Research Article

A Spirulina-Grass Jelly Gel Mitigates Transport Stress and Improves Physiological Status in Day-Old Broiler Chicks

Makbul Siregar[1], Ma'ruf Tafsin[1*], Sumiati[2]

[1]Master Program of Animal Science, Faculty of Agriculture, University of North Sumatra, Medan-Indonesia; [2]Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Bogor-Indonesia.

Abstract | Delayed access to feed and water during chick handling and transportation may induce dehydration, oxidative stress, and deterioration in chick quality. This study evaluated the efficacy of a Spirulina-enriched grass jelly supplement (Cincau Gel *Spirulina platensis*; CGSP) on the physiological responses of day-old broiler chicks (DOC). A total of 500 DOC (44 ± 2 g) were allocated to a completely randomized design with five treatments: P0 (control), P1 (CGSP + 25 g Spirulina/100 chicks), P2 (CGSP + 50 g Spirulina/100 chicks), P3 (CGSP + 75 g Spirulina/100 chicks), and P4 (Spirulina solution, 2 g/L). After a 15-hour transport period, measurements included live weight loss, body temperature, Pasgar score, hematological indicators, and malondialdehyde (MDA) concentration. The P2 treatment significantly reduced live weight loss (4.04%) and better maintained body temperature (40.01 °C) and Pasgar score (9.85) compared with the control ($P < 0.05$). While P4 exhibited the greatest reduction in MDA concentration, its markedly higher variability indicated inconsistent intake among chicks, likely due to uneven consumption of the liquid form. In contrast, CGSP in gel form provided a more uniform and stable supplementation intake, resulting in more consistent physiological responses across replicates. These findings indicate that CGSP containing 50 g Spirulina (P2) offers a practical and effective strategy to mitigate transport-related stress and maintain DOC quality under commercial conditions. Delayed supplementation hydration and nutrient support was associated with greater physiological decline, underscoring the importance of timely and accessible early-life supplementation.

Keywords | DOC, CGSP, Physiological response, Hematological parameters

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***Correspondence** | Ma'ruf Tafsin, Master Program of Animal Science, Faculty of Agriculture, University of North Sumatra, Medan-Indonesia; **Email:** maruf_tafsin@usu.ac.id
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INTRODUCTION

Delayed access to feed and water after chicks are transferred from the incubator is a common condition in commercial poultry production. This delay typically results from post-hatch handling procedures such as selection, sexing, vaccination, counting, packaging, and transportation from the hatchery to the rearing facility. Transportation represents a critical phase in the early life of broiler chicks, during which they are exposed to multiple stressors including feed and water deprivation, dehydration, thermal fluctuation, and physical vibration. These stressors may impair thermoregulation, increase oxidative stress, disrupt hematological homeostasis, and ultimately reduce early growth performance (Batal and Parsons, 2002; Panda *et al*., 2015).

Supplementation during the pre-placement period is therefore used to minimize the impact of feed and water delay. Early nutrient and hydration support has been shown to improve yolk sac utilization efficiency, maintain physiological stability, and support early immune system development (Noy and Sklan, 2002). Spirulina platensis is a microalga rich in protein, vitamins, minerals, and bioactive pigments such as phycocyanin, which possess strong antioxidant and anti-inflammatory properties. Meanwhile, cincau (grass jelly) contains soluble fiber and phenolic compounds with reported antioxidant and lipid-lowering effects (Dhesti and Widyaningsih, 2014).

The primary challenge in devising early chick supplementation strategies extends beyond the mere selection of an appropriate nutrient source. It critically involves the design of a delivery system that simultaneously addresses the key practical requirements of hydration, palatability, and controlled intake during transport. For instance, while a liquid supplement like a Spirulina solution may offer rapid antioxidant absorption, it presents significant difficulties in stabilization and uniform distribution. Conversely, conventional dry feed is often poorly consumed by chicks in a dehydrated state. Thus, the core dilemma lies in creating a delivery format that bridges this gap, ensuring nutritional benefits are effectively and reliably delivered under practical conditions. Therefore, the primary rationale for formulating Spirulina within a cincau-based gel matrix (Cincau Gel *Spirulina platensis*/CGSP) is to create a semi-moist, stabilizing carrier that provides hydration and while maintaining antioxidant integrity.

