Meta-Analysis: The effect of post-hatch delayed feed access on the physiological conditions of broiler chickens during the golden period

Teysar A. Sarjana*, Enjinore E. Putri, Sugiharto Sugiharto, Dwi Sunarti, Luthfi D. Mahfudz

Poultry Production Laboratory, Animal and Agricultural Sciences Faculty, Diponegoro University, Jl. Prof H. Soedarto. S. H., Tembalang District. Semarang – Central Java 50275.

ARTICLE INFO

Recieved: 17 September 2025

Accepted: 17 October 2025

*Correspondence:

Corresponding author: Teysar Adi Sarjana E-mail: teysaradisarjana@lecturer.undip.ac.id

Keywords

Broiler, Physiological condition, Delayed feed access, Golden period

ABSTRACT

This meta-analysis aimed to conduct a systematic review and statistical analysis regarding the effects of post-hatch delayed access feed (DAF) on the physiological conditions of broiler chickens during the golden period. The materials were obtained from international journals, comprising 113 case studies collected from 11 selected and relevant journals. From various relevant parameters, six blood profiles and biochemical parameters were selected for the meta-analysis: glucose levels, cholesterol levels, total protein, heterophil counts, lymphocyte counts, and the heterophil-to-lymphocyte (H/L) ratio. Observations have focused on the impact of DAF during the golden period in chicks aged 1–7 days. The data were tabulated and analyzed using meta-analysis with JASP software version 0.18.3.0. DAF had negative effects, including increased cholesterol levels, heterophil counts, and H/L ratio. However, it did not negatively affect glucose or total protein levels. Publication bias was identified in several DAF variable moderators related to glucose, total protein heterophile, lymphocyte, and H/L parameters, suggesting potential research outcome manipulation or interpretive bias. In conclusion, delayed feed access consistently exacerbates physiological conditions, as indicated by elevated cholesterol levels, heterophil counts, and H/L ratio in broiler chickens during the golden period.

Introduction

Post-hatch hyperplasia in broiler chicks has been reported to occur up to 0-4 days of age, encompassing the development of the digestive tract, respiratory system, and immune system (Noy and Sklan, 1998; Fatmaningsih *et al.*, 2016). The transition from hyperplasia to hypertrophy occurs approximately 7-8 days post-hatch, and the transition to hypertrophy continues from 7-14 days. Hypertrophy is the process of muscle growth through an increase in the size of existing muscle fibers (myofibers) (Halevy, 2020). These two mechanisms operate continuously, especially during the early growth period known as the golden period in chicks up to 14 days post-hatch. This is highly influential in the differentiation of three body systems in chicks: the digestive system, thermoregulation, and body weight gain. These three systems develop during the golden period; thus, nutrition and physiological stimulation of post-hatch chicks must be optimal.

Ideally, no interventions should occur during the golden period that could compromise optimization at this phase, as it is not only broilers that grow well during the golden period that will exhibit optimal performance in subsequent periods. Various interventions and conditions can result in negative post-hatch effects, such as uneven hatching times of chicks, typically within 24-48 hours (Tong *et al.*, 2013). This variation in hatching time (hatch window) was categorized into several timeframes: early, middle, and late hatch. This results in chicks that hatch earlier having access to feed for a longer period until all chicks have hatched. Other variations occur when chicks undergo selection, sexing, vaccination, packaging, and transportation from the hatchery to farms at varying distances, further prolonging the delay in access to feed (Sudding, 2014).

Many research efforts prioritize evaluating performance indicators over conducting comprehensive metabolic studies. Delays in placement and feeding of post-hatch chicks due to varied transportation processes have impacts such as poor response to vaccination, slow development of the digestive tract and immune system, low resistance to disease and

pathogens, and adverse effects on long-term performance of post-hatch chicks (Panda *et al.*, 2015). A 48-hour delay in feed access results in lower body weight, decreased serum glucose, total protein, and triglyceride concentrations up to 56 days (Gaglo-Disse *et al.*, 2010). Delaying access to water and feed for up to 72 h causes dehydration and disrupts physiological development (Prabakar *et al.*, 2016). The negative impacts of delayed feed access on physiological conditions have been detected up to 14 days of age, and the negative impacts reported by some researchers are inconsistent; thus, further evaluation is needed.

Khosravinia (2015) reported that DAF for \leq 12 h post-hatching resulted in a significant 2.16% reduction in blood glucose levels. Similarly, Shinde *et al.* (2015) found that delaying feed access for \leq 12 h led to a substantial 1.52% decrease in the blood glucose levels in chicks. However, Jacobs *et al.* (2017) observed that delaying feed access for \leq 12 h did not significantly affect the blood glucose levels. This discrepancy suggests potential publication bias, necessitating a meta-analysis to derive more precise and robust conclusions.

The purpose of this study was to conduct a systematic review with statistical analysis support regarding the effect of post-hatch feed access delay on the physiological conditions of broiler chickens during the golden period. The benefit obtained from this research is that it recommends a more accurate conclusion from various relevant studies to provide a more comprehensive understanding of the effects of post-hatch feed access delay on the physiological conditions of broiler chickens during the golden period. The hypothesis proposed in this study was that a longer duration of post-hatch feed access delay has a negative effect on the physiological conditions of broiler chickens during the golden period.

Materials and methods

Materials

The materials used in this research originated from international

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made. ISSN: 2090-6277/2090-6269/ © 2011-2025 Journal of Advanced Veterinary Research. All rights reserved.

journals, with 113 case studies obtained from 11 selected and relevant journals (Appendix 1). Secondary data were obtained by searching published articles. The articles used were in English, with a publication period from to 2000-2025, from the ScienceDirect, Scopus, PubMed, ProQuest, and the search engine platforms Google Scholar and ResearchGate databases, which had the potential to be included in the meta-analysis. The equipment used consisted of laptop, software in the form of Microsoft Word 2021, PDF, and Mendeley for journal screening, and data processing applications in the form of Microsoft Excel 2021, PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) for the data selection process created with Revman 5.4 software, while the meta-analysis was conducted using JASP 0.18.3.0 software. Several literature compilations, transliteration, and grammatical arrangement processes during the article writing process were also conducted with the assistance of Al such as Gemini 2.0, Chat GPT 4.0, Scopus Al, and Scispace.

Methods

This study used a quantitative method that employed a random-effects model meta-analysis. The studies included in the meta-analysis were selected based on the inclusion and exclusion criteria. The criteria were based on evaluations of abstracts and full texts, including data presentation in each relevant article. The keywords used in the search were combinations of terms related to the effect of delayed feed access and various conditions causing delays, such as hatch windows, transportation duration, and post-hatch, as the treatment, and their impact on physiological condition parameters (e.g., respiration rate and duration, heart rate, thermoregulation, oxygen consumption, comprehensive blood profile, and biochemical parameters) at various ages, rearing periods, and in broiler chickens. The selected data comprised only physiological-condition data with corresponding controls (non-delayed conditions) for comparison.

From the relevant parameters, six parameters met the data selection criteria for the meta-analysis, specifically those related to blood profile and biochemistry: glucose levels, cholesterol levels, total protein, heterophil count, lymphocyte count, and H/L ratio. In some studies, distance and speed data from transportation distance studies, which are also related to DAF and its effects on physiological conditions, could be converted into feed access delay duration if information on travel distance and speed were available.

In this study, feed access delay was categorized into four duration ranges (≤12 h, >12-24 hours, >24 h, and ≤48 h). We identified that feed access delay durations of ≤12 h, in some studies, begin to cause physiological disturbances and initial adaptation of broiler chickens to environmental changes; however, there is insufficient literature reporting significant changes. The yolk sac provides essential nutrients and energy for chicks during the initial post-hatch hours, with the best utilization efficiency occurring during the first few hours post-hatch until exogenous feed support is available; therefore, immediate access to feed is a key factor for achieving optimal performance in subsequent periods (Elibol et al., 2023). Data presentation in the discussion focuses on the observation point of >12-24 hours delay duration, because at this point negative effects on physiological conditions begin to appear, while other data are presented in (Appendix 2 and 3). At delay durations >12-24 hours, chicks begin to utilize yolk sac reserves, including cholesterol, as the sole source of nutrients for survival before being given access to feed (Osama et al., 2011).

During DAF exceeding 24 h, yolk sac reserves are rapidly depleted within the first three days post-hatch, characterized by high feed energy metabolism and lipid absorption for growth. The yolk sac provides approximately 22% of energy and 30% of protein (Murakami *et al.*, 1992). At this stage, chicks transition from yolk sac utilization to exogenous feed; immediate post-hatch feeding enhances yolk sac utilization compared with longer delays (Bhanja *et al.*, 2009). This duration also allows comparison between extended and shorter delay periods. The categorization of

feed access delay in this study aimed to specify post-hatch physiological responses to varying delay durations. This 4 DAF categorization provides insight into the long-term effects and identifies critical delay points affecting broiler golden period physiology. At the 1–7-day of age group observation point, DAF had already shown negative impacts on physiological conditions. This observation point is particularly important; therefore, we chose to focus on the 1- to 7-day age range, while other data are presented in the appendix in the form of statistical analysis results and data interpretation (Appendices 2 and 3).

