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Optimal Post-Hatch Holding Time Maintains Growth, Gut Integrity, Oxidative Balance, and Immunity in Broilers

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ABSTRACT

Delayed access to feed after hatching can increase oxidative stress and disrupt intestinal function and immunity in broiler chickens. This study evaluated the physiological responses of broilers subjected to different post-hatch holding times under uniform vitamin E–selenium–supplemented diets. A total of 200 Cobb 500 chicks were assigned to four holding time treatments (24, 36, 48, and 60 hours), with five replications per treatment and ten birds per replication, and reared for 35 days under tropical conditions. All birds received a diet supplemented with vitamin E (250 mg/kg) and selenium (1 mg/kg). Observed parameters included growth performance, intestinal histomorphology, oxidative stress indicators (MDA and H/L ratio), and $CD4^+$ and $CD8^+$ T cell expression. Results showed that holding times of ≤36 hours maintained better feed intake, body weight gain, villus height, and oxidative balance, with the highest $CD4^+/CD8^+$ ratio observed at 36 hours. In contrast, prolonged holding times (48–60 hours) increased MDA and H/L ratios, reduced intestinal surface area, and decreased T cell activity despite antioxidant supplementation. These findings indicate that a holding time of ≤36 hours is critical for maintaining intestinal integrity, oxidative stability, and immune competence in broilers reared under tropical conditions.

***Keywords:** broiler; holding time; immunity; oxidative stress; vitamin E and selenium*

INTRODUCTION

Maintaining optimal growth during the early stages of life is a crucial issue in broiler chicken production, as this phase significantly impacts subsequent performance. Post-hatch handling practices, particularly delays in feeding and watering, can induce metabolic stress, reduce environmental adaptability (Wijnen *et al.*, 2022), and accelerate oxidative damage due to exposure to free radicals. This phenomenon is more common in tropical hatcheries, where day-old chick (DOC) distribution is often hampered by long shipping distances and relatively high ambient temperatures. Under these conditions, post-hatch feeding delay becomes a major risk factor for oxidative stress. Oxidative stress occurs when the production of reactive oxygen species (ROS) exceeds the capacity of the endogenous antioxidant system, negatively impacting cell integrity and physiological function (Khelfi, 2024).

Vitamin E and selenium are commonly added to broiler chicken feed as part of a standard nutritional strategy to support antioxidant balance. Vitamin E plays a role in protecting cell membrane structure, while selenium is an essential component of the enzyme glutathione peroxidase. These two nutrients function to support the regulation of oxidative processes, including by suppressing lipid peroxidation and facilitating enzymatic detoxification of free radicals (Çalik *et al.*, 2022). In this study, vitamin E and selenium were administered uniformly to all treatment groups, so they were positioned as having the same nutritional background and not as the main treatment factor.

Oxidative stress associated with delayed post-hatching feeding has the potential to disrupt intestinal tissue integrity and immune response. Increased malondialdehyde (MDA) levels reflect lipid peroxidation, while changes in the proportion of $CD4^+$ and $CD8^+$ T cells indicate changes in adaptive immune status (Yang *et al.*, 2023). Although feed with antioxidant support has been reported to help maintain physiological stability under environmental stress conditions, the extent to which delayed post-hatch feeding itself affects oxidative status, intestinal morphology, and immune response under uniform nutritional conditions has not been widely studied, especially in commercial broiler production systems in tropical regions (Özlü *et al.*, 2022).

Therefore, this study aims to examine the relationship between post-hatch holding time (24–60 hours) and physiological responses related to oxidative status, intestinal morphology, and immune function in broiler chickens reared on uniformly supplemented vitamin E-selenium feed. This approach allows for the evaluation of the effect of holding time duration under supported nutritional conditions, rather than assessing the direct effects of antioxidant supplementation. The working hypothesis of this study is that shorter holding times (≤36 hours) tend to support better physiological adaptation, whereas longer delays may exceed adaptive capacity, even under supported nutritional conditions.

MATERIALS AND METHODS

Ethical Approval

The implementation of this study has been permitted by the Research Ethics Commission of Brawijaya University with the number 063-KEP-UB-2023.

Study Model, Feed, and Maintenance

A total of 200 one-day-old Cobb 500 broiler chicks with an average body weight of 35–40 g, previously vaccinated against Newcastle Disease (ND) and Infectious Bursal Disease (IBD), were obtained from a licensed commercial hatchery (PT. Malindo, South Sulawesi, Indonesia). Post-hatch holding time (HT) was defined as the duration from chick hatching at the hatchery until first access to feed and drinking water at the experimental facility, encompassing hatchery handling and transportation periods. Based on this definition, chicks were allocated into four holding time (HT) treatments: 24, 36, 48, and 60 hours. Each treatment consisted of five replicates with 10 chicks per replicate. The experimental unit was an individual bird. The birds were reared under an open-house floor system using rice husk litter, reflecting common commercial broiler management practices in tropical regions. All groups received dietary supplementation of Vitamin E (α-tocopherol) at 250 mg/kg and Selenium (Na_2SeO_3) at 1 mg/kg, while drinking water was provided ad libitum.

This study did not include a control group without holding time (0 hours) or a group without antioxidant supplementation, because the experimental design focused on evaluating the impact of long holding time after slaughter under applicable conditions with a uniform antioxidant administration strategy, as commonly applied in commercial broiler production systems in tropical regions. Therefore, the 24-hour holding time treatment was used as a practical reference group, representing the shortest and most operationally feasible delay duration in commercial hatchery farm systems.

