Research Article

Optimizing Broiler Chick Management: Interactive Effects of Initial Body Weight and Post-Hatch Feed Deprivation on Growth, Hematological Immune Response, and Intestinal Integrity

Bahri Syamsuryadi[1,4], Eko Widodo[2], Lilik Eka Radiati[2], Irfan Hadji Djunaidi[2], Muhaimin Rifa'I[3], Suyadi Suyadi[2]*

[1]Doctoral Program of Animal Science, Faculty of Animal Science, Brawijaya University, Jl. Veteran, Malang 65145, Jawa Timur, Indonesia; [2]Department of Animal Science, Faculty of Animal Husbandry, Brawijaya University, Jl. Veteran, Malang 65145, Jawa Timur, Indonesia; [3]Departement of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University Jl. Veteran, Malang 65145, Jawa Timur, Indonesia; [4]Program of Animal Science, Faculty of Agriculture, Universitas Muhammadiyah Sinjai Jl. Teuku Umar, North Sinjai, South, Sulawesi, Indonesia.

Abstract | This study investigated the interactive effects of initial day-old chick (DOC) body weight and post-hatch holding time (feed deprivation) on early growth, hematological parameters, and intestinal morphology in broiler chickens. A total of 256 Cobb 500 broiler chicks were allocated in a 2×4 factorial design consisting of two DOC weight groups (light: 41–45 g; heavy: 46–50 g) and four holding durations (24, 36, 48, and 60 hours). Parameters measured included body weight, feed intake, feed conversion ratio (FCR), leukocyte profile, heterophil-to-lymphocyte (H/L) ratio, and duodenal histomorphometry. Results showed that heavier chicks exhibited significantly better early growth and feed intake (P<0.01), as well as improved final body weight and feed efficiency (P<0.05). A significant interaction between DOC weight and holding time was observed for leukocyte count (P=0.028), indicating that heavier chicks demonstrated a more pronounced stress-induced immune activation in response to feed deprivation. Although no statistically significant differences were detected in duodenal morphology, prolonged holding (60 hours) showed a biological trend toward villus atrophy. These findings highlight the importance of limiting holding time to a maximum of 36 hours, particularly for lighter chicks, to ensure optimal broiler performance, immune homeostasis, and intestinal health.

Keywords | Broiler, Chick weight, Post-hatch delay, Leukocytes, Intestinal morphology, Early nutrition

Received | July 15, 2025; **Accepted** | August 04, 2025; **Published** | September 05, 2025
***Correspondence** | Suyadi Suyadi, Department of Animal Science, Faculty of Animal Husbandry, Brawijaya University, Jl. Veteran, Malang 65145, Jawa Timur, Indonesia; **Email:** suyadi@ub.ac.id
Citation | Syamsuryadi B, Widodo E, Radiati LE, Djunaidi IH, Rifa'I M, Suyadi S (2025). Optimizing broiler chick management: Interactive effects of initial body weight and post-hatch feed deprivation on growth, hematological immune response, and intestinal integrity. Adv. Anim. Vet. Sci., 13(9):2096-2102.
DOI | https://dx.doi.org/10.17582/journal.aavs/2025/13.9.2096.2102
ISSN (Online) | 2307-8316

INTRODUCTION

Early post-hatch management represents a critical phase that profoundly determines the long-term growth performance and health status of broiler chickens. This period encompasses several essential factors, including day-old chick (DOC) body weight and the delay in access to feed and water, commonly referred to as holding time. Both factors directly influence physiological status, stress response, immune function, and gastrointestinal tract integrity (Tona *et al*., 2003; Narinç *et al*., 2023).

DOC weight has long been recognized as a practical indicator of chick quality, as it correlates with residual yolk nutrient reserves, organ maturation, and the physiological readiness to adapt to exogenous environments. Heavier chicks generally exhibit superior growth performance, improved feed efficiency, and more stable immune responses (Willemsen *et al.*, 2010; Calik *et al.*, 2023). However, this advantage is not independent, as early management practices such as holding time duration may either enhance or compromise the chick's initial potential.