The research article selection process results are illustrated through the article inclusion stages based on Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA), as presented in Fig. 1.

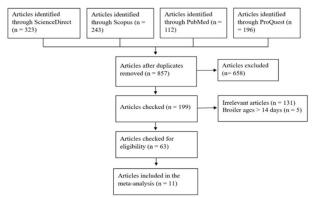


Fig. 1. Article Selection with the PRISMA method (Page et al., 2021).

Data from the selected journals were tabulated into Microsoft Excel, in the form of a coding table containing data coded according to the analysis requirements and arranged, corresponding to the column headings containing study, review, DOI/link, author, year, parameter, control group and experimental group, each consisting of N sample, means, and SD, delay duration, strain, and reasons for changes in parameters. The next step was to group the data according to the age of the chicks: 1-3, 1-7, and 1-14 days. The data that had been grouped by age were then compiled based on delayed duration and further analyzed using JASP software version 0.18.3.0.

Decision making

The meta-analysis stages involved heterogeneity testing, summary effect calculation, funnel plot, forest plot, and publication bias assessment. Heterogeneity testing was performed using a random-effects model, acknowledging heterogeneity among studies. The random-effects model assumes that true effects vary across studies due to inter-study heterogeneity. The summary effect was measured by comparing experimental and control groups to estimate the effect size and determine the overall summary effect. According to Cohen (1988), effect size is classified into four categories: weak, moderate, strong, and very strong. Effect size classification was used to determine the magnitude of feed access delay's influence on broiler post-hatch physiological condition parameters and performance. These classifications are listed in Table 1.

Table 1. Effect Size classification according to Cohen (1988).

Effect Size	Interpretation
ES ≤ 0,20	Weak
$0,20 < ES \le 0,50$	Moderate
$0.50 < ES \le 1.00$	Strong
ES > 1,00	Very Strong

Forest plot graphical representations in meta-analysis display individual study data, the overall meta-analysis estimate, summary visual effect size, and confidence intervals (CI) from observed studies, enabling researchers to quickly assess consistency and effect magnitude across studies (Patole, 2021); Sarkar and Baidya (2025). Since forest plots do not precisely account for bias or confounding factors necessary for accurate meta-analysis interpretation, additional complementary analysis is required to ensure publication bias measurement accuracy (Brush *et al.*, 2024; Sarkar and Baidya, 2025). Publication bias in the meta-analysis was identified using funnel plots and Egger's test. In funnel plots, the X-axis represents effect size, and the Y-axis represents sample size/variance/standard error. Larger sample size studies appear at the top of the graph, clustered around the summary effect (M), whereas smaller sample size studies appear at the bottom because of their larger standard error in effect size and tendency to scatter across effect size values. Funnel plot results are presented in Fig. 2.

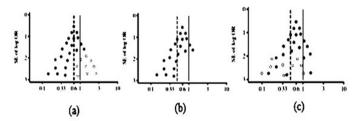


Fig 2. Plot (a) symmetry; (b) asymmetry; (c) asymmetry (Retnawati et al., 2018).

Fig. 2. (a) shows no publication bias, with smaller sample size studies at the bottom distributed symmetrically. (b) shows publication bias, with smaller sample size studies at the bottom distributed asymmetrically. This

condition indicates that studies with smaller sample sizes with statistically significant results have not been published. (c) It is similar to (b), but publication bias occurs because of excessive intervention in smaller studies with low methodological quality. In some cases, excessive intervention is beneficial in smaller studies, as these are more comprehensive or careful than larger studies.

Publication bias testing using funnel plot asymmetry is potentially subjective and requires verification with Egger's test to confirm publication bias. Changes between data groups can be observed through coefficient results in the JASP 0.18.3.0 tabulation; if the P-value < 0.05, there is a change between data groups; conversely, if the P-value > 0.05, there is no change between data groups. Decision-making criteria are also based on confidence interval (CI) calculations; if the CI includes 0, feed access delay does not affect the parameter; conversely, if the CI does not include 0, DAF affects the parameter (Kim and Peiris, 2019).

Results

The tabulation of the meta-analysis results on the effect of post-hatch DAF on broiler physiological condition during the golden period is presented in Table 2, Appendices 2 and 3. The analysis results in Table 2, Appendices 2 and 3 present heterogeneity, summary effect, P-value of the effect of post-hatch DAF, and Egger's Test, which are used to determine the effect of DAF on the physiological condition of broiler chickens during the golden period. The data presentation includes the inclusion of data on the impact of DAF on physiological conditions at the ages of 1-3, 1-7, and 1-14 days under moderators of different feed access delay durations (\leq 12 hours, >12-24 hours, >24 hours, and \leq 48 hours). The variation

Table 2. Meta-Analysis Results: The Effect of Delayed Access to Post-Hatch Feeding on the Physiological Condition of Broiler Chicks during the Golden Period (Ages 1-7 Days).

Parameter	DAF (hour)	Test of Residual Hetero- geneity			Estimation	CE	7	p-value	95% Confidence Intervals		Egger 's Test Fail-		Rosenthal		Fail- Safe N
1 arameter	DAF (nour)	Q	df	p-value	Esumation	SE	Z	p-value	Lower	Upper	p-value	Safe N	**Value	p-value	vs Rosenthal
-	≤12	99.94	14	<. 001	-0.16	0.09	-1.71	0.09	-0.33	0.02	<. 001	104,00	85	<. 001	>
Glucose	>12-24	229.91	8	<. 001	-0.68	0.73	-0.94	0.35	-2.11	0.74	*0.590	277,00	55	<. 001	>
	>24	129.05	7	<. 001	-0.72	0.90	-0.80	0.42	-2.48	1.04	0.00	8,00	50	0.01	***<
	≤48	531.81	31	<. 001	-0.58	0.34	-1.70	0.09	-1.24	0.09	<. 001	975,00	170	<. 001	>
	≤12	39.69	9	<. 001	0.30	0.27	1.11	0.27	-0.23	0.84	*0.929	15,00	60	0.01	NA
	>12-24	79.91	6	<. 001	1.39	0.64	2.18	0.03	0.14	2.64	*0.095	159,00	45	<. 001	NA
Cholesterol	>24	10.53	3	0.02	1.15	0.44	2.60	0.01	0.28	2.01	0.00	30,00	30	<. 001	=
	≤48	152.41	20	<. 001	0.82	0.27	3.01	0.00	0.28	1.35	0.01	539,00	155	<. 001	>
Total Protein	≤12	22.71	6	<. 001	0.58	0.31	1.87	0.06	-0.03	1.19	*0.348	28,00	45	<. 001	NA
	>12-24	53.37	4	<. 001	1.24	0.79	1.57	0.12	-0.30	2.79	<. 001	52,00	35	<. 001	>
	>24	66.89	7	<. 001	-0.31	0.38	-0.83	0.41	-1.05	0.42	<. 001	54,00	50	<. 001	>
	≤48	219.65	19	<. 001	0.38	0.29	1.32	0.19	-0.19	0.94	<. 001	12,00	110	0.02	***<
	≤12	6.68	4	0.15	0.83	0.32	2.59	0.01	0.2	1.46	0.02	16,00	35	<. 001	***<
TT 4 1.11	>12-24	2.43	2	0.30	2.80	0.48	5.86	<. 001	1.87	3.74	*0.856	45,00	25	<. 001	NA
Heterophil	>24	18.42	3	<. 001	4.62	1.26	3.67	<. 001	2.15	7.09	<. 001	118,00	30	<. 001	>
	≤48	70.20	11	<. 001	2.52	0.59	4.27	<. 001	1.36	3.67	<. 001	494,00	70	<. 001	>
	≤12	9.00	5	0.11	-0.30	0.28	-1.05	0.29	-0.85	0.26	0.04	0,00	40	0.07	***<
T14	>12-24	41.69	3	<. 001	-1.22	1.12	-1.09	0.28	-3.41	0.97	<. 001	12,00	30	<. 001	***<
Lymphocyte	>24	72.86	5	<. 001	-1.98	1.30	-1.52	0.13	-4.52	0.57	<. 001	40,00	40	<. 001	=
	≤48	125.38	15	<. 001	-1.12	0.53	-2.11	0.04	-2.16	-0.08	<. 001	148,00	90	<. 001	>
	≤12	4.93	4	0.29	0.80	0.26	3.09	0.00	0.29	1.30	0.05	17,00	35	<. 001	***<
II/I Datia	>12-24	12.86	2	0.00	2.42	1.04	2.33	0.02	0.38	4.45	<. 001	32,00	25	<. 001	>
H/L Ratio	>24	16.11	3	0.00	4.4	1.11	3.95	< .001	2.22	6.58	< .001	117,00	30	< .001	>
	≤48	69.82	11	<. 001	2.37	0.58	4.07	<. 001	1.23	3.51	<. 001	452,00	70	<. 001	>

^{*}No indication of publication bias

The calculation of the Rosenthal value is performed by using the following equation:

^{**}K = df + 1; Rosenthal value = 5(K) + 10

^{***}Fail-Safe N value < Rosenthal = publication bias occurs, which affects the overall study results and data interpretation.