The birds were reared under an open-house system in South Sulawesi with 24-hour lighting, consisting of approximately 12 hours of natural daylight and 12 hours of artificial light provided by incandescent bulbs. Daily ambient temperature ranged between 28–32 °C with relative humidity of 70%–85%. Environmental factors were not strictly controlled but were recorded as part of the natural tropical conditions. Continuous lighting was applied to promote early feed and water intake and to minimize adaptation stress, thereby allowing the response of broilers to antioxidant supplementation to be observed under practical field conditions.

The feed used was a commercial ration that had been supplemented with antioxidants, according to the study model. Feed in the form of granules (crumble) was given after holding time (HT) in the starter phase until the age of 14 days. For the finisher phase, aged 15-35, feed in the form of pellets was given. The composition and nutrient profile of the starter and finisher diets are presented in Table 1.

The treatments provided were HT of 24, 36, 48, and 60 hours, respectively. Slaughter was carried out at the ages of 14 and 35 to collect samples of the small intestine. Broiler chickens were randomly selected for weighing in each treatment unit. Before slaughter, blood was taken using a syringe in the brachial vein and transferred to a vacuum tube containing ethylene diamine tetraacetic acid anticoagulant.

Measured Variables

Growth performance. The weight of broiler chickens was measured for each treatment unit at the ages of 14 and 35 days. Growth indicators are shown from the average feed consumption, body weight gain, and feed conversion in the starter and finisher phases (Quintana-Ospina *et al*., 2023).

Blood sampling. Blood sampling of 2 mL per bird was carried out randomly from each treatment unit (3 birds per replicate) using a 23–25G needle with a 2 mL syringe via the brachial vein (AVMA, 2020) at the end of the rearing period. The collected blood was transferred into test tubes containing Ethylenediaminetetraacetic Acid (EDTA) as an anticoagulant.

Table 1. Composition and calculated analysis of the starter and finisher diet

Nutrients	Starter diet	Finisher diet
Water content	14%	14%
Crude protein	23%-24%	20%-22%
Crude fat	5%	5%
Crude fiber	4%	5%
Ash	8%	8%
Calcium	0.8%-1%	0.8%-1.1%
Phosphorus total	0.5%	0.5%
Aflatoxin	40 μg/kg	50 μg/kg
Amino acids		
Lysine	1.3%	1.2%
Methionine	0.5%	0.45%
Methionine+Cystine	0.9%	0.8%
Tryptophan	0.2%	0.19%
Threonine	0.8%	0.75%

Note: Source: Analysis results of PT. Perkasa Agung Sejati.

The sample tube was then gently inverted and stored at a cool temperature (18 °C) to prevent coagulation. Blood samples used for hematology analysis were placed in a blood cooler, and parameter examinations were performed according to established standard procedures.

Hematology. Differential leukocyte analysis was performed using 1 mL of blood sample collected in EDTA-anticoagulant tubes. Thin and thick blood smears were prepared, then fixed with methanol and stained with Giemsa–Wright solution. Each smear was observed using a light microscope at 1000× magnification with oil immersion. A total of 100 leukocytes were counted sequentially to determine the proportion of heterophils, lymphocytes, eosinophils, basophils, and monocytes according to the method reported by Valladão *et al.* (2019). The heterophil to lymphocyte ratio (H/L) was then determined based on the results of the count.

Intestinal histology. A total of 40 broiler chickens were selected for intestinal histology analysis, consisting of 20 14-day-old chickens and 20 35-day-old chickens. The experimental animals were then euthanized for tissue collection. Prior to the procedure, the chickens were fasted for 4 hours with ad libitum access to drinking water. Each chicken was manually restrained, and the slaughter process was carried out by severing the carotid artery and jugular vein using a sharp knife in accordance with halal slaughter principles. All procedures were carried out in accordance with AVMA (2020) guidelines to minimize pain and stress. After complete exsanguination, duodenal tissue was removed and cut into several sections approximately 2 cm long for histopathological evaluation.

Intestinal tissue was first fixed in 10% formalin solution, then underwent a gradual dehydration process using alcohol with a concentration of 70% to 100%. Residual alcohol was removed from the tissue by immersion in Xylol I, II, and III, before being infiltrated with liquid paraffin. After the tissue block was used, histological sections were made by using a microtome with a thickness of approximately 5 μm and stretched in an air bath at 50 °C for approximately 15 minutes. Next, the tissue sections were stained using the hematoxylin–eosin method, mounted on glass slides, and analyzed using a digital light microscope with the help of ImageJ software (Mahalingashetti *et al.,* 2016).

Villus surface area was calculated using the formula:

$$\text{Villus surface area} = [(\text{villus height} + \text{apical villus width}) / (\text{apical villus width})] + \text{villus height}$$

where villus height and apical villus width were obtained from histomorphometric measurements, following the method described by Iji *et al.* (2001).

Malondialdehyde (MDA) levels. Malondialdehyde (MDA) levels were measured using the thiobarbituric acid reactive substances (TBARS) method with recent modifications to improve accuracy and reduce interference. Serum samples (0.2–0.5 mL) were mixed with 1 mL of 20% trichloroacetic acid (TCA) and 1 mL of 0.67% thiobarbituric acid (TBA) in acetic acid, then heated at 95–100 °C for 15 minutes to form an MDA–TBA complex. After cooling and centrifugation (3000 rpm, 10 minutes), the absorbance of the supernatant was measured at 532 nm using a spectrophotometer. MDA levels were calculated based on a tetramethoxypropane (TMP) standard curve and expressed in μmol/mL. This procedure refers to a modification of the modern method by Elibol *et al.* (2023), Wang *et al.* (2022), Wu *et al.* (2024), and Rizzo (2024), which emphasizes increasing the selectivity and reproducibility of the TBARS test.

