg weight of 85.76 g, shell thickness of 0.31 mm, an average hatchability of 89.45%, the response to histopathology showed a significant difference ($P <0.01$), namely the number of ileal villi (per field of view) 10x10 cm was 12.97, the average height of the ileal villi was 385.76 µm, the number of Peyer's plaques was 8.11, the height of Peyer's plaques was 38.93 µm, and the expression of HSP and 3β-HSD mRNA, namely HSP was 2.25 ng/dL, 3β- HSD mRNA was 7.86 ng/dL and Catalase was 2.06 µL/dL. The feed additive primarily provides heat-stress resilience by boosting antioxidant defenses, thereby reducing oxidative damage at the cellular level and maintaining intestinal integrity and hormonal function, ultimately supporting productivity during thermal stress.

Keywords | Blood biochemistry, Ducks, Histopathology, Performance, Metabolism

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INTRODUCTION

West Java boasts a wealth of local livestock commodities that can be developed to achieve high productivity. One such commodity is ducks. Currently, the Indonesian government has identified the local Cihateup and Pajajaran duck strains. However, livestock productivity is influenced by genetic factors to the extent of 30% and by environmental factors to 70%, although both factors must work together (Mushawwir *et al*., 2023).

Environmental factors that often pose challenges to local poultry farming include feed and physical conditions. Indonesia, as a tropical country, has relatively high average temperatures (ranging from 25 to 35°C), with some areas reaching 38°C (Adriani *et al*., 2024a). Laying ducks living above their comfort zone (19 to 22°C) experience oxidative stress (Muhammad *et al*., 2023), which can impact their ability to achieve optimal egg production. Likewise, improved feed intake is expected to increase the productivity of these local West Javanese ducks (Mushawwir *et al*., 2023).

Local West Javanese ducks are a dual-purpose duck (in addition to being a good source of meat, they also have relatively high egg production). Given this, a strategy is needed to mitigate the negative impacts of these environmental factors (Mushawwir *et al*., 2024). One approach is the "Three in One" strategy. This strategy involves a single feed formulation containing a feed additive that can provide three benefits simultaneously: (1) mitigating oxidative stress resulting from high environmental temperatures, (2) improving the performance of local West Javanese ducks, and (3) producing eggs rich in antioxidants (Petrilla *et al*., 2022).

Previous research has identified several active substances and micronutrients with potential as additives to address these three aspects: Flavonoids, methionine, oleic acid, linoleic acid, and allicin (Purwanti *et al*., 2024; Sahara *et al*., 2025). The results of the first phase of research demonstrated the effectiveness of flavonoids and linoleic acid in preventing increased oxidative stress, as evidenced by histological morphometry of the liver, blood biochemical profiles, and free radical levels. Allicin fights bacteria by attaching to certain groups in their proteins, stopping important enzymes needed for their survival. This helps keep a healthy balance of microbes in the gut. When chickens are given garlic, which contains allicin, it helps increase good bacteria like lactobacilli and decreases bad bacteria like coliforms, especially when the chickens are under heat stress. This leads to better gut health and improved ability to absorb nutrients (Chae *et al*., 2023).

Heat stress in chickens causes more free radicals and increases oxidative stress, which harms their production and damages the intestines. Garlic has bioactive compounds, especially allicin, that act as antioxidants. These compounds help get rid of harmful oxygen molecules, lower oxidative stress, and reduce the bad effects of heat stress (Ryu *et al*., 2017). The combination of allicin's ability to fight bacteria and its antioxidant effects helps improve the overall health of laying hens, resulting in better egg production and quality. These results are expected to improve the performance of ducks raised in dryland conditions.

MATERIALS AND METHODS

Animal samples, experimental design, and basal diet

Ninety Chihateup ducks in the laying phase were used as samples in an in vivo test of feed supplements, consisting of a combination of flavonoids, methionine, linoleic acid, and allicin, with a composition of 25% each. The experimental ducks were divided into two large groups, each consisting of 45 samples. The experimental ducks were placed in litter system pens lined with rice husks, each measuring 4 x 5 m. They were equipped with feeding and drinking troughs.