NA: further statistical test was not applicable to perform since there was no potential publication bias

in feed access delay durations in this study is generally caused by differences in hatch time, selection, sexing, health program implementation, packaging, and transportation duration. We used the determination perspective based on the summary effect (estimation) value as an indicator of the degree of severity or the severity level of the impact of feed access delay duration, considering the limitations of the available data.

Glucose

The summary effect of DAF on blood glucose levels in broiler chickens during the golden period (aged 1-7 days) for feed access delay durations of \leq 12 hours, >12-24 hours, >24 hours, and \leq 48 hours is -155, -0.682, -0.720, and -0.576, respectively. However, it should be noted that a P-value >0.05 indicates that the summary effect is likely influenced by factors other than the duration of DAF.

Meanwhile, if we examine more closely by including only samples with observations up to 3 days (Appendix 2), delayed access to feed has a negative effect (P<0.05) not only on glucose levels but also on other parameters, namely: 1.) a decrease in glucose levels for delays >24 hours and \leq 48 hours; 2.) a decrease in lymphocyte levels for delays >12-24 hours, >24 hours, and \leq 48 hours; 3.) an increase in cholesterol levels for delays >12-24 hours and \leq 48 hours; 4.) an increase in total protein for delays of \leq 12 hours and a decrease with delays >24 hours in chicks aged 1-3 days. This means that in chicks aged 1-3 days, the determinant point of delayed feed access that begins to impact total protein is \leq 12 hours, compared to delays of other durations.

The CI results for glucose levels (Table 2) as a result of various delay durations ≤12 hours, >12-24 hours, >24 hours, and ≤48 hours have CI values that include the number 0 (zero), which indicates that the length of post-hatch DAF does not affect changes in glucose levels in golden period chicks (7 days old). Ideally, the results of the glucose funnel plot (Appendix 4) show the distribution of effect size dots symmetrically forming a triangle and centered on the y-axis (standard error). In the attached funnel plot illustration, we find it objectively difficult to conclude whether the distribution of effect size dots is symmetric or asymmetric, and therefore, further testing with Egger's Test is needed to evaluate funnel plot symmetry and detect any publication bias in the analysis results. The results of Egger's Test analysis show that delays of >12-24 hours have P values >0.05, which means there is no indication of publication bias and the funnel plot is symmetrical in the research results, so no further testing is needed. Conversely, delays of ≤12 hours, >24 hours, and ≤48 hours in the Egger's Test have P values < 0.05, indicating publication bias that may intervene in the research results and the interpretation of meta-analytic data. Therefore, the effect of delayed post-hatch feed access on glucose changes in broiler chicks during the golden period (1-7 days old) has an asymmetric funnel plot. Based on this, further Rosenthal testing was conducted for the delays of ≤12 hours, >24 hours, and ≤48 hours. The Fail-Safe N > Rosenthal values for delayed feed access of ≤12 hours and ≤48 hours show that the existing publication bias does not intervene in the overall research results and data interpretation, except for the > 24hour delay. When moderators are grouped for the observation impact of ≤12 hours to 24 hours, there is, in principle, no publication bias, so experts agree that increasing the duration of the DAF does not have an effect on glucose levels.

When we collected moderator variables at the observation point of delays >24 hours, significant differences on Egger's test confirmed with fail – safe N value < Rosenthal were found, or publication bias was detected. This means that experts do not agree that delays longer than 24 hours lead to a decrease in glucose levels; in some conditions, there was no significant change reported, but in certain studies, a significant decrease in glucose levels was observed. The reasons why glucose levels remain unchanged or significantly decrease in some studies will be discussed in the section on publication bias. Several meta-analyses related to the impact of DAF duration have shown a decrease in glucose levels at observation

points with data inclusion up to 7 days. However, this predominantly occurs when data inclusion is limited to up to 3 days (see Appendix 2 and 3).

Cholesterol

Data in Table 2 shows that the summary effect of DAF duration on cholesterol levels for delays \leq 12 hours, >12-24 hours, >24 hours, and \leq 48 hours is 0.303, 1.387, 1.145, and 0.815, with P>0.05 for the delay of \leq 12 hours, indicating that cholesterol levels are likely influenced by factors other than the duration of delayed feed access. The summary effect values within the CI range also indicate that the effect of DAF on cholesterol falls within the moderate category (0.20 < ES \le 0.50) for delays \le 12 hours, strong category (0.50 < ES \le 1.00) for \le 48 hours, and very strong category (ES > 1.00) for delays of >12-24 hours and >24 hours. Delaying feed access by>12-24 hours has the strongest effect on changes in cholesterol levels, except when compared to delays of \le 12 hours, >24 hours, and \le 48 hours.

The data distribution characteristics in the funnel plot of broiler cholesterol levels up to 7 days of age make it difficult to interpret the distribution of effect size points (Appendix 5), so Egger's Test was performed to assess distribution characteristics and detect publication bias. Egger's Test results for delay durations of ≤12 hours and >12-24 hours showed P>0.05, indicating no evidence of publication bias and a symmetrical funnel plot. In contrast, for delays of >24 hours and ≤48 hours, the Egger's test had P<0.05. This value indicates the presence of publication bias that may affect research results or meta-analysis data interpretation, as reflected by an asymmetrical funnel plot. Further Rosenthal testing for delays >24 hours and ≤48 hours showed a Fail-Safe N value > Rosenthal, so the existing publication bias does not significantly affect the overall research findings and data interpretation. When moderators are combined for the observation period of ≤12 to 48 hours, in principle, there is no publication bias, meaning that experts agree that increasing DAF duration results in increased cholesterol levels.

Total protein

The data in Table 2 show that DAF durations of \leq 12 hours, >12-24 hours, >24 hours, and \leq 48 hours contributed 0.582, 1.240, -0.312, and 0.379, respectively, to changes in total protein. However, the summary effect value with P>0.05 indicates that changes in total protein are likely influenced by factors other than the duration of delayed access to feed. The CI results for the total protein parameter, which include the value 0 during DAF durations of \leq 12 hours, >12-24 hours, >24 hours, and \leq 48 hours, indicate that DAF duration does not affect changes in total protein in golden period chicks (1-7 days old).

The results of further analysis using a funnel plot for total blood protein with Egger's Test to detect possible publication bias in analyses with a delay duration of ≤ 12 hours indicated no evidence of publication bias (P>0.05), as demonstrated by the symmetrical characteristic of the funnel plot in the study findings (Appendix 6). Meanwhile, for delays of >12–24 hours, >24 hours, and ≤ 48 hours, the Egger's Test yielded (P<0.05), thus indicating an asymmetrical funnel plot and the presence of publication bias (P<0.05) that may influence the overall research results and interpretation of the meta-analysis data. When confirmed with further Rosenthal analysis for delays of >12–24 hours, >24 hours, and ≤ 48 hours, the Fail-Safe N value > Rosenthal for delays of >12–24 hours and >24 hours indicated that the observed publication bias did not significantly influence the overall study results and data interpretation.

Nevertheless, publication bias intervention occurred in the ≤48-hour delay (Table 2). This means that experts do not agree that a delay in access of ≤48 hours does not have a significant impact on total protein; in fact, in some cases, a significant increase in total protein was reported. Several meta-analysis results regarding the impact of delayed feed access showed an increase in total protein at observation points with data

inclusion up to 7 days; however, this predominantly occurred when data inclusion was limited to only up to 3 days.

Heterophils, lymphocytes, and H/L ratio

The data in Table 2 show the summary effect values of the impact of delayed feed access, indicating a very strong and significant contribution (P<0.05) to the increase in heterophil levels and the H/L ratio in broiler chickens during the golden period (ages 1-7 days), whether the delay was \leq 12 hours, >12-24 hours, >24 hours, or \leq 48 hours. For the lymphocyte parameter, DAF did not make a significant contribution (P>0.05) except for the moderator variable of \leq 48 DAF, which made a significant contribution (P<0.05) to the reduction in lymphocytes.

The CI results for heterophile levels and the H/L ratio in broiler chickens that do not involve 0 indicate that the duration of DAF has an effect on increasing heterophile levels and the H/L ratio during the golden period of chicks. This confirms the summary effect of the lymphocyte parameter with P>0.05; the CI values for DAF impact <12 hours, 12-24 hours, and >24 hours on the lymphocyte parameter that include the number 0 indicate that DAF also does not result in changes in lymphocyte levels. Although it has a very strong effect size, changes in lymphocytes occur due to factors outside of DAF.