CD4 and CD8 levels. Analysis of $CD4^+$ and $CD8^+$ T cell expression was performed using flow cytometry (FCM) to precisely identify lymphocyte subpopulations. Venous blood samples of 100–200 μL were collected into EDTA-containing tubes, then treated with RBC lysis buffer and incubated for 10 minutes to remove erythrocytes. Remaining cells were then rinsed with phosphate-buffered saline (PBS), centrifuged, and resuspended in FACS buffer. Staining was performed with CD4-FITC and CD8α-PE monoclonal antibodies at a dilution of 1:20 to 1:100, followed by incubation for 20–30 minutes at 4 °C in the dark. After washing, cells were resuspended in 500 μL of FACS buffer and analyzed using a flow cytometer. Gating was performed on the lymphocyte population based on FSC/SSC characteristics, while $CD4^+$ and $CD8^+$ expression was determined based on fluorescence signal intensity. This procedure followed recent immunophenotyping protocols developed for chickens and other avian species (Härtle *et al.,* 2024).

Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) with IBM SPSS Statistics (version 21, IBM, Armonk, NY, USA). The confidence level was set at 95%, and differences were considered statistically significant at $p<0.05$. The mathematical model used was:

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

where Y_{ij} was the response of broiler chickens to the i-th holding time (HT) treatment with j-th replication, μ was the average observation, τ_i was the effect of maintenance treatment, and ε_{ij} was the effect of experimental error from the i-th holding time (HT) treatment and j-th replication.

RESULTS

Performance

Post-hatch holding time (HT) had a significant effect ($p<0.05$) on broiler chicken performance, particularly during the early growth phase. The longer the delay in feed access, from 24 to 60 hours, the more significant the decrease in feed intake and weight gain at 1–15 days of age, followed by an increase in feed conversion ratio. Chickens that had earlier access to feed (HT 24–36 hours) were able to maintain better growth than those with HT ≥48 hours, indicating that excessive feed delay limits early growth potential. In the next phase (16–35 days of age), performance differences between treatments became less apparent, but consistently, HT 36 hours resulted in higher final body weight, while HT 48–60 hours continued to show lower final weight and less optimal feed efficiency. Overall, these results confirm that earlier feeding after hatching provides clear benefits for broiler chicken adaptation and performance, while excessive delay has a negative impact on long-term growth.

Histological analysis of the duodenum showed that the combination of vitamin E and selenium significantly affected the development of the intestinal mucosa. Broiler chickens with 24 hours of HT had the highest villus height, epithelial width, and mucosal surface area at 14 and 35 days, whereas 60 hours of HT showed a significant decrease in all parameters. A decrease in crypt depth was observed with increasing post-hatch (HT) time, indicating a regenerative response to intestinal mucosal damage. These findings suggest that delayed feeding exceeding 36 hours negatively impacts intestinal absorptive architecture, while feed access within ≤36 hours plays a role in maintaining mucosal integrity and supporting optimal absorptive capacity.

Based on Figure 1, the heterophil to lymphocyte (H/L) ratio in broiler chickens remained at a low level at 24- and 36-hours post-hatch (HT), indicating a relatively mild physiological stress condition. In contrast, a significant increase in the H/L ratio occurred at 48 and 60 hours post-hatch, reflecting higher stress levels despite vitamin E and selenium supplementation. These findings indicated that prolonging HT beyond 36 hours triggers a systemic stress response, thus emphasizing the importance of providing feed and air within 24–36 hours after hatching to maintain the stability of the broiler chicken immune system.

Heterophil/Lymphocyte

Based on Figure 1, the heterophil/lymphocyte (H/L) ratio in broiler chickens was at a low level at 24 hours (0.10) and 36 hours (0.12) post-hatch culling, reflecting a relatively stable physiological condition with minimal stress levels. In contrast, there was a significant increase in the H/L culling ratio at 48 hours (0.36), with the highest value recorded at 60 hours (0.40). These findings indicate that delays in feeding and air supply beyond 36 hours trigger increased physiological stress, even though the chickens have received vitamin E and selenium supplementation. These results highlight that the protective effect of antioxidant supplementation is only optimal within a holding time of ≤36 h, whereas prolonged delays (48–60 h) disrupt leukocyte balance and may compromise immune competence.

Melanodealdehyde (MDA) Levels

Figure 2 shows the MDA levels based on HT variations after supplementation with vitamin E and selenium. Malondialdehyde (MDA) concentrations remained low and relatively constant at 24 and 36 hours post-hatch (HT) at approximately 0.80 nmol/mL, indicating minimal oxidative stress. Conversely, MDA levels increased at 48 hours post-hatch (0.88 nmol/mL) and reached their highest value at 60 hours post-hatch (0.97 nmol/mL). This increase indicates that increasing HT beyond 36 hours leads to a higher accumulation of reactive oxygen species (ROS), thus exceeding the protective effect of antioxidant supplementation. These findings highlight the importance of providing feed and air within an optimal timeframe (≤36 hours) to maintain antioxidant system function and suppress oxidative stress in the early stages of broiler growth.

The Percentage of CD4 and CD8

Flow cytometry analysis of blood lymphocytes showed that the percentage of helper T cells ($CD4^+$) and cytotoxic T cells ($CD8^+$) remained relatively low (<2%) at 24 hours of imbibition. The proportion of both T cell subtypes then increased, reaching a peak at 36 hours of imbibition, before declining again at 48 and 60 hours. These findings suggest that feeding within the first 24–36 hours post-hatch is crucial for supporting the development of adaptive immunity. Conversely, delayed feed access beyond 36 hours resulted in a decline in the $CD4^+$ and $CD8^+$ T cell populations, despite vitamin E and selenium supplementation.