Prolonged holding time (>36 hours) without access to feed and water has been shown to induce oxidative stress, reduce body temperature, delay intestinal epithelial development, and elevate the heterophil-to lymphocyte (H/L) ratio an established marker of chronic stress in poultry (Juul-Madsen *et al.*, 2021). These physiological disturbances also impact leukocyte composition, immunocompetence, and impair mucosal integrity of the gut (Uni and Ferket, 2004).

Although numerous studies have independently investigated the effects of day-old chick (DOC) body weight on broiler performance and physiological status (Willemsen *et al.*, 2010; Calik *et al.*, 2023), and the impact of post-hatch holding time on oxidative stress and intestinal morphology (Juul-Madsen *et al.*, 2021; Uni and Ferket, 2004), there remains a gap in the literature concerning the interactive influence of these two critical factors on early adaptation responses. Few studies have comprehensively examined how the synergy between physiological status at hatch and delayed access to feed and water shapes immunological, hematological, and gut histomorphological outcomes in broiler chickens (Shini *et al.*, 2019). This lack of integrated analysis is particularly relevant given the growing demand for precision nutrition and adaptive resilience in modern poultry production systems. Therefore, this study was designed to address this knowledge gap through a holistic, cross-system physiological approach.

Study design, feed and management

A total of 256 Cobb 500 broiler chicks were obtained from a commercial hatchery and had been vaccinated against Newcastle Disease (ND) and Infectious Bursal Disease (IBD). The chicks were randomly assigned to 32 floor pens, each measuring 100 cm × 100 cm with a height of 75 cm. Each pen housed 8 chicks and was equipped with a 5 watt incandescent bulb, a feeder, and a drinker. The experimental design consisted of a combination of DOC weight (light: 41–45 g; heavy: 46–50 g) and post-hatch holding times of 24, 36, 48, and 60 hours, and 60 hours, each replicated four times.

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	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 %

Source: Analysis Results of PT. Perkasa Agung Sejati

The feed used in this study consisted of a commercial ration, provided in crumble form during the starter phase (from the end of holding time until 14 days of age), and in pellet form during the finisher phase (15 to 35 days of age).

At 35 days of age, selected birds were slaughtered for small intestinal sampling. Chickens were randomly selected from each experimental unit for body weight measurement prior to slaughter.

Measured variables

Growth performance

Production performance was assessed by measuring body weight at days 1, 7, and 35, average daily gain (ADG), cumulative feed intake (FI) and feed conversion ratio (FCR) as described by Noruzi *et al.* (2023). The evaluation aimed to identify the impact of treatments on early phase growth efficiency in broiler chickens (Narinç *et al.*, 2023).

Hematology

On day 35, blood samples were collected from two birds per replicate via the brachial vein using sterile 1 mL syringes and transferred into EDTA-coated tubes, then stored at 4 - 8°C until analysis. Erythrocyte and leukocyte counts were determined using Natt-Herrick diluent and a Neubauer hemocytometer under a light microscope at 400× magnification. Hematocrit was measured using microhematocrit capillary tubes centrifuged at 12.000 rpm for 5 minutes. Hemoglobin concentration was determined using the Sahli method, in which whole blood was added to 0.1 N hydrochloric acid, and the resulting brown acid hematin color was visually matched against a calibrated color comparator on the Sahli hemometer.

The heterophil-to-lymphocyte (H/L) ratio was calculated from blood smears stained with Giemsa by counting 100 leukocytes under oil immersion at 1000× magnification. All procedures followed standard poultry hematology protocols (Campbell and Ellis, 2007; Juul-Madsen *et al*., 2021; Shini *et al*., 2020).