The first group received feed additives (P1), and the second group did not (P2). Group P1 received 0.5 mL/kg bw of feed additives per duck per day, added to the ration. All experimental ducks received a basal ration with a crude protein content of 17.5% and a metabolizable energy of 2850 kcal/kg.

Analytical methods

HSP, 3β- HSD protein analysis

HSP (heat shock protein) concentration: The assay was performed using spectrophotometry. 100 µL of standard, blank, or sample was added to each well, and the mixture was incubated for 2 hours at 37°C. Unadhered portions were discarded, and 100 µL of reagent A was added, and the mixture was incubated for 1 hour at 37°C. Unadhered portions were discarded, washed with 350 µL of wash solution, and left for 1-2 minutes. The residual solution was removed using blotting paper. Washes were repeated three times. After the final wash, the wash buffer was removed by decantation. 100 µL of reagent B was added, and the mixture was incubated for 30 minutes at 37°C. Unadhered portions were discarded, washed with 350 µL of wash solution, and left for 1-2 minutes. The remaining solution was removed using blotting paper, and the sample was washed 5 times. After the final wash, the wash buffer was removed by decantation. 90 µL of substrate solution was added to each well. The plate was covered and incubated for 15-25 minutes at 37 0C (do not exceed 30 minutes). The mixture was protected from light, and the solution turned blue with the addition of the substrate solution. Stop solution was added to each well, and the solution turned yellow. If the colour change was not homogeneous, the solution was shaken carefully. Absorbance was measured at 450 nm using a plate reader.

Level of 3β-hydroxysteroid dehydrogenase

The spectrophotometer was set to 340 nm at 25°C. The following was pipetted into each cuvette: 0.6 mL sodium pyrophosphate; 0.2 mL 0.0043 M NAD; 2.0 mL reagent-grade water; and 0.1 mL enzyme. The solution was incubated in the spectrophotometer for 3-4 minutes to reach temperature equilibrium, and a blank measurement was performed. At time zero, 0.01 mL of testosterone solution was added. Absorbance was recorded for 3-4 minutes. ΔA340 per minute was calculated, starting from the linear portion of the curve. The procedure was repeated with androsterone as the substrate.

$$Unit/mg = \frac{\Delta A340/min}{6{,}22\ x\ mg\,\frac{enzym}{mL}\ reaction\ mixture}$$

Blood biochemistry and haematology analysis

Blood samples will be analysed using a haematology analyser to determine the haematology profile. To determine their biochemical profile (cholesterol, triglycerides, glucose, and others), spectrophotometric methods will be used. Haematology will be analysed using a Mindray BC-2800 haematology analyser.

In vivo antioxidant assay (protein concentration)

Plasma preparation

Blood is collected using an anticoagulant such as heparin, citrate, or EDTA. The blood is then separated by centrifugation at 700 to 1,000x g for 10 minutes at 4 degrees Celsius. The upper yellow plasma layer is separated from the white blood cell layer by pipetting without disturbing the white blood cell layer. The plasma is stored in a cool container. If not used the same day, it should be frozen at -80 degrees Celsius. Plasma samples remain stable for 1 month. Before testing for SOD activity, the plasma must be diluted 1:5 with the sample buffer.

Catalase (707002)

Formaldehyde standard wells: Each well was filled with 100 µL of test buffer, 30 µL of methanol, and 20 µL of the standard (tubes A-G). Positive control wells (bovine liver catalase): Each well was filled with 100 µL of test buffer, 30 µL of methanol, and 20 µL of catalase (as a control).