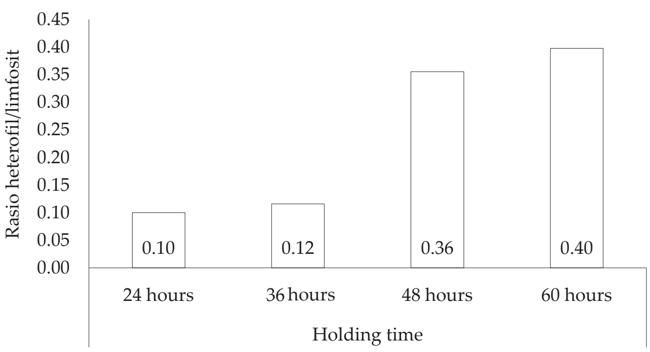


Figure 1. Changes in the ratio of heterophils to lymphocytes in broiler chickens at various post-hatch holding time stages after administration of Vitamin E and Selenium in feed

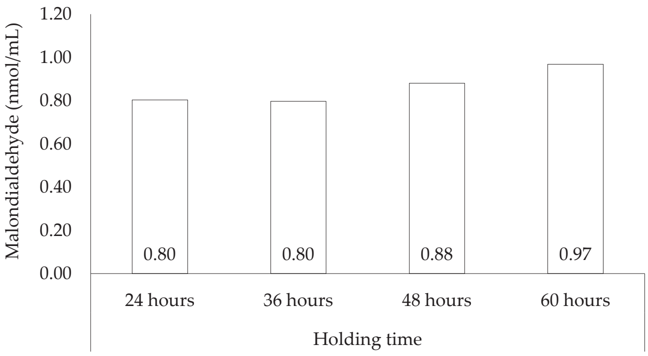


Figure 2. Malondialdehyde levels in broiler chickens with a combination of Vitamin E and Selenium in feed, with variations in post-hatch holding time

DISCUSSION

Post-hatch (HT) time is a crucial factor affecting the initial performance, immune status, and physiological balance of broiler chickens. During HT up to 36 hours, the embryo's energy reserves are sufficient to support the initial adaptation process. However, as the duration is extended, glycogen and lipid reserves are depleted, triggering metabolic stress and increasing the accumulation of reactive oxygen species (ROS), which can potentially damage cell membranes and functional proteins (Gregrova *et al.*, 2024). Under these conditions, the observed physiological responses reflect the interaction between post-hatch holding time and the birds' antioxidant-supported adaptive capacity, rather than a direct protective effect of vitamin E and selenium alone. Vitamin E protects membrane phospholipids from lipid peroxidation, while selenium supports the activity of the glutathione peroxidase enzyme, thereby contributing to the modulation of oxidative balance when nutritional access occurs within a tolerable holding time window (Khelfi, 2024).

In terms of production performance, post-hatch (HT) removal for ≥48 hours causes decreased feed consumption, low body weight gain (BWG), and a worsening feed conversion ratio (FCR), as shown in Table 2. This condition is related to slow enterocyte maturation and impaired nutrient absorption due to increased oxidative stress in the digestive tract (Desai *et al.*, 2025). Within this context, antioxidant supplementation was associated with numerical improvements in several intestinal histological parameters when the holding time was 24–36 hours, including villus height, crypt depth balance, and absorptive surface area (Table 3) (Boyner *et al*., 2025; Marcato *et al.*, 2024). However, these differences were not statistically significant for some parameters and should therefore be interpreted as indicative trends rather than definitive biological effects. These tendencies were not maintained at prolonged holding times (≥48–60 hours), suggesting limited physiological compensation despite antioxidant-supported nutrition.

The numerical decrease in intestinal surface area and crypt depth with increasing HT (Table 3) suggests that delayed feed access may constrain intestinal structural development. Early access to feed and water at 24 hours HT was associated with a tendency toward improved villus maturation and nutrient absorption efficiency, as reflected by a higher villus to crypt ratio. These findings suggest that early post-hatch feeding facilitates intestinal adaptation, while antioxidant supplementation functions as a supportive factor rather

Table 2. Performance of broiler chickens with a combination of Vitamin E and Selenium in feed with variations in post-hatch holding time

Variables	Holding time (hour(s))				p Value
	24	36	48	60	
Age 1 - 15 days					
DOC weight (g/bird)	49.33±2.08^{a}	45.33±2.89^{ab}	45.04±1.03^{ab}	41.00±1.73^{b}	0.008
FC (g/bird)	639.8±20.8^{a}	589.4±13.9^{ab}	542.0±33.4^{bc}	513.1±17.9^{c}	0.000
BWG (g/bird)	536.30±3.24^{a}	485.97±20.75^{b}	445.30±11.39^{c}	421.37±12.71^{c}	0.001
FCR	1.17±0.05	1.21±0.04	1.22±0.07	1.22±0.01	0.467
Age 16 - 35 days					
FC (g/bird)	2257.9±158.9	2140.5±97,52	2123.1±143.7	2035.7±41.0	0.233
BWG (g/bird)	1396.6±49.31	1517.83±226.28	1351.77±97.91	1339.6±101.1	0.410
FCR	1.51±0,07	1.48±0.10	1.61±0.12	1.60±0.05	0.243
FW (g/bird)	1982.23±46.83	2049.13±229.2	1842.10±108.1	1802.00±111.77	0.158

Note: a,b,c Different letters following the mean value in the same row indicate a significant effect ($p<0.05$), DOC (day-old chick), FC (feed consumption), BWG (body weight gain), FCR (feed conversion ratio), and FW (final weight).