The results of follow-up tests through Egger's Test to identify the characteristics of the effect size funnel plot and detect publication bias in the analysis results show an asymmetric plot characteristic and the occurrence of publication bias in the effect of DAF on heterophile, lymphocyte, and H/L ratio parameters (P<0.05) (see also Appendix 7,8 and 9), except for the heterophile parameter with a delay moderator of >12-24 hours (P>0.05), which shows a symmetrical plot and is not indicated to have publication bias.

Referring to Table 2, the results of the follow-up Rosenthal test and the Fail-Safe N values for the heterophil, lymphocyte, and H/L ratio parameters indicate that publication bias which may influence the research results and overall data interpretation only occurs in the heterophil parameter due to DAF <12 hours; lymphocytes due to DAF <12 and >12-24 hours; as well as the H/L ratio parameter due to DAF <12 hours, while no intervention in results or overall data interpretation occurs with other DAF moderators. Therefore, in general, for DAF moderation >12 hours, experts agree that increasing the duration of feed access delay results in increased heterophil and H/L ratio levels, as well as decreased lymphocyte levels. Several meta-analysis results related to the impact of delayed feed access show that the decrease in lymphocyte levels predominantly occurs when data inclusion is limited to only up to 3 days (Appendix 2).

We identified forms of publication bias other than the previously mentioned increase in heterophil and H/L ratio levels accompanied by a decrease in lymphocytes. In addition, some research findings were also found that reported no significant impact of DAF on heterophil, H/L, and lymphocyte values.

Discussion

Several other factors causing changes in glucose levels aside from DAF duration are: 1.) box density of DOC (Qaid *et al.*, 2016); 2.) microclimate fluctuations (Jacobs *et al.*, 2017); 3.) DOC handling methods; 4.) strain and gene expression factors; 5.) sex (Qin *et al.*, 2022).

DAF duration of >12-24 hours does not have a significant effect on changes in glucose levels in broiler chickens during the golden period (age 1-7 days). This condition is suspected to be caused by at least two factors: First, chicks have adaptation mechanisms and bodily regulation by responding physiologically or genetically to detect threats in post-hatch chicks. As stated by Mafruchati (2023), adaptive response mechanisms consist of three stages: the chick detects a threat, then responds either physiologically or genetically to present the threat, and the chick then starts preparing its body for future threats.

The second factor, DAF does not affect glucose levels because the process of gluconeogenesis is already underway. This is in line with Murwani (2010) who stated that the nonsignificant difference in glucose levels is suspected to be achieved through gluconeogenesis, which is the formation of new glucose. Poultry maintain blood glucose levels through gluconeogenesis and also through the glycogenesis pathway (Purba *et al.*, 2021). Shorter delays may have temporary impact on glucose or be offset as the chicks gel older, as long as DAF isn't too extensive (Shinde *et al.*, 2015)

In some studies, the decrease in glucose levels was reported. Shinde *et al.* (2015) stated that a decrease in glucose levels in the chick, particularly after prolonged feed deprivation for more than 24 hours can be attributed to the body's response to lack of external nutrient intake. This leads to the utilization of internal energy reserves and eventually, a shit in metabolic process. These involve the utilization and eventual depletion of internal energy reserves (glycogen depletion and metabolic adjustments during fasting such as insufficient gluconeogenesis and protein catabolism (Wang *et al.*, 2014)

A summary effect with P>0.05 indicates the contribution of other factors that may cause fluctuations in cholesterol beyond the duration of DAF. According to some research findings, 1) Genetic selection for rapid growth and high muscle accretion shows altered somatotropic gene expression, which can affect cholesterol metabolism (Vaccaro *et al.*, 2024). 2. Stress conditions and the administration of anti-stress measures can affect cholesterol levels (Koza *et al.*, 2007).

The CI results for the cholesterol parameter in Table 2 show a CI range that does not include zero (0) for delayed feed access > 12-24 hours, >24 hours, and \leq 48 hours, thus having an effect on changes in cholesterol levels. In contrast, delayed feed access for \leq 12 hours has a CI value that includes zero, meaning that delayed feed access for \leq 12 hours has no differing effect on changes in cholesterol levels in chicks during the golden period (7 days old).

The negative impact of DAF in the form of increased cholesterol levels may be caused by: 1.) acute physiological stress due to feed not being provided immediately, which triggers the release of corticosterone hormone and increases cholesterol synthesis in the liver; 2.) chicks utilizing fat-rich yolk reserves as their main energy source during the DAF period, resulting in increased blood lipid levels, including cholesterol; 3.) DAF can disrupt the natural maturation process of the digestive system and lipid metabolism (the process of converting fat into energy), resulting in increased cholesterol levels in chicks.

In almost all previous studies, when newly hatched chicks are not immediately given feed, their bodies respond by releasing corticosterone hormones as an acute physiological stress response, which can increase cholesterol synthesis in the liver and raise blood cholesterol levels (Thaxton and Puvadolpirod, 2000; Post *et al.*, 2003; Khosravinia, 2015). Gonzales *et al.* (2003) reported that chicks rely on fat reserves from the yolk to survive during fasting periods, which leads to fluctuations in fat levels, including cholesterol, in the blood and or fat reserve mobilization (Shinde *et al.*, 2015). This is supported by the opinion of Noy and Sklan (1999) who stated that during the feed deprivation period, chicks depend on yolk reserves that are rich in fat, potentially increasing blood lipid levels.

Delaying access to feed hinders the development of the digestive system, as well as the absorption and metabolism of chicks after hatching, which can increase the total lipids in the blood. Proszkowiec-Weglarz et al. (2022) stated that delayed feeding disrupts the normal development of the digestive tract, which plays a vital role in lipid metabolism. This alteration in lipid metabolism also causes shifts in metabolism due to changes in the expression of genes responsible for lipid metabolism and absorption in liver cells and intestinal epithelial cells (Tsunekage and Ricklefs, 2015; Metzler-Zebeli et al., 2019; Zaytsoff et al., 2019; Prasad et al., 2022).

We did not find publication bias regarding DAF \leq 12 hours, and within this delay duration, cholesterol levels did not change significantly due

to: 1.) chicks utilizing yolk reserves, which provide energy and nutrients, including lipids and cholesterol, thereby helping maintain metabolism; 2.) yolk reserves help minimize stress and aid chick survival during feed delay or prolonged transportation, thus preventing drastic changes in cholesterol; 3.) chicks rely more on other energy sources (glucose and fatty acids) to overcome stress, enabling them to maintain stable cholesterol levels as it is used more for hormone production and cell membrane formation, not as the main energy source.

Yolk reserves are an essential source of nutrients for newly hatched chicks in maintaining physiological functions before they have access to feed (Kang *et al.*, 2018). The nutrients from the yolk provide an energy source while minimizing stress caused by delayed access to feed and transportation processes, thereby helping to keep cholesterol levels stable (Dibner *et al.* (1998) and Bigot *et al.* (2003)). This finding may explain the stability of cholesterol levels during transportation or delayed feeding. This aligns with the opinion of Kang *et al.* (2018, who stated that insignificant changes in cholesterol levels are likely due to the resilience of newly hatched chicks and the efficient use of yolk reserves, supporting cholesterol regulation through metabolic adaptation.

According to several researchers, other factors that may cause changes in total protein after hatching are: 1) Heat stress compromising broiler performance and affecting blood total protein level (Hosseini-Vashan and Piray, 2021). 2) Thermal manipulation can modify physiological parameters including blood total protein level (Zaboli *et al.*, 2017). 3) Hatching weight variation, with higher hatching weight associated with higher concentrations of total protein, globulin, and albumin in the early post-hatch period (Chen *et al.*, 2015). 4.) Stress sensitivity, broiler exhibit hinger blood total protein indicating hinger stress sensitivity (Smolentsev *et al.*, 2024).

Regarding the CI value that indicates no DAF effect on total protein, according to Bigot et al. (2003), there are at least four reasons why total protein levels do not always follow the pattern of DAF: 1. Protein synthesis activation: Delayed feeding can initiate protein synthesis, though levels may remain lower than in early-fed chicks. 2. Genetic factors: Starvation can minimize the genetic control of growth, masking the full potential for protein synthesis and leading to varied effects on protein levels. 3. Nutrient availability: While early feeding improves growth and protein synthesis, delayed feeding only stunts-but does not completely halt-these processes. 4. Compensatory growth: The absence of compensatory growth in the early post-hatching days can stabilize total protein levels despite initial feeding delays. Kang et al. (2018) generally state that delayed feeding primarily affects body weight gain and feed intake, not necessarily total protein levels. Furthermore, it is explained that physiological adaptations in the small intestine post-hatch may lessen the impact of delayed feeding on protein utilization.

An increase in blood total protein has been reported by several researchers, including Hassan *et al.* (2023), who showed that the stress response due to DAF for 24 hours and the metabolic adjustments that occur during the fasting period can lead to increased protein synthesis in chicks as they adapt to nutrient availability. Meanwhile, in the study by Shinde *et al.* (2015), nutrients from the yolk sac can also contribute to protein synthesis, particularly during the initial post-hatch period, thus potentially resulting in a non-significant difference in total protein.