Sample wells: Each well was filled with 100 µL of test buffer, 30 µL of methanol, and 20 µL of sample. The amount of catalase added should exhibit an activity between 2 and 35 nmol/min/mL to obtain consistent results. The reaction was initiated by adding 20 µL of hydrogen peroxide to all wells. The plate was covered and shaken for 20 minutes at room temperature. Then, 30 µL of potassium hydroxide was added to each well to stop the reaction, along with 30 µL of Purpald's Catalase (dye). The plate was covered and shaken for 10 minutes at room temperature. Next, 10 µL of catalase potassium periodate was added to each well, the plate was covered, and the plate was shaken for 5 minutes at room temperature. Absorbance was then measured at 540 nm using a plate reader.

$$CAT\ activity = \frac{\mu M\ sample}{20\ mit}\ x\ sample\ dilution = \frac{nmol}{min}/mL$$

One unit is defined as the amount of enzyme that results in the formation of 1.0 nmol of formaldehyde per minute at 25 °C.

Performance recording

Performance recording includes recording egg production, average egg weight, and hatching biology.

Statistical analysis

All data were collected and tabulated in Microsoft Excel 2023, and the effects and differences of the treatments applied to the two groups of Cihateup ducks were analyzed. A two-sample Student's t-test was used to assess treatment effects and differences, with a 95% confidence level (p<0.05).

RESULTS AND DISCUSSION

Blood biochemistry

The physiological and biochemical conditions of the blood of ducks that received feed additives and those without feed additives are presented in the following table.

Table 1: Average blood biochemical profile of ducks.

Response	Duck group response	
	Without feed additive	**Feed additive**
Urid Acid (g/dL)	23.45[a]	10.67[b]
Urea-NH3 (g/dL)	5.51[a]	1.89[b]
Albumin (g/dL)	5.26[a]	2.75[b]
Total Protein (g/dL)	2.12[a]	1.94[b]
Glucose (g/dL)	243.89[a]	244.18[a]
Tryglycerides (g/dL)	118.64[a]	101.89[b]
Reticulocytes count (%)	39.92[a]	6.15[b]
MDA (nm/$M^{-1}cm^{-1}$)	3.86[a]	1.15[b]

[a,b]different notation between different columns on the same parameter, indicating a significant difference (P<0.05).

Based on the research results to date, ducks receiving the best combination of feed additives in the first year of the study showed a favourable biochemical profile. The overall blood biochemical profile of the ducks receiving the feed additives, except for glucose levels, differed from that of the group not receiving the feed additives. This indicates that the group receiving the feed additives was able to cope with stress even when reared on dry land without standing water.

The increase in blood urea levels in ducks is due to increased glucose synthesis from amino acids under environmental heat stress (Nurfauziah *et al.*, 2024). Environmental heat stress causes the hypothalamus to secrete corticotropin-releasing factor (CRF) and stimulates the anterior pituitary to increase adenocorticotropin (ACTH) synthesis. Increased ACTH can increase glucocorticoid secretion (Rodenburg *et al.*, 2023), and increased glucocorticoids can increase gluconeogenesis (Rusmana *et al.*, 2025).

Increased gluconeogenesis can increase blood urea. Gluconeogenesis is formed from the main glucogenic substances, namely, amino acids. Amino acids can be converted into α-keto acids by deamination and transamination reactions. These α-keto acids then enter the citric acid cycle. Some keto acids, such as pyruvate, oxaloacetate, and α-ketoglutarate, can be converted into glucose and glycogen (Mushawwir *et al.*, 2025a). When entering the citric acid cycle, the enzymes glutamate and glutamate dehydrogenase play a specific role in amino acid metabolism (Teke *et al.*, 2024).

Glutamate and glutamate dehydrogenase are activated by the positive modulator ADP and inhibited by GTP, a product of the succinyl-CoA synthase reaction in the citric acid cycle. Increased gluconeogenesis can increase blood urea because, when the liver requires more precursors to produce more ATP, glutamate and glutamate dehydrogenase activity increase, making α-ketoglutarate available for the citric acid cycle and releasing NH_3.