Table 3. Histology of the intestine (duodenum) of broiler chickens with a combination of Vitamin E and Selenium with variations in post-hatch holding time

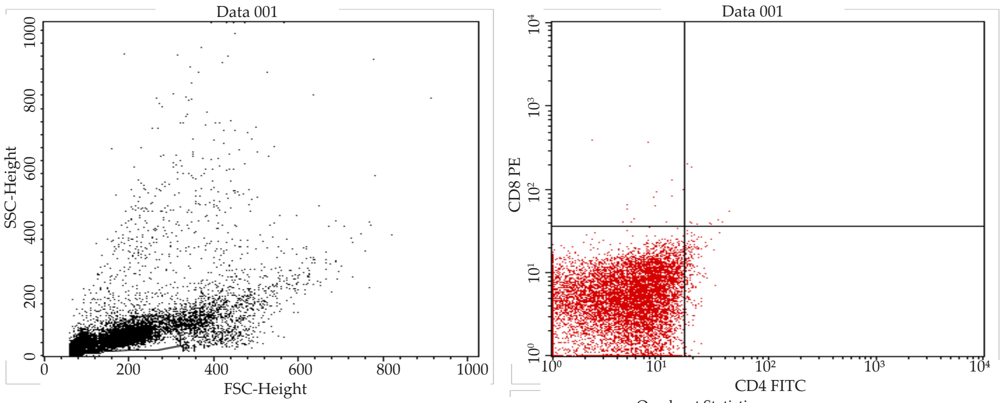
Variables	Holding time (hour(s))				p value
	24	36	48	60	
Age 14 (days)					
Villus height (μm)	870.00±92.21	822.72±123.14	617.15±105.47	784.67±50.76	0.053
Apical width (μm)	68.60±10.04	59.48±2.52	53.08±17.05	60.73±13.08	0.502
Basal width (μm)	119.67±24.08	129.20±12.29	113.06±18.24	129.50±4.29	0.576
Crypt depth (μm)	112.03±21.09	119.90±23.91	114.30±7.81	132.72±34.69	0.723
Intestinal surface area (μm^2)	887.82±91.99^{a}	837.51±124.68^{ab}	630.26±104.39^{ab}	697.22±49.71^{b}	0.073
Age 35 (days)					
Villus height (μm)	904.67±210.83	852.97±11.65	832.32±255.92	552.18±423.27	0.425
Apical width (μm)	71.93±16.93	76.14±21.17	84.89±18.59	85.82±6.81	0.701
Basal width (μm)	112.73±36.07	112.28±9.31	122.32±33.45	139.82±23.48	0.604
Crypt depth (μm)	246.33±16.50^{a}	220.00±39.05^{a}	192.67±13.61^{ab}	152.67±18.15^{b}	0.008
Intestinal surface area (μm^2)	918.00±210.76	865.33±5.03	842.67±256.43	694.40±427.84	0.470

Note: a,bDifferent letters following the mean value in the same row indicate a very significant effect ($p<0.01$).

than a sole determinant of intestinal integrity. This pattern is consistent with previous reports confirming that early post-hatch feeding improves broiler intestinal morphology, function, and overall health (Proszkowiec-Weglarz *et al*., 2022; Syamsuryadi *et al*. 2025b; Zhang *et al*., 2025; Boyner *et al*., 2025).

An increase in the H/L ratio in HT ≥48 hours indicates activation of the systemic stress response associated with stimulation of the hypothalamic pituitary adrenal (HPA) axis, as illustrated in Figure 1. Prolonged feed deprivation increases heterophil numbers and decreases lymphocytes through increased corticosterone secretion, further prolonging the immune status and inflammatory response (Madej *et al*., 2024; Wlaźlak, 2023). Conversely, earlier access to food and air (≤36 hours) helps suppress the physiological stress response by maintaining energy and hydration balance. This stress-mitigating effect appears to be conditional on the duration of holding time, even under antioxidant supplementation, as longer delays still amplified oxidative stress and stress hormone production (Figures 1 and 2).

From an immunological perspective, shorter post-hatch feeding times (≤36 hours) increased $CD4^+$ and $CD8^+$ T lymphocyte populations, reflecting more effective activation of adaptive immunity (Figures 3 and 4). It should be emphasized that the $CD4^+$ and $CD8^+$ values reported in this study represent relative proportions of lymphocyte subpopulations within peripheral blood, rather than absolute cell counts.

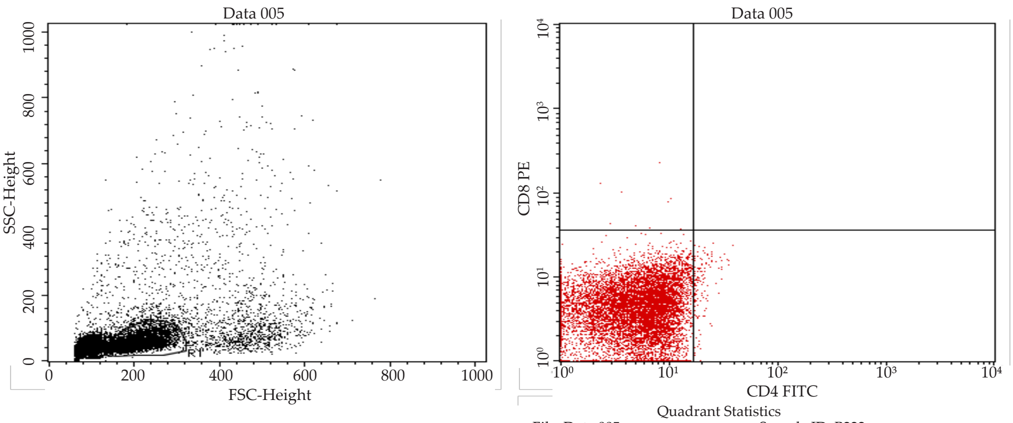


Quadrant Statistics

File: Data 001 Sample ID: B211
Acquisition Date: 04-Apr-24 Gate: G1
Gated Events: 9790 Total Events: 22439
X Parameter: CD4 FITC (Log) Y Parameter: CD8 PE (Log)
Quad Location: 17.36