Intestinal histology

A total of 32 birds were slaughtered at day 35, and 2 cm segments of the duodenum were excised for histological examination. Samples were stained with hematoxylin and eosin, fixed in 10% formalin, and dehydrated through a graded alcohol series (70% to 100%). Dehydrated tissues were cleared in xylol (I, II, III) and infiltrated with molten paraffin. Once solidified, tissue blocks were sectioned at 5 m using a microtome, floated on a 50°C water bath for 15 minutes, and stained again with hematoxylin eosin. The histological slides were examined under a light microscope equipped with an OptiLab camera and connected to a computer system. Photomicrographs were captured at 10× ocular and 4× objective magnification to measure villus height and width, crypt depth (Prakatur *et al*., 2019), basal width, and surface area of the intestine (Noruzi *et al*., 2022). Measurements were conducted using ImageJ software (Calik *et al*., 2023).

Statistical analysis

The data were analyzed using a Completely Randomized Design (CRD) in a 2 × 4 factorial arrangement with four replications (Gasperz, 1991). The following statistical model was applied:

$$\text{Yijk} = \mu + \text{ai} + \text{bj} + (\text{ab})\text{ij} + \mathcal{E}\text{ijk}$$
$$i = 1,2\,;\ j = 1,2,3,4\,;\ k = 1,2,3,4$$

Where; Yijk: The response of broiler chickens in the k-th replicate receiving the ij combination of treatments, where the i-th level represents the DOC weight factor and the j-th level corresponds to the holding time factor (24, 36, 48, or 60 hours). μ: Overall mean of the observations. ai: Additive effect of the i-th level of the DOC weight factor. bj: Additive effect of the j-th level of the holding time factor. (ab)ij: Interaction effect between the i-th level of DOC weight and the j-th level of holding time. Ɛijk: Experimental error effect in the k-th trial receiving the ij treatment combination.

RESULT

Performance

Initial chick weight (DOC weight) had a significant effect on early broiler performance. Heavier chicks exhibited higher body weight and feed intake on day 7 compared to lighter chicks. However, this difference did not persist through day 35, as body weight, feed intake, and feed conversion ratio (FCR) were not significantly different (P > 0.05), indicating a potential partial recovery mechanism in lighter chicks.

Holding time significantly affected final body weight (P < 0.001) and feed intake at day 35. Broilers subjected to a 24-hour holding period achieved the highest body weight and feed consumption, whereas a 60-hour delay resulted in the poorest performance. Although no significant differences were observed in FCR among treatments, a downward trend in feed efficiency was evident with increasing holding time. No significant interaction between DOC weight and holding time was detected; however, there was a biological indication that heavier chicks were more resilient to delayed feed access.

Table 2: Growth performance of broiler chickens after post hatch management.

Treatment		Body Weight (gram)		Feed Intake (gram)		FCR	
	Holding	Day 7	Day 35	Day 7	Day 35	Day 7	Day 35
DOC weight (B)							
Light	41.1±1.1^{a}	148.9±22.9^{a}	1831.6±44.6	160.6±22.3^{a}	2831.7±171.1	1.08±0.03	1.55±0.11
Heavy	47.5±0.9^{b}	159.7±24.9^{b}	1781.5±193.1	174.6±25.6^{b}	2959.1±241.2	1.10±0.06	1.67±0.16
P-value	0.000	0.018	0.448	0.001	0.093	0.370	0.070
Holding time (H)							
24 hours	44.0±3.8	182.9±11.4^{a}	1976.8±104.4^{a}	193.1±13.1^{a}	3103.1±214.8^{a}	1.05±0.06	1.57±0.12
36 hours	44.9±3.3	162.6±8.5^{b}	1831.5±146.6^{ab}	182.4±6.5^{a}	2915.8±119.4^{ab}	1.12±0.04	1.60±0.11
48 hours	43.9±4.2	146.5±16.2^{b}	1795.4±221.7^{b}	157.9±14.0^{b}	2826.7±96.2^{ab}	1.08±0.04	1.60±0.20
60 hours	44.2±3.3	125.1±6.1^{c}	1620.5±82.8^{b}	137.1±9.8^{c}	2736.1±232.1^{b}	1.08±0.04	1.67±0.15
P-value	0.375	0.000	0.009	0.000	0.014	0.136	0.696
P-value (BxH)	0.430	0.502	0.607	0.681	0.778	0.391	0.838

Description: $^{a, b, c}$ Different superscript letters the same column indicate statistically significant differences (P < 0.05), DOC weight: light (41–45 g), heavy (46–50 g), FCR (feed conversion ratio).