A summary effect with P>0.05 indicates that changes in heterophil levels, lymphocytes, and the H/L ratio in broiler chicks post-hatch are influenced by factors other than delayed access to feed. According to several studies, these factors may include: 1.) Environmental Stressors, such as elevated air temperatures (Skomorucha *et al.*, 2010), exposure to cold temperatures (Olfati *et al.*, 2018), and light exposure (Campo *et al.*, 2007). 2) Transportation and handling (Ghareeb and Böhm, 2009). 3) Genetic Factors (Skomorucha *et al.*, 2010), and 4) Immune Response and Disease Resistance (Thiam *et al.*, 2021).

The negative impact of DAF in the form of increased heterophil levels and H/L ratio, as well as decreased lymphocyte levels, can be attributed

to at least three causes as follows: First, prolonged DAF increases physiological stress, which negatively affects the development of the immune system in newly hatched chicks. Tamboli *et al.* (2018) reported that chicks deprived of feed for 24 and 36 hours showed inhibited immune system development, resulting in higher heterophil counts and H/L ratios and, conversely, lower lymphocyte counts compared to controls. These findings also confirm the opinions of Gross and Siegel (1986), and Cengiz *et al.* (2012). The increase in H/L Ratio is caused by stress in chicks, as a result of progressively delayed access to feed (Shinde *et al.*, 2015; Shakeel *et al.*, 2016).

Second, the bursa of fabricius does not function properly when chicks are stressed, causing an increase in heterophil counts, followed by a decrease in lymphocytes with or without it, which leads to an increased H/L ratio. The H/L ratio has been reported as a good indicator of stress, including situations such as feed deprivation (Gross and Siegel, 1983; Tamboli *et al.*, 2018), as well as immune resistance as a form of the chicken's response and adaptation to poor environmental conditions (Fajrih *et al.*, 2014). Furthermore, explained that under stress conditions, lymphocyte production by the immune organ (bursa of Fabricius) is insufficient, resulting in increased heterophil levels and H/L ratio (Fajrih *et al.*, 2014).

With regard to publication bias, some studies have reported no significant changes. Shakeel *et al.* (2016) reported that short-term delayed access to feed (first 12 hours) has not been enough to cause significant changes in stress indicators, and levels of heterophils and the H/L ratio remain stable due to the availability and utilization of residual yolk, so chicks have good resilience to short-term feed shortages. In another study, Tamboli *et al.*, (2018) reported the influence of sex, where female chicks were able to tolerate short-term DAF stress better than males without showing significant changes in H/L ratio parameters. Furthermore, it was explained that differences in stress responses between male and female chicks during the same duration of DAF may be due to variations in physiological, hormonal, or immune system development responses between sexes.

In the study by Kang *et al.* (2018), it was explained that the stability of lymphocyte levels occurs because, in the early post-hatch period of gut development, growth and anticipation of short-term stress become important priorities. Goessling *et al.* (2015) reported that the initial stress response is usually indicated by the mobilization of heterophils, while lymphocyte levels are maintained at stable levels, and their response is slower, thus remaining relatively stable.

Based on the results of a meta-analysis on the effects of DAF on these physiological condition parameters, there are still many inconsistencies in the study outcomes, as evidenced by indications of publication bias in several parameters. Therefore, due to the limited number of studies, there is a need for more observational support and comprehensive research on this subject. In this study, we also revealed that not all parameters that are dogmatically used and/or considered as indicators of the problem of delayed feed access are, in fact, ideal for use. Based on the results of our research, DAF generally does not have sufficient determination on changes in glucose levels, total blood protein, and lymphocytes; conversely, it has strong determination on blood cholesterol, heterophils, and the heterophil/lymphocyte ratio during the golden period. Specifically, the DAF moderator group <12 hours, consistently in immune parameters such as heterophils, H/L, and lymphocytes (<12 and >12-24 hours), cannot be used as indicators of the negative impact/stress of DAF because there is publication bias that can intervene with both the results and the interpretation of the data.

If we narrow the impact of observation to just 3 days after DAF, then the strong determinant effect of DAF on changes in blood glucose, cholesterol, total protein, and lymphocytes occurs more intensely compared to the observation phase during the golden period. On the other hand, if we extend the observation to 14 days, the strong determinant effect on several mentioned parameters becomes less intense than at 3 days or during the golden period. Regarding this matter, we suspect that, physi-

ologically, the negative impact of DAF can be recovered after the golden period phase.

Conclusion

The duration of delayed feed access consistently worsens physiological conditions as indicated by increased cholesterol levels, heterophil counts, and the H/L ratio, while several other parameters cannot be fully used as indicators of the negative impacts of DAF. There is a need to re-evaluate the negative physiological indicators of DAF by specifically narrowing down and focusing on physiological markers such as cholesterol levels, heterophil counts, and the H/L ratio. Further exploration of the potential recovery time from the negative impacts of DAF is recommended.

Acknowledgments

The authors are thankful to Ronaldo Marpangidoan, and Silvi Susanti for their assistance and discussion regarding data collection.

Conflict of interest

The authors have no conflict of interest to declare.

References

- Bhanja, S.K., Devi, C.A., Panda, A.K., Sunder, G.S., 2009. Effect of post hatch feed deprivation on yolk-sac utilization and performance of young broiler chickens. Asian Australas J Anim Sci. 22,1174-1179.
- Bigot, K., Mignon-Grasteau, S., Picard, M., Tesseraud, S., 2003. Effects of delayed feed intake on body, intestine, and muscle development in neonate broilers. Poultry Science 82, 781-788.
- Brush, P.L., Sherman, M., Lambrechts, M.J., 2024. Interpreting meta-analyses: a guide to funnel and forest plots. Clin. Spine. Surg. 37, 40-42.
- Campo, J.L., Gil, M.G., Dávila, S.G., Muñoz, I. 2007. Effect of lighting stress on fluctuating asymmetry, heterophil-to-lymphocyte ratio, and tonic immobility duration in eleven breeds of chickens. Poult. Sci. 86, 37-45.
- Cengiz, O., Koksal, B.H., Tatli, O., Sevim, O., Avci, H., Epikmen, T., Beyaz, D., Buyu-kyoruk, S., Boyacioglu, M., Uner, A., Onol, A.G., 2012. Influence of dietary organic acid blend supplementation and interaction with delayed feed access after hatch on broiler growth performance and intestinal health. Vet. Med. 57, 515 528.
- Chen, Y., Wen, C., Zhuang, S., Zhou, Y., 2015. A comparison of growth, immunity and oxidative status of broilers that differ in hatching weight at early age. J. Poult. Sci. 52, 137-144.
- Cohen, J., 1988. Statistical Power Analysis for the Behavioral Sciences. Lawrence Erlbaum Associates, New York.
- Dibner, J.J., Knight, C.D., Kitchell, M.L., Atwell, C.A., Downs, A.C., Ivey, F.J., 1998. Early feeding and development of the immune system in neonatal poultry. J. Appl. Poult. Res. 7, 425 436.
- 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, https://doi.org/10.3390/ani13243827
- Fajrih, N., Suthama, N., Yunianto, V.D., 2014. Body resistance and productive performances of crossbred local chicken fed inulin of dahlia tubers. Media Peternakan 37, 108 114.
- Fatmaningsih, R., Riyanti, R., Nova, K., 2016. Performa ayam pedaging pada sistem brooding konvensional dan thermos. J. Ilmiah Peternakan Terpadu. 4, 222 229.
- Gaglo-Disse, A., Tona, K., Aliou, S., Debonne, M., Aklikokou, K., Gbeassor, M., Decuypere, E., 2010. Effect of delayed feed access on production and blood parameters of layer-type chicks. Acta Vet Hung. 58, 211 219.
- Ghareeb, K., Böhm, J., 2009. Stress indicators to pre-slaughter transportation of broiler chickens fed diets supplemented with a symbiotic. Int. J. Poult. Sci. 8, 621 – 625.
- Goessling, J.M., Kennedy, H., Mendonça, M.T., Wilson, A.E., 2015. A meta-analysis of plasma corticosterone and heterophil: lymphocyte ratios is there conservation of physiological stress responses over time?. Funct. Ecol. 29, 1189–1196.
- Gonzales, E., Kondo, N., Saldanha, E.S., Loddy, M.M., Careghi, C., Decuypere, E., 2003. Performance and physiological parameters of broiler chickens subjected to fasting on the neonatal period. Poult. Sci. 82, 1250–1256.
- Gross, W.B., Siegel, H.S., 1986. Effects of initial and second periods of fasting on heterophil/lymphocyte ratios and body weight. Avian Dis. 30, 345 346.
- Gross, W. B., Siegel, H.S., 1983. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. Avian Dis. 27, 972 979.
- Halevy, O., 2020. Timing Is Everything: The high sensitivity of avian satellite cells to thermal conditions during embryonic and post hatch periods. Front. Physiol. 11, 1–7
- Hassan, M.I., Khalifah, A.M., El Sabry, M.I., Mohamed, A.E., Hassan, S.S., 2023. Performance traits and selected blood constituents of broiler chicks as influenced by