Quad	Events	%Gated	%Total
UL	16	0.16	0.07
UR	10	0.10	0.04
LL	9588	97.94	42.73
LR	176	1.80	0.78

Figure 3. CD4 and CD8 values of broiler chickens with a 24-hour holding time. The left panel (black dots) shows FSC–SSC distribution for lymphocyte gating, while the right panel (red dots) shows $CD4^+$ (FITC) and $CD8^+$ (PE) expression in the gated lymphocyte population.



Quadrant Statistics

File: Data 005 Sample ID: B222
Acquisition Date: 04-Apr-24 Gate: G1
Gated Events: 9618 Total Events: 23637
X Parameter: CD4 FITC (Log) Y Parameter: CD8 PE (Log)
Quad Location: 17.36

Quad	Events	%Gated	%Total
UL	9	0.09	0.04
UR	1	0.01	0.00
LL	9476	9852	40.09
LR	132	1.37	0.56

Figure 4. CD4 and CD8 values of broiler chickens with a 36-hour holding time. The left panel (black dots) shows FSC–SSC distribution for lymphocyte gating, while the right panel (red dots) shows $CD4^+$ (FITC) and $CD8^+$ (PE) expression in the gated lymphocyte population.

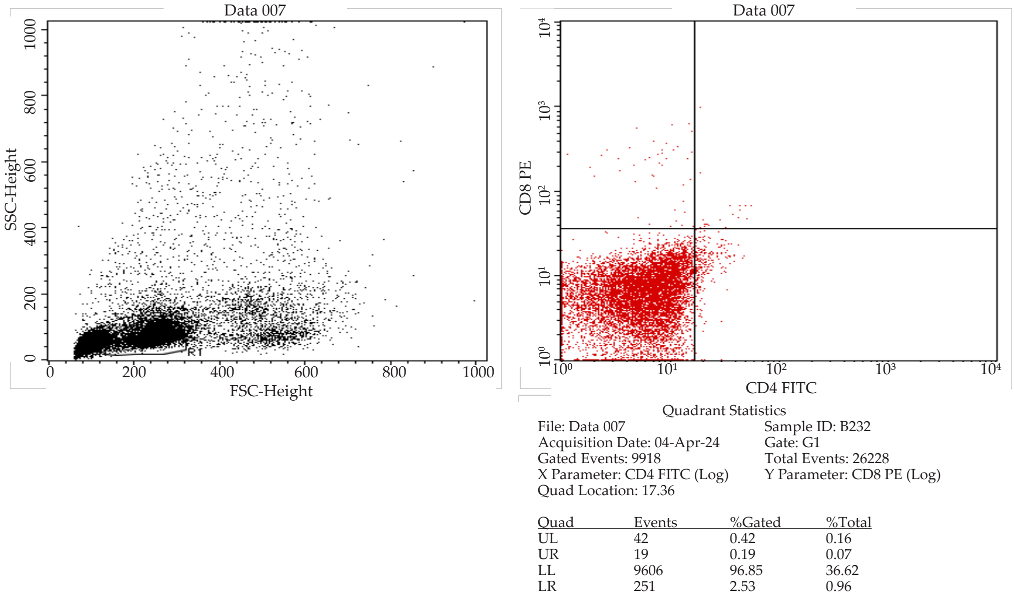


Figure 5. CD4 and CD8 values of broiler chickens with a 48-hour holding time. The left panel (black dots) shows FSC–SSC distribution for lymphocyte gating, while the right panel (red dots) shows CD4+ (FITC) and CD8+ (PE) expression in the gated lymphocyte population.

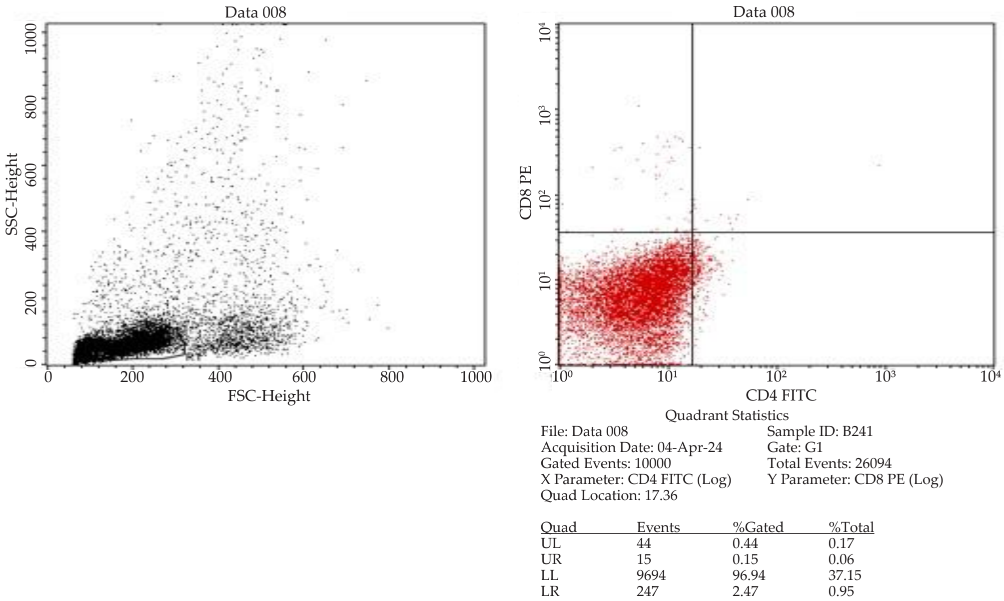


Figure 6. CD4 and CD8 values of broiler chickens with a 60-hour holding time. The left panel (black dots) shows FSC–SSC distribution for lymphocyte gating, while the right panel (red dots) shows CD4+ (FITC) and CD8+ (PE) expression in the gated lymphocyte population.