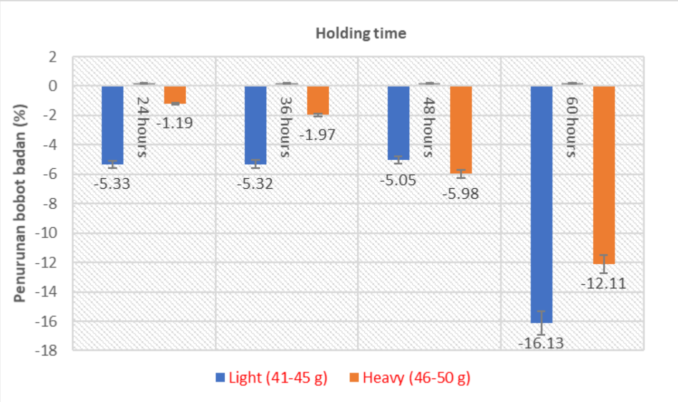

Figure 1: Percentage reduction in body weight of broiler chicks relative to initial DOC weight across different post-hatch holding times.

Figure 1 illustrates that the percentage loss in relative body weight increased with longer holding times, with greater reductions observed in light DOCs (41–45 g) compared to heavy DOCs (46–50 g). At 60 hours of holding, the weight loss reached 16.13% in light chicks and 12.11% in heavy chicks. Chicks with higher initial body weights were better able to retain their weight during periods without feed and water. This pattern reinforces the notion that lighter chicks are more vulnerable to feed and water deprivation stress, as evidenced by consistently greater relative weight loss across all holding durations.

Figure 2 illustrates that after a 24 hour holding period, heavier chicks maintained a higher relative body weight (96%) compared to lighter chicks (91%). This difference persisted through days 7 and 35, during which heavier chicks consistently exhibited greater relative body weights (75% and 89%) than their lighter counterparts (71% and 86%). This pattern aligns with the findings in Figure 1, which showed more pronounced body weight loss in lighter chicks, as well as Table 2, which confirmed the superior early performance of heavier DOCs. These results suggest that heavier chicks are more resilient to body weight loss and possess a greater capacity for partial recovery following delayed feed access.

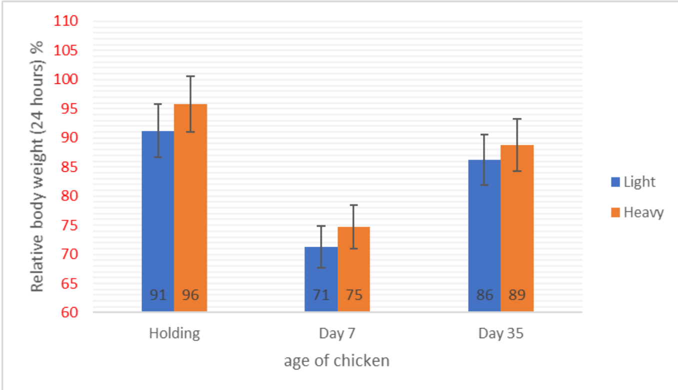

Figure 2: Relative body weight (24-hour holding time) of broiler chickens.

Hematology

Hematological analysis revealed that the combination of initial day-old chick (DOC) body weight and post-hatch holding time significantly influenced the physiological responses of broiler chickens, particularly leukocyte counts, with a significant interaction observed (P<0.05). The highest leukocyte count was recorded in heavier chicks subjected to a 60 hour holding time, indicating immune system activation in response to prolonged physiological stress due to delayed feed access. Although the heterophil to lymphocyte (H/L) ratio did not differ significantly, its increasing trend from 0.40 to 0.67 with longer holding times remains biologically relevant, as values approaching or exceeding 0.50 are commonly associated with chronic stress in poultry (Shini *et al.*, 2008).

Table 3: Hematological parameters of broiler chickens following post-hatch handling treatments.