- early access to feed post-hatch. Anim. Biotechnol. 34, 2855–2862.
- Hosseini-Vashan, S.J., Piray, A.H., 2021. Effect of dietary saffron (Crocus sativus) petal extract on growth performance, blood biochemical indices, antioxidant balance, and immune responses of broiler chickens reared under heat stress conditions. Ital. J. Anim. Sci. 20, 1338–1347.
- Jacobs, L., Delezie, E., Duchateau, L., Goethals, K., Ampe, B., Buyse, J., Tuyttens, F.A.M., 2017. Impact of transportation duration on stress responses in day-old chicks from young and old breeders. Res. Vet. Sci. 112, 172–176.
- Jhetam, S., Shynkaruk, T., Buchynski, K., Van Kessel, A. G., Crowe, T. G., Schwean-Lardner, K., 2024. Stocking density within chick transport boxes: effects on leghorn chick stress and box microclimate. Journal of Applied Poultry Research. 33, 1–10.
- Kang, H.K., Bang, H.T., Kim, C.H., Jeon, J.J., Kim, H.S., Suh, S.W., Hong, E.C., Kim, S.H., Park, S.B., 2018. Effects of early posthatch feeding on growth, organ development, and blood biochemical profile in broiler chickens. Canadian J. Anim. Sci. 99, 418 – 424.
- Khosravinia, H., 2015. Physiological response of newly hatched broiler chicks to increasing journey distance during road transportation. Italian J. Anim. Sci. 14, 519–523.
- Kim, S., Peiris, T.B., 2019. Meta-analysis of regression: a review and new approach with application to linear-circular regression model. Communications in Statistics Theory and Methods 50, 2723-2731.
- Koza, G.A., Mussart, N.B., Coppo, J.A., 2007. Effect of a lipotropic agent with choleretic-cholagogue properties on metabolic indicators in chickens submitted to stress. Rev. Med. Ved. Zoot. 18, 124 – 129.
- Mafruchati, M., 2023. Perbedaan Masa Inkubasi terhadap Perkembangan Embrio. Zifatama Jawara, Sidoarjo.
- Metzler-Zebeli, B.U., Siegerstetter, S.C., Magowan, E., Lawlor, P.G., O'Connell, N.E., Zebeli, Q., 2019. Feed restriction reveals distinct serum metabolome profiles in chickens divergent in feed efficiency traits. Metabolites. 9, 38.
- Murakami, H., Akiba, Y., Horiguchi, M., 1992. Growth and utilization of nutrients in newly hatched chick with or without removal of residual yolk. Growth Dev. Ageing 56, 75 84.
- Murwani, R. 2010. Broiler Modern. Se- marang: Widya Karya.
- Noy, Y., Sklan, D., 1998. Yolk utilization in the newly hatched poult. Br. Poult. Sci. 39, 446 451.
- Noy, Y., Sklan, D., 1999. Energy utilization in newly hatched chicks. Poult. Sci. 78, 1750 1756.
- Olfati, A., Mojtahedin, A., Sadeghi, T., Akbari, M., Martínez-Pastor, F., 2018. Comparison of growth performance and immune responses of broiler chicks reared under heat stress, cold stress, and thermoneutral conditions. Spanish Journal of Agricultural Research. 16, 1 7.
- Osama, H.A., Huwaida, A., Malik, E.E., Elhadi, H.M., 2011. Changes in the Concentrations of Liver Total Lipids, Serum Total Lipids, and Serum Cholesterol During Early Days Post-hatch in Broiler Chicks. Asian Journal of Poultry Science 5, 51–55
- Page, M.J., McKenzie, J.E., Bossuyt, P.M., Boutron, I., Hoffmann, T.C., Mulrow, C.D., Shamseer, L., Tetzlaff, J.M., Akl, E.A., Brennan, S.E., Chou, R., Glanville, J., Grim-shaw, J.M., Hro 'bjartsson, A., Lalu, M.M., Li, T., Loder, E.W., Mayo-Wilson, E., McDonald, S., McGuinness, L.A., Stewart, L.A., Thomas, J., Tricco, A.C., Welch, V.A., Whiting, P., Moher, D., 2021. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. The BMJ. 372, 1 – 8.
- Panda, A. K., S. Bhanja, and G. S. Sunder. 2015. Early post-hatch nutrition on immune system development and function in broiler chickens. Worlds. Poult. Sci. J. 7, 285 – 296.
- Patole, S., 2021. Forest Plots in a Meta-Analysis. In: Principles and Practice of Systematic Reviews and Meta-Analysis. Springer, Cham. pp. 79–88
- Post, J., Rebel, J.M., Ter Huurne, A.A., 2003. Physiological effects of elevated plasma corticosterone concentrations in broiler chickens. An alternative means by which to assess the physiological effects of stress. Poult. Sci. 82, 1313 1318.
- Prabakar, G. Pavulraj, S., Shanmuganathan, S., Kirubakaran, A., Mohana, N., 2016. Early nutrition and its importance in poultry: a review. Indian J. Animal Nutrition 33. 245 – 252.
- Prasad, A.R., Bhattacharya, T.K., Chatterjee, R.N., Divya, D., Bhanja, S.K., Shanmugam, M., Sagar, N.G., 2022. Silencing acetyl-CoA carboxylase A and sterol regulatory element-binding protein 1 genes through RNAi reduce serum and egg cholesterol in chicken. Sci. Rep. 12, 1191.
- 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 afects the development and maturation of gastrointestinal tract microbiota in broiler chickens. BMC Microbiology 22, 2 20.
- Purba, D.H., Marzuki, I., Dailami, M., Saputra, H.A., Mawarti, H., Gurning, K., Yesti, Y., Khotimah, K., Purba, S.R.F., Unsunnidhal, L., Situmorang, R.F.P., and Purba, A.M.F., 2021. Biokimia. Yayasan Kita Menulis, Medan. [Indonesia]
- Qaid, M., Albatshan, H., Shafey, T., Hussein, E., Abudabos, A.M., 2016. Effect of stocking Density on the performance and immunity of 1- to 14-d- Old Broiler Chicks. Brazilian J. Poult. Sci. 18, 683 692.
- Qin, Z., Shah, A. M., Zhu, Q., Wang, Y., Li, D., Shu, G., Tian, Y., Zhao, X., 2022. Glucose Homeostasis Differed in the Fast- and Slow-Growing Chickens (Gallus domestics). Pak. J. Zool. 54, 953 – 956.
- Retnawati, H., Apino, E., Kartianom, K., Djidu, H., Anazifa, R.D., 2018. Pengantar Analisis Meta (Ed ke-1). Parama Publishing, Yogyakarta.
- Sarkar, S., Baidya, D.K., 2025. Meta-analysis interpretation of forest plots: A wood for the trees. Indian J. Anaesth. 69, 147–152.
 Shakeel, I., Khan, A.A., Qureshi, S., Adil, S., Wani, B.M., Din, M.M., Amin, U., 2016.
- Shakeel, I., Khan, A.A., Qureshi, S., Adil, S., Wani, B.M., Din, M.M., Amin, U., 2016. Stress levels, mortality, intestinal morphometry and histomorphology of chabro broiler birds subjected to varying degrees of post hatch delay in feeding. Pak. J. Biol. Sci. 19, 331 – 337.
- Shinde, A. S., Goel, A., Mehra, M., Rokade, J., Bhadauria, P., Mandal, A.B., Bhanja, S.K., 2015. Delayed post hatch feeding affects performance, intestinal morphology and expression pattern of nutrient transporter genes in egg type chickens. J. Nutr. Food. Sci. 5, 1 11.

- Skomorucha, I., Sosnówka-Czajka, E., Muchacka, R., 2010. Effect of thermal conditions on welfare of broiler chickens of different. Annals of Animal Science 10, 489 497.
- Smolentsev, S., Holodova, L., Rozhentsov, A., Bryukhacheva, S., Belyakova, K., Gracheva, O., Gasanov, A., Medetkhanov, F., Mukhutdinova, D., Tamimdarov, B., Isupova, N., Milaev, V., 2024. Comparative characteristics of metabolism in stress-sensitive and stress-resistant broiler chickens (Conference Paper). BIO Web of Conferences. 130, 5.
- Sudding, H.A.F., 2014. Pengaruh penundaan penanganan dan pemberian pakan sesaat setelah menetas terhadap performans ayam ras pedaging. J. Ilmu dan Industri Peternakan 1, 253 262. [Indonesia]
- Tamboli, A.S., Goel, A., Mehra, M., Rokade, J.J., Bhadauria, P., Yadav, A.S., Bhanja, S.M. S.K., 2018. Delayed post-hatch feeding affects the performance and immunocompetence differently in male and female broiler chickens. J. Applied Animal Research 46, 306–313.
- Thaxton, J. P., Puvadolpirod, S., 2000. Model of physiological stress in chicken 5. Quantitative Evaluation. Poultry Science 79, 391 – 395.
- Thiam M., Wang Q., Sánchez A.L.B., Zhang J., Zheng M., Wen J., Zhao G., 2021. Association of heterophil/lymphocyte ratio with intestinal barrier function and immune response to salmonella enteritidis infection in chicken. Animals 11, 3498.
- Tong, Q., Romanini, C.E., Exadaktylos, V., Bahr, C., Berckmans, D., Bergoug, H., Eterradossi, N., Roulston, N., Verhelst, R., McGonnell, I.M., Demmers, T., 2013. Embryonic developmment and the physiological factors that coordinate hatching in domestic chickens. Poult Sci. 92, 620 628.
- Tsunekage, T., Ricklefs, R.E., 2015. Increased lipid peroxidation occurs during development in Japanese quail (Coturnix japonica) embryos. Br. Poult. Sci. 56, 262–266.