Consequently, the relatively low percentages (<2%) observed are consistent with flow cytometry–based immunophenotyping studies in poultry, in which T-cell subsets are typically reported as fractions of total leukocytes or lymphocytes. In contrast, at HT ≥48 hours, both subpopulations significantly decreased (Figures 5 and 6), suggesting impaired T cell differentiation associated with excessive oxidative stress that could not be fully compensated by antioxidant-supported nutrition (Madej *et al.*, 2024; Wlaźlak, 2023). Prolonged feed deprivation is known to inhibit cytokine signaling and lymphocyte proliferation, thereby suppressing the adaptive immune response (Syamsuryadi *et al.*, 2025a). Overall, these findings indicate that the benefits of vitamin E and selenium are expressed optimally when early nutritional access is ensured, highlighting a time-dependent interaction rather than a direct causal immunoprotective effect.

The results of this study indicate that post-hatch holding time is a critical determinant of broiler performance, physiological balance, and immune development. When nutritional access occurs within 36 hours after hatching, the birds can still adapt physiologically to antioxidant-supported nutrition, resulting in lower oxidative stress and better overall responses. In contrast, prolonged holding time (≥48 hours) exceeds the birds' adaptive capacity, leading to impaired intestinal condition, performance, hematological stability, and immune responses, even with antioxidant supplementation. These findings highlight that early nutritional access, rather than antioxidant supplementation alone, is essential for achieving optimal broiler growth under tropical production conditions.

CONCLUSION

The results of this study indicated that the duration of post-hatch shedding significantly determines the production performance, physiological homeostasis, and immune system development of broiler chickens. Vitamin E and Selenium supplementation effectively suppressed oxidative stress and maintained physiological integrity, but were only optimal at HT ≤36 hours. Delays of up to ≥48 hours reduced protection effectiveness, worsening intestinal conditions, performance, hematology, and immune responses. The study also reported that early nutritional access was crucial to optimizing the benefits of antioxidants and supporting optimal broiler chicken growth. These findings imply that minimizing post-hatch holding time to 36 hours or less should be prioritized in commercial broiler management, particularly under tropical conditions, to enhance production efficiency and maintain physiological and immune stability.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, the author(s) used ChatGPT only to improve grammar, language clarity, and formatting. No AI tools were used for data analysis, interpretation, or drawing scientific conclusions. The authors take full responsibility for the content of this article. After using this tool/service, the author(s) reviewed and edited the content as needed and take full responsibility for the content of the publication.

REFERENCES

American Veterinary Medical Association. (2020). AVMA guidelines for the euthanasia of animals: 2020 edition. American Veterinary Medical Association. https://www.avma.org/resources-tools/avma-guidelines-euthanasia-animals

Boyner, M., Ivarsson, E., Wattrang, E., Sun, L., Wistedt, A., & Wall, H. (2025). Effects of early access to feed and water in hatchers on growth performance in broiler chickens. Animal, 19(6), 101519. https://doi.org/10.1016/j.animal.2025.101519

Çalik, A., Emami, N. K., White, M. B., Walsh, M. C., Romero, L. F., & Dalloul, R. A. (2022). Influence of dietary vitamin E and selenium supplementation on broilers subjected to heat stress, Part I: Growth performance, body composition and intestinal nutrient transporters. Poultry Science, 101(6), 101857. https://doi.org/10.1016/j.psj.2022.101857

Desai, D. M., Vahora, S. G., Rajpura, R. M., Patel, M. M., Panchal, D. P., & Raut, L. B. (2025). Effect of early post-hatch feeding on nutrient digestibility, carcass characteristics, gut morphology and livability of commercial broiler chicken. International Journal of Bio-Resource and Stress Management, 16(July 7), 01–08. https://doi.org/10.23910/1.2025.6082

Elibol, O., Özlü, S., Erkuş, T., & Nicholson, D. (2023). Effects and interactions of incubation time and preplacement holding time on mortality at placement, yolk sac utilization, early feeding behavior and broiler live performance. Animals, 13(24), 3827. https://doi.org/10.3390/ani13243827

Gregrova, M., Lichovnikova, M., Foltyn, M., Tvrdon, Z., & Hampel, D. (2024). Interactions between broiler parent stock age and egg pre-incubation duration: effects on embryo development, hatchability, day-old chick weight, and yolk sac weight. Animal, 18(11), 101333. https://doi.org/10.1016/j.animal.2024.101333

Härtle, S., Sutton, K., Vervelde, L., & Dalgaard, T. S. (2024). Delineation of chicken immune markers in the era of omics and multicolor flow cytometry. Frontiers in Veterinary Science, 11. https://doi.org/10.3389/fvets.2024.1385400

Iji, P. A., Saki, A. A., & Tivey, D. R. (2001). Intestinal structure and function of broiler chickens on diets supplemented with a mannan oligosaccharide. Journal of the Science of Food and Agriculture, 81(12), 1186–1192. https://doi.org/10.1002/jsfa.925