Free ammonia is highly toxic to poultry and cannot be transported through the bloodstream. Therefore, ammonia is converted to glutamine by the enzyme glutamine synthase, which travels through the bloodstream to the liver, where it is catalysed by glutaminase to form glutamate and ammonia, which is then converted to urea in a cyclic mechanism via the urea cycle. Several studies have shown that administering amino acids increases N-ammonia levels (Tanuwiria *et al.*, 2022, 2023; Saneyasu *et al.*, 2017). The results of this study suggest that protein metabolism, specifically amino acid transamination reactions, will increase blood ammonia levels. Mushawwir *et al.* (2010) demonstrated an increased rate of ammonia (NH_3) synthesis into ammonia urea through the urea cycle, involving mitochondrial ornithine. However, in poultry, most N production is converted to uric acid.

Glutamine is the primary ammonium transporter from peripheral tissues to the liver. Besides glutamine, alanine also transports ammonia from muscles to the liver. Alanine works by transferring its amino group to α-ketoglutarate via alanine transaminase, producing glutamate (Muller *et al.*, 2022), which is then deaminated to regenerate α-ketoglutarate and ammonia. Glutamate acts to convert the resulting ammonia to urea via the urea cycle in the liver (Manin *et al.*, 2024).

The rate of transamination reactions will continue to increase as amino acids are increasingly utilised to form glutamate-aspartate-fumarate, which is involved in the Krebs cycle and compensates for their role in the gluconeogenesis pathway for energy production. It is known that water deprivation during maintenance increases heat stress. This means that the rate of amino acid breakdown via transamination reactions ultimately increases, leading to greater involvement in the urea cycle. Kharazi *et al.* (2022) reported that urea is a good indicator of amino acid utilisation in the gluconeogenic pathway for energy production. The higher the urea level, the greater the utilisation of amino acids through transamination reactions and the urea cycle. This is the main reason for high blood urea levels.

The increase in blood uric acid in experimental ducks raised in a low-water system without feed additives places a heavy burden on their heat-stress response, particularly during evaporative, radiative, and conductive heat loss. This impacts the activation of the neurogenic system, which stimulates corticotropin-releasing hormone (CRH), which, in turn, stimulates the anterior pituitary to release adrenocorticotropic hormone (ACTH). The adrenal medulla then secretes epinephrine, which functions as a second messenger for adenylate cyclase. Adenylate cyclase catalyses the formation of cAMP, which then activates protein kinase A (Lawal *et al.*, 2020). Protein kinase A regulates metabolic enzymes and gene transcription, including triggering glycogenolysis.

Increased cAMP can increase uric acid production. This is because increased cAMP can increase AMP synthesis, a purine nucleotide. AMP is then deaminated into inosine, which is then hydrolysed to produce hypoxanthine and D-ribose, hypoxanthine to xanthine, and then uric acid by xanthine oxidase.

Hypoxanthine-Guanine Phosphoribosyl Transferase (HGPRT) is an enzyme involved in the conversion of purine bases into nucleotides. This enzyme catalyses the conversion of purines into purine nucleotides, which can be reused as components of DNA and RNA. If this enzyme is deficient, purine levels in the body can increase because purines are not metabolised by HGPRT, leading them to be metabolised by xanthine oxidoreductase (XOR) to uric acid. The increase in environmental temperature and heat stress experienced by experimental ducks triggers increased XOR enzyme activity (Dudi *et al.*, 2023; Gurnin *et al.*, 2024). This condition leads to elevated circulating uric acid levels.

Short and prolonged stress triggers epinephrine secretion, thereby increasing the activation of several protein pathways, including cAMP and cGMP. These pathways increase purine base levels (Firmansyah *et al.*, 2024; Sahara *et al.*, 2025). Concurrently, heat stress is known to decrease XOR enzyme activity; thus, this condition will undoubtedly lead to increased blood uric acid levels.