Treatment	Hematology				
	Erythrocytes (10^6/mm^3)	Leukocytes (10^5/mm^3)	Hematocrit (%)	Hemoglobin (g/dL)	H/L
DOC weight (B)					
Light	1.62±0.28	5.67±0.85^a	27.33±1.88	10.70±1.26	0.57±0.22
Heavy	1.83±0.48	7.47±1.81^b	27.92±2.75	9.63±2.54	0.52±0.26
P-value	0.118	0.000	0.481	0.184	0.636
Holding time (H)					
24 hours	2.12±0.45^a	5.60±0.71^a	27.67±2.25	10.40±1.83	0.40±0.20
36 hours	1.68±0.18^b	6.69±0.94^a	27.00±2.28	9.57±2.18	0.46±0.27
48 hours	1.62±0.34^b	5.77±1.05^a	28.50±2.95	10.75±0.63	0.64±0.21
60 hours	1.47±0.33^b	8.21±2.25^b	27.33±2.07	10.95±0.93	0.67±0.19
P-value	0.015	0.000	0.603	0.152	0.181
P-value (BxH)	0.270	0.028	0.020	0.433	0.950

Description: ^{ab} Different superscript letters within the same column indicate a statistically significant difference (P< 0.05). DOC weight: light (41–45 g), heavy (46–50 g).

Table 4: Duodenal histomorphology of broiler chickens following post-hatch handling.

Treatment	Parameter				
	Villus height (µm)	Apical width (µm)	Basal width (µm)	Crypt depth (µm)	Villus surface area (µm²)
DOC weight (B)					
Light	751.43±94.05	70.52±33.14	101.78±50.81	196.35±101.76	765.38±178.69
Heavy	793.76±252.5	76.97±15.76	115.10±23.41	201.49±48.40	805.32±255.23
P-value	0.630	0.490	0.370	0.866	0.649
Holding time (H)					
24 hours	778.77±336.1	90.33±29.19	140.33±46.91	265.17±115.35	778.83±339.99
36 hours	873.12±150.9	75.85±15.74	110.13±25.24	212.92±54.35	885.90±152.05
48 hours	747.17±138.7	64.28±22.41	92.38±25.81	169.22±41.83	76080±138.88
60 hours	692.45±194.4	64.50±29.41	90.92±40.81	148.27±18.12	705.88±192.41
P-value	0.516	0.185	0.094	0.063	0.528
P-value (BxH)	0.158	0.079	0.319	0.821	0.167

Description: DOC weight: light (41–45 g), heavy (46–50 g),

Histology

Table 4 shows that, statistically, neither initial day-old chick (DOC) body weight nor post-hatch holding time had a significant effect ($P > 0.05$) on the microscopic structure of the duodenum, including villus height, apical and basal width, crypt depth, and villus surface area. Nevertheless, a noteworthy biological observation was made: Chicks subjected to a 60 hour holding period exhibited lower mean villus height and villus surface area compared to other groups. Although this difference was not statistically significant, such a pattern may serve as an early indication of mild physiological stress in the intestinal mucosa due to delayed access to feed and water.

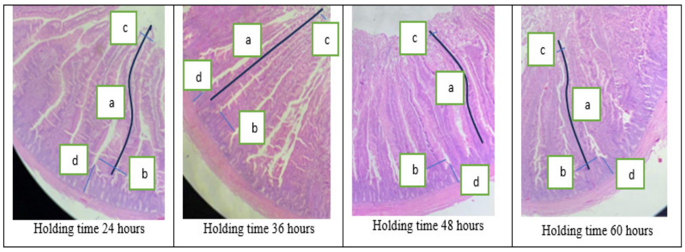

Figure 3: Histology of broiler chicken intestine: a (villus height), b (basal width), c (apical width), d (crypt depth)

Figure 3 presents histological images of the duodenum in broiler chickens, supporting the data in Table 4. Overall, no striking differences are observed between treatments; however, there is a visual trend suggesting that chicks held for 60 hours tend to exhibit shorter and narrower villi compared to those held for 24 or 36 hours, which show relatively taller and broader villi structures. Crypt depth appears relatively consistent across groups. Although these differences are not statistically significant, the trend may indicate that prolonged post-hatch feed deprivation is associated with less optimal mucosal development, without clear pathological alterations.