- Vaccaro L.A., Herring K., Wilson A., England E., Smith A.L., Ellestad L.E. 2024. Dynamic changes in insulin-like growth factor binding protein expression occur between embryonic and early post-hatch development in broiler chickens. Poultry Science 103, 104174.
- Wang, Y., Li, Y., Willems, E., Willemsen, H., Franssens, L., Koppenol, A., Guo, X., Tona, K., Decuypere, E., Buyse, J., Everaert, N. 2014. Spread of hatch and delayed feed access affect post hatch performance of female broiler chicks up to day 5. Animal Journal 8, 610–617.
- Zaboli, G.R., Rahimi, S., Shariatmadari, F., Torshizi, M.A.K., Baghbanzadeh, A., Mehri, M., 2017. Physiology and reproduction: Thermal manipulation during pre and post-hatch on thermotolerance of male broiler chickens exposed to chronic heat stress. Poultry Science 96, 478 485.
- Zarrinkavyani, K., Khosravinia, H., Shahsavari, G., Biranvand, Z., 2019. Effect of injection of diazepam, melatonin, methocarbamol and glucose on physiological responses of neonate chicks subjected to road transportation stress. J. Bas. Res. Med Sci. 6, 1–10.
- Zaytsoff, S.J.M., Brown, C.L.J., Montina, T., Metz, G.A.S., Abbott, D.W., Uwiera, R.R.E., Inglis, G.D., 2019. Corticosterone mediated physiological stress modulates hepatic lipid metabolism, metabolite profiles, and systemic responses in chickens. Sci. Rep. 9, 19225.
- Zhao, X., Sumners, L., Gilbert, E.L., Siegel, P.B., Zhang, W., Cline, M., 2014. Delayed feeding after hatch caused compensatory increases in blood glucose concentration in fed chicks from low but not high body weight lines. Poult Sci. 93, 617 624.

APPENDIX

Appendix 1. Journal Sources Included in the Meta-Analysis Calculation.

Researcher	Year	Parameter	DAF (hour)
Bigot et al.	2003	Total protein	48
Khosravinia	2015	Glucose, cholesterol	4, 8, 12, 14, 16
Shakeel et al.	2016	Heterophil, lymphocyte, and H/L ratio	12, 24, 48, 72
Wang et al.	2014	Glucose	48
Kang et al.	2018	Cholesterol, total protein, and lymphocyte	12, 24, 36, 48
Tamboli <i>et al</i> .	2018	Heterophil, lymphocyte and H/L rasio	6, 12, 24, 36
Shinde et al.	2015	Glucose, cholesterol, total protein	6, 12, 24, 36
Zhao et al.	2014	Glucose	16
Jacobs et al.	2017	Glucose	1,5, 11
Zarrinkavyani et al.	2019	Glucose, cholesterol, total protein	6, 12, 18, 24
Jhetam et al.	2024	Glucose	7

Appendix 2. Meta-Analysis Results: The Effect of Delayed Access to Post-Hatch Feeding on the Physiological Condition of Broiler Chickens during the Golden Period (1-3 Days Old).

Parameter	DAF (hour)		Residua geneit	al Hetero- y	Summary Effect				95% Confidence Intervals		Egger 's Test	Fail-Safe N Value	Rosenthal		Fail-Safe N vs Rosenthal
		Q	df	p-value	Estimation	SE	Z	p-value	Lower	Upper	p-value	N value	**Value	p-value	Rosentilal
Glucose	≤12	99.62	12	<. 001	-8.76	4.94	-1.77	0.08	-18.45	0.92	<. 001	105	75	<. 001	>
	>12-24	196.12	7	<. 001	-0.93	0.77	-1.20	0.23	-2.44	0.59	*0.753	341	50	<. 001	NA
	>24	2.32	3	0.51	-2.96	0.32	-9.30	< .001	-3.59	-2.34	*0.129	127	30	<. 001	NA
	≤48	419.08	24	<. 001	-1.09	0.39	-2.81	0.01	-1.85	-0.33	<. 001	1653	135	<. 001	>
Cholesterol	≤12	28.71	6	<. 001	0.56	0.34	1.65	0.10	-0.11	1.21	*0.602	34	45	< .001	NA
	>12-24	47.67	4	<. 001	1.98	0.70	2.83	0.01	0.61	3.35	0.04	170	30	<. 001	>
	≤24	102.26	11	<. 001	1.14	0.39	2.92	0.00	0.37	1.9	0.01	370	70	<. 001	>
	≤48	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	≤12	10.76	3	0.01	0.99	0.40	2.48	0.01	0.21	1.77	0.02	30	30	<. 001	>
Total Protein	>12-24	35.28	2	<. 001	2.03	1.14	1.79	0.07	-0.20	4.26	0.00	51	25	<. 001	>
Iotal Protein	>24	17.30	3	<. 001	-0.83	0.33	-2.55	0.01	-1.47	-0.19	*0.627	57	30	<. 001	NA
	≤48	167.27	10	<. 001	0.59	0.48	1.24	0.22	-0.35	1.53	<. 001	18	65	0.00	***<
	≤12	6.98	4	0.14	-0.45	0.32	-1.42	0.16	-1.08	0.17	*0.157	2	35	<. 001	NA
Lymphocyte	>12-24	14.48	2	<. 001	-2.22	0.70	-3.19	0.00	-3.58	-0.86	*0.154	71	25	<. 001	NA
	>24	34.73	3	<. 001	-3.45	1.5	-2.30	0.02	-6.39	-0.51	< .001	76	30	<. 001	>
	≤48	71.25	11	<. 001	-1.83	0.58	-3.16	0.00	-2.97	-0.69	<. 001	291	70	<. 001	>

NA = available data does not meet the rules of Meta-Analysis; the heterophil parameter and H/L ratio have no available data at all in the Meta-Analysis or further statistical test was not applicable to perform since there was no potential publication bias

Delayed access to feeding has a negative effect (p<0.05) in the form of: 1.) decreased glucose levels with a delay of >24 hours and \leq 48 hours; 2.) decreased lymphocyte levels with a delay of >12-24 hours, >24 hours, and \leq 48 hours; 3.) increased cholesterol levels with a delay of >12-24 hours and \leq 48 hours; 4.) increased total protein for \leq 12 hours and decreased total protein for >24 hours in chicks aged 1-3 days. The extent of the impact contribution of delayed access to feeding on the observed parameters can be seen in the estimation column. In chicks aged 1-3 days, the critical point that starts to have an impact is a delay of \leq 12 hours.

^{*}There is no indication of publication bias that could intervene in the research results and overall data interpretation.

Rosenthal value calculation is performed by calculating the following equation:

^{**}K = df + 1; Rosenthal value = 5(K) + 10

^{***}Fail-Safe N value < Rosenthal = publication bias occurs that intervenes in the research results and overall data interpretation.

Appendix 3. Meta-Analysis Results: The Effect of Delayed Access to Feed Post-Hatching on the Physiological Condition of Broiler Chickens During the Golden Period (Aged 1-14 Days).