Production performance

The production performance of ducks receiving feed additives and those without feed additives is as follows (Table 2).

Table 2: Average duck production performance.

Respon	Duck group response	
	Without feed additive	Feed additive
Average HDP (%)	64,83[a]	83,97[b]
Average Weight of Eggs (g)	69,51[a]	85,76[b]
Shell thickness (mm)	2.21[a]	3,11[b]
Average hatching power (%)	67.23[a]	89,45[b]
Income Over feed duck cost (IDR)	1,745	3,755

[a,b]different notation between different columns on the same parameter, indicating a significant difference (P<0.05).

The results showed that the performance of ducks receiving different feed additives was significantly higher (P<0.05). These preliminary results confirm that the given feed additives improve metabolic conditions during egg biosynthesis.

Histopathology

The histological response of the ileum of Cihateup ducks fed with and without feed additives is presented in the following Table 3 and Figure 1.

Table 3: Average histopathology of ducks.

Respon	Duck group response	
	Without feed additive	Feed additive
Number of ileal villi (per 10x10 field of view)	7.83[a]	12.97[b]
Average height of ileal villi (μm)	269.51[a]	385.76[b]
Number of Peyer's plaques	5.21[a]	8.11[b]
Peyer's plaque height	26.45[a]	38.93[b]

[a, b] different notation between different columns on the same parameter, indicating a significant difference (P<0.05).

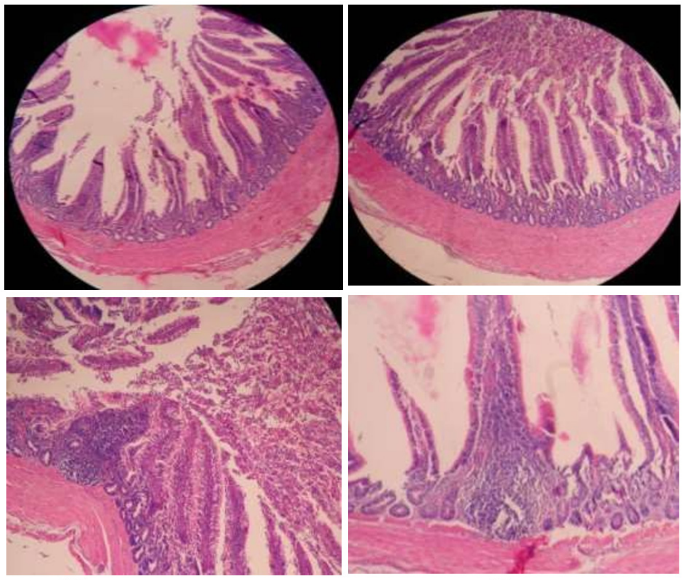

Figure 1: Ileum villi and Peyer's plaque in the laying phase duck with (P1) and without feed additives (P2).

Low Water levels in ducks reduce their thermoregulatory abilities. This is exacerbated by hot environmental temperatures, which require homeostasis to balance heat intake and output. Heat stress triggers the hypothalamic pituitary gland to release CRF, which in turn stimulates the anterior pituitary to produce ACTH. High levels of ACTH stimulate the adrenal cortex to produce cortisol. Cortisol aids in gluconeogenesis and is often referred to as an anti- anabolic hormone.

During heat stress, the continuous production of cortisol increases free radical levels, which can reduce lymphocyte levels. Lymphocytes recognise and destroy various antigenic determinants, such as free radicals. Lymphocytes destroy these antigens, forming plasma cells that produce immunoglobulins. Peyer's patches play a crucial role in the immune system. Peyer's patches are collections of lymphocyte nodules. Lymphocyte nodules are collections of lymphocyte cells. Under stress, many lymphocytes in Peyer's plaques transform into plasma cells, thereby reducing their numbers and altering their distribution (Gurnin *et al.*, 2024). This reduction in lymphocyte numbers leads to fewer lymphatic nodules and smaller ileal Peyer's plaques.