DISCUSSION

Initial body weight of day old chicks (DOC) significantly influences broiler performance during the early growth phase. Heavier chicks exhibit greater body weight and feed intake by day 7, consistent with the findings of Moghaddam *et al.* (2022), who emphasized the critical role of yolk reserves particularly lipids and proteins in supporting early metabolism (Muwakhid *et al.* 2025). Physiologically, heavier DOCs possess more developed muscle tissue, a more mature digestive system, and greater thermoregulatory capacity, enabling them to initiate feeding and growth more effectively. Nevertheless, lighter chicks show potential for partial recovery, characterized by higher relative growth rates after the early phase, provided they are offered high quality feed and optimal environmental conditions (Gawel *et al.*, 2022). However, potential for partial recovery in lighter chicks may be accompanied by higher maintenance energy requirements relative to body weight, as basal metabolism, thermoregulation, and stress adaptation demand a greater proportion of energy (Noy and Sklan, 2001; Syamsuryadi *et al.*, 2017). As a result, a larger share of consumed nutrients is allocated to sustaining vital functions rather than tissue accretion, leading to lower metabolic efficiency compared to heavier chicks with more stable physiology. Supporting this, van der Klein *et al.* (2017) reported that chicks with lower adaptive capacity tend to exhibit increased heat production and respiratory quotient, indicating greater energy expenditure and maintenance costs. Therefore, although initial body weight is not the sole determinant of broiler performance, it remains a key predictor during the early adaptation phase, particularly in terms of energy utilization efficiency and nutrient partitioning under environmental stress.

Holding time duration is also a critical factor influencing broiler performance. Delayed access to feed and water for ≥48 hours resulted in a significant reduction in body weight by day 35, as presented in Table 2 and Figure 1. A holding time of 60 hours led to a 10.5% decrease in final body weight compared to the 24-hour holding group, reinforcing the adverse impact of delayed nutritional access. Previous studies Geyra *et al*. (2001) and Uni *et al*. (2020) demonstrated that nutrient deprivation during the crucial post-hatch period impairs intestinal maturation, induces villus atrophy, and reduces digestive enzyme activity. Physiologically, this delay hampers the transition from utilizing residual yolk nutrients to exogenous digestion, thereby slowing weight recovery and tissue development. Figure 2 shows that heavier DOCs exhibited greater resilience, with a lower relative weight loss (5.3%) and faster performance recovery compared to light-weight chicks, which experienced a 9.7% reduction after 48 hours of holding. This finding underscores the importance of maintaining optimal initial body weight and minimizing holding time to support early adaptation and performance in broiler production.

A significant interaction between DOC body weight and holding time was observed for leukocyte count and hematocrit, indicating that the hematological responses of broiler chickens are strongly influenced by the combination of initial physiological status and the duration of post-hatch feed deprivation. The highest leukocyte count was recorded in heavier chicks held for 60 hours, reflecting stress-induced immune activation and potential systemic inflammation mediated by hypothalamic pituitary adrenal (HPA) axis activation and proinflammatory cytokine release (Shini *et al*., 2020; Calik *et al*., 2022). The H/L ratio also increased progressively with longer holding durations, from 0.40 at 24 hours to 0.67 at 60 hours, with values above 0.50 being recognized as indicators of chronic stress in poultry (Gross and Siegel, 1983; Shini *et al*., 2008). In contrast, hematocrit differences observed under certain treatment combinations may reflect adaptive mechanisms such as hemoconcentration or increased erythropoiesis in response to oxidative stress and mild dehydration (Juul-Madsen *et al*., 2021). Overall, these findings emphasize the importance of jointly considering DOC body weight and holding duration in early broiler management to prevent excessive physiological stress that may impair performance and health status.