Khelfi, A. (2024). Future directions, pitfalls, and solutions in oxidative stress assessment. Future directions, pitfalls, and solutions in oxidative stress assessment. In Biomarkers of oxidative stress. Springer. https://doi.org/10.1007/978-3-031-60738-7_23

Madej, J. P., Graczyk, S., Bobrek, K., Bajzert, J., & Gaweł, A. (2024). Impact of early posthatch feeding on the immune system and selected hematological, biochemical, and hormonal parameters in broiler chickens. Poultry Science, 103(3), 103366. https://doi.org/10.1016/j.psj.2023.103366

Mahalingashetti, P. B., Reddy, Y. J. V., Vijay, A., & Gali, S. C. (2016). A histomorphological study of intestine resections at a rural tertiary care centre. Scholars Journal of Applied Medical Sciences, 4(7), 2636–2642. https://doi.org/10.21276/sjams.2016.4.7.71

Marcato, F., Schokker, D., Kar, S. K., Bossers, A., Harders, F., Rebel, J. M. J., Jansen, C. A., van der Valk, E., Kruijt, L., te Beest, D. E., & de Jong, I. C. (2024). Effects of breed and early feeding on intestinal microbiota, inflammation markers, and behavior of broiler chickens. Frontiers in Veterinary Science, 11. https://doi.org/10.3389/fvets.2024.1492274

Özlü, S., Erkuş, T., Kamanlı, S., Nicholson, A. D., & Elibol, O. (2022). Influence of the preplacement holding time and feeding hydration supplementation before placement on yolk sac utilization, the crop filling rate, feeding behavior and first-week broiler performance. Poultry Science, 101(10), 102056. https://doi.org/10.1016/j.psj.2022.102056

Proszkowiec-Weglarz, M., Miska, K. B., Ellestad, L. E., Schreier, L. L., Kahl, S., Darwish, N., Campos, P., & Shao, J. (2022). Delayed access to feed early post-hatch affects the development and maturation of gastrointestinal tract microbiota in broiler chickens. BMC Microbiology, 22(1). https://doi.org/10.1186/s12866-022-02619-6

Quintana-Ospina, G. A., Alfaro-Wisaquillo, M. C., Oviedo-Rondon, E. O., Ruiz-Ramirez, J. R., Bernal-Arango, L. C., & Martinez-Bernal, G. D. (2023). Data analytics of broiler growth dynamics and feed conversion ratio of broilers raised to 35 d under commercial tropical conditions. Animals, 13(15), 2447. https://doi.org/10.3390/ani13152447

Rizzo, M. (2024). Measurement of malondialdehyde as a biomarker of lipid oxidation in fish. American Journal of Analytical Chemistry, 15(09), 303–332. https://doi.org/10.4236/ajac.2024.159020

Syamsuryadi, B., Widodo, E., Radiati, L. E., & Suyadi, S. (2025a). Interactive effects of chick hatch weight and dietary antioxidant supplementation on physiological, hematological, and intestinal responses in broilers. Journal of Animal Health and Production, 13(3), 845-853. https://doi.org/10.17582/journal.jahp/2025/13.3.845.853

Syamsuryadi, B., Widodo, E., Radiati, L. E., Djunaidi, I. H., Rifa'I, M., & Suyadi, S. (2025b). Optimizing broiler chick management: interactive effects of initial body weight and post-hatch feed deprivation on growth, hematological immune response, and intestinal integrity. Advances in Animal and Veterinary Sciences, 13(9), 2096-2102. https://doi.org/10.17582/journal.aavs/2025/13.9.2096.2102

Valladão, G. M. R., Gallani, S. U., Kotzent, S., Assane, I. M., & Pilarski, F. (2019). Effects of dietary thyme essential oil on hemato-immunological indices, intestinal morphology, and microbiota of Nile tilapia. Aquaculture International, 27(2), 399–411. https://doi.org/10.1007/s10499-018-0332-5

Wang, W., Zhang, Z., Liu, X., Cao, X., Wang, L., Ding, Y., & Zhou, X. (2022). An improved GC-MS method for malondialdehyde (MDA) detection: Avoiding the effects of nitrite in foods. Foods, 11(9), 1176. https://doi.org/10.3390/foods11091176

Wijnen, H. J., Molenaar, R., Kemp, B., van Roovert-Reijrink, I. A. M., van den Brand, H., & van der Pol, C. W. (2022). Effects of late incubation temperature and moment of first post-hatch feed access on neonatal broiler development, temperature preference, and stress response. Poultry Science, 101(10), 102088. https://doi.org/10.1016/j.psj.2022.102088

Wlaźlak, S., Pietrzak, E., Biesek, J., & Dunislawska, A. (2023). Modulation of the immune system of chickens a key factor in maintaining poultry production—a review. Poultry Science, 102(8), 102785. https://doi.org/10.1016/j.psj.2023.102785

Wu, H., Kong, Y., Zhao, W., & Wang, F. (2024). Measurement of cellular MDA content through MTBE-extraction based TBA assay by eliminating cellular interferences. Journal of Pharmaceutical and Biomedical Analysis, 248, 116332. https://doi.org/10.1016/j.jpba.2024.116332

Yang, W., Liu, X., & Wang, X. (2023). The immune system of chicken and its response to H9N2 avian influenza virus. Veterinary Quarterly, 43(1), 1–14. https://doi.org/10.1080/01652176.2023.2228360

Zhang, Y., Zhu, L., Luo, Z., & Feng, J. (2025). The effect of lysolecithin supplementation on growth performance, nutrient digestibility, intestinal morphology, and lipid metabolism in yellow-feathered broilers fed diets with different fat levels. Animals, 15(18), 2752. https://doi.org/10.3390/ani15182752