This increase occurs due to the role of feed additives in stimulating immunity. IgA levels are higher in livestock fed FOS, reducing the amount of IgA naturally produced by the animal's body. This prevents a decrease in lymphocytes and the number of Peyer's plaques (Chrystal *et al.*, 2020). Peyer's plaques are composed of numerous B lymphocytes. Psychological stress and heat reduce the number of Peyer's plaques because the B lymphocytes in the plaques transform into multiple plasma cells.

The length and width of the ileal Peyer's plaques increase with the administration of feed additives. Feed additives have been shown to improve the integrity of the small intestinal epithelium, as PUFAs and methionine are essential for intestinal epithelial cell metabolism, improving digestive tract function and nutrient absorption. This fermentation is closely related to the development of Gut-Associated Lymphoid Tissue (GALT), the largest tissue in the immune system, accounting for approximately 60% of the total lymphocytes in the body, thus increasing the growth of Peyer's plaques in the ileum of Cihateup ducks (Adriani *et al*., 2024b; Aritonang *et al*., 2025).

Increased PUFAs induce endocrine L-cells in the cecum and colonic mucosa to produce GLP-1 (Glucagon-Like Peptide-1). GLP-1 is a type of incretin hormone that functions to repair pancreatic β-cells and increase insulin production. Insulin is an anabolic hormone that promotes lymphocyte proliferation, leading to increased length and width of Peyer's plaques. Ahmad-Farid *et al*. (2021) also stated that increasing ileal size with a combination of methionine, linoleic acid, flavonoids, and allicin may enlarge Peyer's plaques and other cells. This is why the ileal morphometrics appear to improve markedly.

HSP and 3β- HSD protein concentration

The responses of experimental ducks raised under conditions of minimal water intake and without feed additives are shown in the following Table 4.

Table 4: Average HSP and 3β-HSD protein concentration.

Respon	Group of ducks	
	Without feed additive	Feed additive
HSP (ng/dL)	3,72[a]	2,25[b]
3β-HSD (ng/dL)	4,84[a]	7,86[b]
Catalase (μL/dL)	3,75[a]	2.06[b]

[a,b] different notation between different columns on the same parameter, indicating a significant difference (P<0.05).

The study showed that the highest HSP was observed in the treatment without feed additives, with an average of 3.72 ng/dL. In contrast, HSP gene expression was lower in the group given feed additive treatment.

Ducks fed without feed additives showed higher levels of HSP (P<0.05). The mechanism of HSP occurs when excessive stress, whether due to temperature or psychological stress (loss of water immersion), causes the central nervous system (CNS) to remain continuously activated, sending signals to target organs to maintain homeostasis (Mushawwir *et al*., 2025b). Hormones are signalling compounds that trigger a series of reactions (signal cascades) in target organs (Rosiyanti *et al*., 2025).

During the study, the ducks were placed in conditions with minimal water, resulting in stress that affected cellular components and increased bioenergetic potential (Selim *et al*., 2021). These conditions led to a sharp increase in the reduction and oxidation of molecules and H+ ions. Consequently, livestock experience psychological stress and hypoxia (Teke *et al*., 2024), a condition accompanied by increased activity of cytochrome and ATPase enzymes (Tanuwiria *et al*., 2023) as well as increased free radicals (OH-, O2-, H2O2) (Mushawwir *et al*., 2025b).

Several studies have also shown that small heat shock proteins (<70 kDa) can protect actin filaments from heat-induced damage (Rodenburg *et al*., 2023). Recent findings highlight the crucial roles of blood vessels and the endothelium in protecting the heart, demonstrating a link between HSP70 in blood vessels and functional recovery after heat-shock protein treatment. Petrilla *et al*. (2022) first reported that a protein with a molecular weight of approximately 71,000 accumulates in the microvascular fraction of blood vessels when the forebrain (telencephalon) is incubated with vinblastine. The protein is induced and is a member of the HSP gene family.