Although no statistically significant differences were observed in intestinal histological parameters, trends indicated a reduction in villus height, apical width, and villus surface area in broilers held for 60 hours. Studies by Gilbert *et al*. (2019) and Xu *et al*. (2021) demonstrated that delayed nutrient access during early life reduces epithelial cell proliferation and increases apoptosis, resulting in shorter, blunter villi and decreased absorptive surface area. Physiologically, this compromises nutrient absorption and transfer, potentially lowering growth efficiency in the long term. While not statistically significant, this trend suggests the presence of subclinical impacts that warrant consideration in early chick management evaluations.

Overall, these findings underscore the importance of integrating optimal DOC body weight with controlled holding time to support broiler performance, physiological health, and intestinal integrity. Implementing appropriate early nutritional strategies such as early feeding or immediate access to feed can accelerate gastrointestinal maturation and mitigate the stress effects of holding. From a hatchery management perspective, limiting holding time to a maximum of 36 hours, particularly for lighter DOCs, is a strategic approach to minimize performance losses and enhance production efficiency. This recommendation aligns with the principles of Early Nutrition Programming and modern hatchery management practices that prioritize sustainable poultry welfare and productivity (Uni and Ferket, 2004; Calik *et al*., 2023).

CONCLUSION

Initial DOC body weight and holding time duration play crucial roles in determining broiler performance and physiological status. Heavier DOCs demonstrated superior early growth and recovery capacity, yet exhibited greater immunological sensitivity under extended holding. Holding time ≥48 hours negatively affected performance and tended to compromise intestinal integrity, although not always significantly. Therefore, precision hatchery management should limit holding time to a maximum of 36 hours especially for lighter chicks to optimize early adaptation, gut health, and overall broiler productivity.

ACKNOWLEDGEMENT

This work was supported by the Beasiswa Pendidikan Indonesia (BPI) program from the Indonesia Endowment Fund for Education (LPDP) through the Center for Higher Education Funding and Assessment (PPAPT), Ministry of Higher Education, Science, and Technology of Republic Indonesia.

NOVELTY STATEMENT

This study is the first to comprehensively assess the interactive effects of DOC body weight and post-hatch holding time on broiler physiological, hematological, and intestinal responses. The findings highlight that a 36-hour maximum holding time is critical to prevent subclinical gut damage and immune stress, especially in lighter chicks.

AUTHOR'S CONTRIBUTION

BS and S: conducted the study, data analysis, and statistical analysis, and wrote the original manuscript. EW, LEW, IHD and MR: formulated the methodology, supervised, reviewed, and edited the manuscript.

Generative AI or AI-assisted Technology Statement

The author(s) declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Calik A, Emami NK, White MB, Walsh MC, Romero LF, Dalloul RA (2022). Effects of dietary vitamin E and selenium supplementation on proinflammatory cytokines and immune responses in broiler chickens. Animals, 12(2): 205.

Calik A, Emami NK, White MB, Walsh MC, Romero LF, Dalloul RA (2023). Influence of dietary vitamin E and selenium supplementation on broilers subjected to heat stress: Performance, gut health, and gene expression. Animals, 13(3): 642.

Campbell TW, Ellis CK (2007). Avian and exotic animal hematology and cytology. 3rd ed. Ames (IA): Blackwell Publishing.

Gasperz V (1991). Metode perancangan percobaan. Jakarta: Gramedia.

Gaweł A, Madej JP, Kozak B, Bobrek K (2022). Early Post-Hatch Nutrition Influences Performance and Muscle Growth in Broiler Chickens. Animals, 12(23):3281. https://doi.org/10.3390/ani12233281

Geyra A, Uni Z, Sklan D (2001). The effect of fasting at different ages on growth and tissue dynamics in the small intestine of the young chick. Br. J. Nutr., 86(1): 53–61.

Gilbert ER, Li H, Emmerson DA, Webb KE, Wong EA (2019). Dietary protein and energy intake modulate growth and intestinal morphology in broiler chickens. Poult. Sci., 98(3): 1109–1117.

Gross WB, Siegel HS (1983). Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. Poult. Sci., 62(1): 1454–1458.