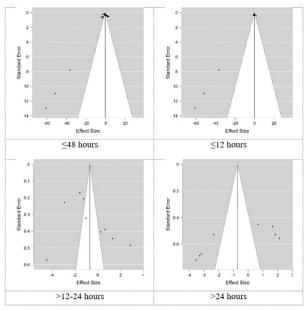
Parameter	DAF (hour)	Test of Residual Hetero- geneity			Estima-	SE	z	p-value	95% Confidence Intervals		Egger 's Test	Fail-Safe	Rosenthal		Fail-Safe N vs
		Q	df	p-value	tion				Lower	Upper	p-value	N Value	**Value	p-value	Rosenthal
Glucose	≤12	102.07	16	<. 001	-0.15	0.09	-1.68	0.09	-0.31	0.02	<. 001	105	95	<. 001	>
	>12-24	414.62	12	<. 001	-0.61	0.68	-0.90	0.37	-1.95	0.72	*0.066	630	75	<. 001	NA
	>24	139.14	8	<. 001	-0.48	0.82	-0.59	0.56	-2.09	1.13	<. 001	0	55	0.12	***<
	≤48	778.03	38	<. 001	-0.50	0.33	-1.54	0.12	-1.14	0.14	<. 001	1442	205	<. 001	>
	≤12	40.36	11	<. 001	0.27	0.23	1.21	0.23	-0.17	0.71	*0.882	17	70	0.01	NA
cholesterol	>12-24	96.32	7	<. 001	1.14	0.60	1.89	0.06	-0.05	2.32	*0.070	136	50	<. 001	NA
cnotesteroi	>24	11.15	4	0.03	1.04	0.35	3.00	0.00	0.36	1.71	0.00	42	35	<. 001	>
	≤48	164.99	24	<. 001	0.69	0.24	2.95	0.00	0.23	1.15	0.01	556	135	<. 001	>
	≤12	30.08	8	<. 001	0.66	0.28	2.38	0.02	0.12	1.20	*0.067	52	55	<. 001	NA
Total Protein	>12-24	53.82	5	<. 001	1.24	0.64	1.94	0.05	-0.02	2.50	<. 001	80	40	<. 001	>
	>24	83.54	8	<. 001	-0.15	0.37	-0.42	0.68	-0.88	0.57	<. 001	30	55	<. 001	***<
	≤48	250.29	23	<. 001	0.49	0.25	1.95	0.05	-0.00	0.98	<. 001	94	130	<. 001	***<

Description

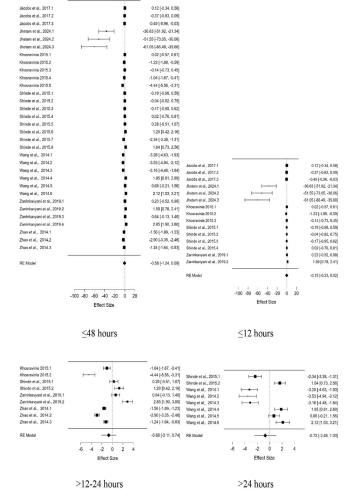
NA = available data does not meet the criteria for Meta-Analysis; the heterophil, lymphocyte, and H/L ratio parameters have no available data at all in the Meta-Analysis, or further statistical test was not applicable to perform since there was no potential publication bias

Delayed access to feeding has a negative effect (p<0.05) in the form of: 1.) increased cholesterol levels with delays of \geq 14 hours, and \geq 48 hours; 2.) increased total protein with delays of \leq 12 hours, \geq 12-24 hours, and \geq 24 hours in chicks aged 1-14 days. The magnitude of the impact of delayed access to feeding on the observed parameters can be seen in the estimation column. In chicks aged 1-14 days, the critical point where the effect begins is a delay of \leq 12 hours.

Appendix 4. Funnel and Forest Plot of Glucose Parameters Age 1-7 Days.



Funnel Plot of the Effect of Delayed Access to Feeding After Hatching on Blood Glucose Changes in Broiler Chickens During the Golden Period



Funnel Plot of the Effect of Delayed Access to Post-Hatch Feeding on Blood Glucose Changes in Broiler Chickens During the Golden Period

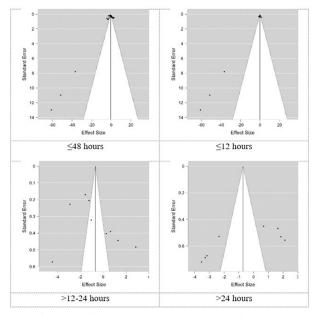
^{*}No indication of publication bias that could intervene in the research results and overall data interpretation.

The calculation of the Rosenthal value was performed using the following formula:

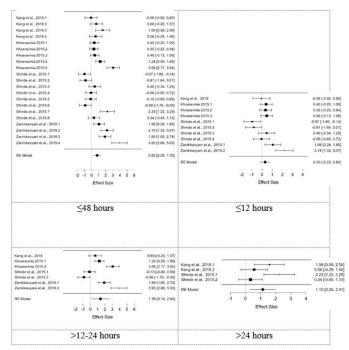
^{**}K = df + 1; Rosenthal value = 5(K) + 10

^{***}Fail-Safe N value < Rosenthal value = publication bias occurs that intervenes in the research results and overall data interpretation.

Appendix 5. Funnel and Forest Plot of Blood Cholesterol Parameters Ages 1-7 Days.

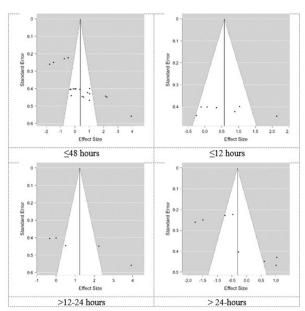


Funnel Plot of the Effect of Delayed Access to Feeding Post-Hatch on Blood Cholesterol Changes in Broiler Chickens during the Golden Period.

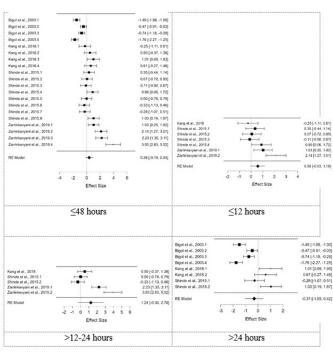


Forest Plot of the Effect of Delayed Access to Feeding After Hatching on Changes in Blood Cholesterol in Broiler Chickens During the Golden Period.

Appendix 6. Funnel and Forest Plot of Total Blood Protein Parameters Ages 1-7 Days

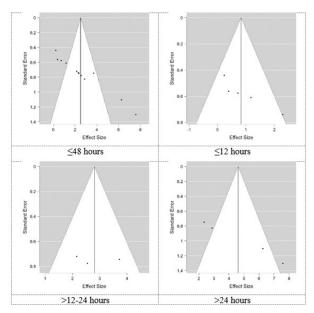


Funnel Plot of the Effect of Delayed Access to Feeding Post-Hatch on Changes in Blood Total Protein in Broiler Chickens During the Golden Period.

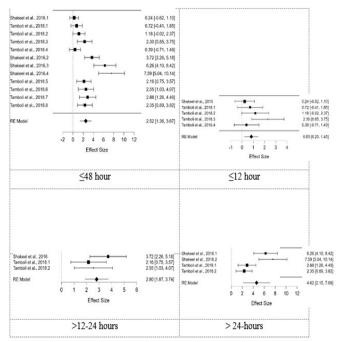


Forest Plot of the Effect of Delayed Access to Post-Hatch Feeding on Changes in Total Blood Protein of Broiler Chickens during the Golden Period.

Appendix 7. Funnel and Forest Plot of Heterophile Parameters for Ages 1-7 Days.

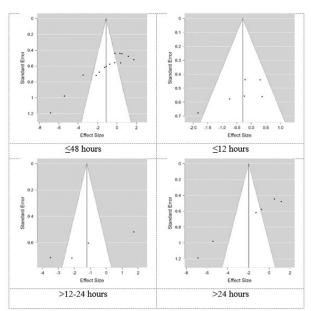


Funnel Plot of the Effect of Delayed Access to Feeding Post-Hatching on Heterophil Changes in Broiler Chickens During the Golden Period.

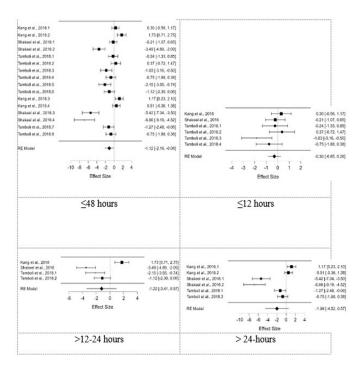


Forest Plot of the Effect of Delayed Access to Post-Hatch Feeding on Heterophil Changes in Golden Period Broiler Chickens.

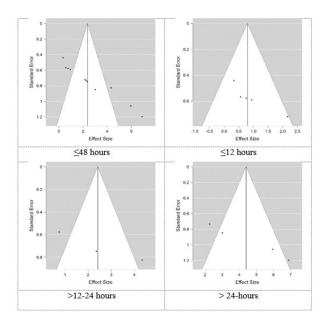
Appendix 8. Funnel and Forest Plot of Lymphocyte Parameters Aged 1-7 Days.

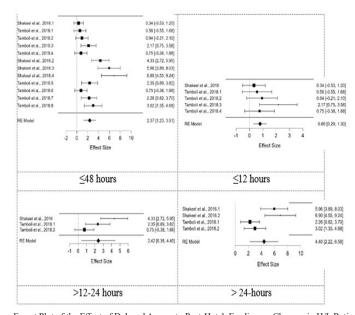


Funnel Plot of the Effect of Delayed Access to Feeding Post-Hatch on Changes in Lymphocytes of Golden Period Broiler Chickens.



Appendix 9. Funnel and Forest Plot of H/L Ratio Parameter Age 1-7 Days.





Funnel Plot of the Effect of Delayed Access to Feeding Post-Hatch on Changes in the H/L Ratio of Broiler Chickens During the Golden Period.

Forest Plot of the Effect of Delayed Access to Post-Hatch Feeding on Changes in H/L Ratio of Broiler Chickens during the Golden Period.