A previous study by Mushawwir *et al*. (2010) reported that HSP70 expression in rat liver hepatocytes was localised around the central vein, suggesting that this distribution is related to blood supply. Positive signals in the vascular endothelial cells of the liver's central vein suggest that HSP70 expression may be associated with hepatic blood supply and functional recovery from stress-induced damage.

Lawal *et al*. (2020) also found that HSP70 was present in rat liver cells around the central vein, suggesting that this distribution of HSP70 may be related to blood flow. Positive signals in endothelial cells of the central vein suggest that HSP70 level may be associated with hepatic blood supply and functional recovery after stress-induced damage.

Imbalanced homeostasis can lead to the accumulation of metabolic products or disturbances in fluid, electrolyte, and acid-base balance. Consequently, physiological functions can be disrupted, potentially endangering the organism's life. The high expression of HSP70 in renal tubular cells and glomerular capillary endothelial cells may be related to the kidney's ability to repair heat-stress-induced damage.

Research by Kharazi *et al*. (2022) showed that hybridisation signals for HSP70 were detected in the cytoplasm of myocardial cells, liver cells, and renal tubular epithelial cells in all tissues examined (liver, kidney, and heart), indicating possible HSP70 induction in all three cell types.

The localisation of HSP70 coincided with the distribution of HSP70 protein determined by IHC. However, Kharazi *et al*. (2022) and Mushawwir *et al*. (2023) noted that HSP70 mRNA expression was undetectable in cardiac, liver, renal, and glomerular capillary endothelial cells, as determined by IHC for HSP70 protein.

Broiler chickens develop heat tolerance after several hours of heat exposure or after a lack of organic matter in feed intake during prolonged heat stress. Chrystal *et al.* (2020) reported that caloric restriction increases HSP70 transcription and improves thermotolerance. It has been reported that inorganic phosphate (Pi) deficiency can affect key cellular biochemical pathways that control HSP protein expression (Dudi *et al*., 2023; Manin *et al*., 2024; Mushawwir *et al*., 2025a). Increased HSP inhibits 3β-HSD expression, but stimulates the antioxidant catalase.

CONCLUSION

Based on the research results, it can be concluded that administering a feed additive containing methionine, allicin, flavonoids, and linoleic acid can improve blood biochemical profiles, production performance, ileal histopathology, and HSP and 3β-HSD under heat stress and water shortages, thereby mitigating oxidative stress.

The researchers strongly recommend further research, particularly regarding variations in the administration of the additive produced in this study, which will require several experimental groups. In addition, studies on the expression of genes related to production traits need to be highlighted for future research.

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NOVELTY STATEMENT

This research focuses on developing an innovative feed additive that combines isolated flavonoids and linoleic acid to synergistically improve physiological performance and egg production in ducks under heat stress and limited water availability. This study not only assesses its effects on performance and productivity but also examines cellular response mechanisms, particularly those related to oxidative stress and metabolic adaptation. Through this approach, this research is expected to develop effective functional nutrition strategies to enhance egg antioxidant content and increase ducks' resistance to heat stress.

AUTHORS' CONTRIBUTION

The authors listed in this article all contributed equally, collaborated to develop the research proposal and study design, participated in scientific discussions to improve research quality, and wrote the article.

Ethical approval

The Animal Ethics Committee of the Research Licence Management Directorate, Indonesia, has investigated the preparation and implementation of this research and has determined that it is feasible in accordance with the principles of animal welfare and research ethics, as stated in decision No. 462/Kep.04/SKE/01/2025.

Generate AI and AI-assisted technology statement

The authors solemnly declare and affirm that during the preparation and writing of this article, they did not use AI technology or any related or similar facilities.

Conflict of interest

All authors state that they have no conflicts of interest with any group related to the data, funding, or any other aspect of the research output.

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