Juul-Madsen HR, Jørgensen PH, Handberg KJ, Norup LR (2021). Cytokine responses and immune cell populations in chickens after infectious bursal disease virus infection. Vet. Immunol. Immunopathol., 234: 110200.

Moghaddam HN, Seidavi A, Asadpour L (2022). Role of egg yolk nutrients in chick embryo development and chick quality: A review. World's Poult. Sci. J., 78(1): 59–76.

Muwakhid B, Ali U, Rifa'i, Nugraha P (2025). Utilization of Industrial Waste (Fish Meal Oil Sludge and Cassava Dregs) as an Alternative to Commercial Broiler Feed. Journal of Animal Health and Production, 13(3). https://doi.org/10.17582/journal.jahp/2025/13.3.682.687

Narinç D, Aygun A, Karaman E, Aksoy T (2023). The effect of hatching weight and brooding period on performance and immune parameters in broiler chickens. Poult. Sci., 102(2): 102377.

Noruzi S, Mahdavi AH, Samie AH, Torshizi MAK (2022). The influence of dietary supplementation with synbiotics on intestinal histomorphology and immune response of broilers. J. Anim. Physiol. Anim. Nutr., 106(1): 65–74.

Noruzi S, Mahdavi AH, Samie AH, Torshizi MAK (2023). Evaluating the performance and immune responses of broilers fed diets supplemented with phytogenic feed additives under stress. Anim. Feed Sci. Technol., 299: 115591.

Noy Y, Sklan D (2001). Yolk and hepatic lipids and serum metabolites during the posthatch transition in chicks. Poult. Sci., 80(6): 719–726.

Prakatur I, Pavic M, Miskulin M, Miskulin I, Crnkovic S, Jurcevic Z (2019). Influence of probiotic supplementation on intestinal morphology and immune status in broiler chickens. Animal, 9(3): 118.

Shini S, Kaiser P, Shini A, Bryden WL (2008). Differential expression of cytokines and chemokines in response to LPS injection in chickens. Vet. Immunol. Immunopathol., 219: 109972.

Shini S, Kaiser P, Shini A, Bryden WL (2019). Effects of delayed nutrient access on immune response and gut health in broiler chicks. Vet. Immunol. Immunopathol., 219: 109972.

Shini S, Kaiser P, Shini A, Bryden WL (2020). Differential expression of cytokines and chemokines in response to LPS injection in chickens. Vet. Immunol. Immunopathol., 219: 109972.

Syamsuryadi B, Afnan R, Arief I, Ekastuti D (2017). Higher Productivity Performance of Male Broilers Reared in The Highlandof South Sulawesi. Jurnal Veteriner, 18(1), 160–166. https://doi.org/10.19087/jveteriner.2017.18.1.160

Tona K, Onagbesan O, Bruggeman V, Mertens K, Decuypere E (2003). Effects of hatching conditions on the growth of broiler chicks to thirty-five days of age. Poult. Sci., 82(3): 329–334.

Uni Z, Ferket PR (2004). Methods for early nutrition and their potential. World's Poult. Sci. J., 60(1): 101–111.

Uni Z, Tako E, Gal-Garber O, Sklan D (2020). Morphological, molecular, and functional changes in the chicken small intestine of the immediate posthatch period. Poult. Sci., 99(4): 1918–1926.

Van der Klein SA, Silva FA, Zuidhof MJ, Betti M (2017). The effect of selection for feed efficiency on metabolism and energetics in broilers. Poult. Sci., 96(2): 398–409.

Willemsen H, Debonne M, Swennen Q, Everaert N, Careghi C, Han H, Bruggeman V, Tona K, Decuypere E (2010). Delay in feed access and spread of hatch: Importance of early nutrition. World's Poult. Sci. J., 66(2): 177–188.

Xu Y, Tian Y, Dai W, Li X, Wang J, Wu S, Zhang H, Qi G (2021). Effects of early feed restriction on performance, nutrient digestibility, and gene expression in broilers. Anim. Feed Sci. Technol., 275: 114889.