Despite the known benefits of Spirulina and cincau individually, the physiological effects of Spirulina delivered through a cincau gel matrix in broiler chicks under transport stress have not been previously evaluated. The present study was therefore designed to determine whether CGSP could maintain physiological stability in day-old broiler chicks experiencing delayed access to feed and water during transportation. Specifically, we hypothesized that CGSP supplementation would (1) reduce body weight loss and dehydration, (2) stabilize body temperature and chick quality scores, and (3) modulate hematological and oxidative stress indicators during transport.

MATERIALS AND METHODS

Material and location

The study was conducted at the Hatchery of PT Berdikari, Lebak, Banten, for chick hatching and collection. Chick transport simulation and physiological measurements were carried out at the Animal Laboratory Management Unit (UPHL), School of Veterinary Medicine and Biomedical Sciences (SKHB), IPB University. Laboratory analyses were performed at the Laboratory of Meat and Work Animal Nutrition (Department of Animal Nutrition and Feed Technology) and the Fish Nutrition Laboratory (Department of Aquaculture), Faculty of Fisheries and Marine Science, IPB University. A total of 500 day-old broiler chicks (44 ± 2 g) were used in the experiment.

Research procedures

Fresh cincau leaves (45 g) were blanched in 750 mL boiling water for 1 minute, then combined with 5 g fresh leaves, squeezed, and filtered to obtain gel extract. *Spirulina platensis* powder was added to the extract according to treatment levels (25 g, 50g and 75 g) and mixed thoroughly. The CGSP mixture was then allowed to set for 2–5 hours and refrigerated before use. To ensure consistent dosage relative to flock size, the CGSP gel was portioned into 80 g per 20 chicks (one square) as seen in Figure 1.

Figure 1: Experimental transport box setup showing a single compartment as one experimental unit. The compartment contains 20 chicks with the CGSP gel treatment portion placed inside for intake during the transport simulation.

For P4, a Spirulina solution (2 g/L) was prepared and dispensed onto an absorbent hydropad placed inside the transport box to allow voluntary intake. 500 DOCs (44 ± 2 g) were evaluated for rectal temperature and Pasgar score before placement in partitioned chick boxes. Chicks were placed into transport boxes according to treatment 20 chicks (one square) and subjected to a 15-hour transport simulation at 25–29°C. No feed or water was provided during this period apart from the assigned supplement. Five chicks/treatment were sampled with 0.5 to 1 mL of blood drawn from the wing vein and euthanized. Euthanasia was performed by cervical dislocation.

Experimental treatment

The experiment used a Completely Randomized Design (CRD) consisting of five treatments, each with five replications, and 20 chicks per replication:

- P0: Control (no supplementation)
- P1: CGSP + 25 g Spirulina/100 chicks
- P2: CGSP + 50 g Spirulina/100 chicks
- P3: CGSP + 75 g Spirulina/100 chicks
- P4: Spirulina solution (2 g/L)

Observation parameters

Measurements were taken immediately after transport, including:

- Physiological indicators: body temperature (cloacal), live weight loss (%), Pasgar score
- Hematological profile: erythrocytes, leukocytes, hematocrit, hemoglobin, H/L ratio (EDTA blood; automated analyzer)
- Oxidative stress marker: malondialdehyde (MDA) concentration (TBARS method)

Experimental design and statistical analysis

Data were analyzed using ANOVA based on the CRD model:

$$Yij = \mu + Ti + \varepsilon ij$$

Where Yij = observation of the i^{th} treatment and j^{th} replication, μ = overall mean, T_i = the effect of the i^{th} treatment, and εij = experimental error (Steel and Torrie, 1995).

Observation parameters included physiological (body temperature, body weight loss, residual yolk, Pasgar score) and hematological indicators (erythrocyte, leukocyte, hematocrit, hemoglobin, H/L ratio) as well as oxidative stress marker (MDA concentration).

RESULT AND DISCUSSION

Based on Table 1, In treatment P4, the Cincau Gel Spirulina (CGSP) supplement was completely consumed by the chicks. Feed consumption in P1 and P2 treatments was almost identical, with P2 showing a slightly higher intake (a difference of 0.2 g), whereas P3 exhibited the highest feed residue (16.2 g). The remaining feed in P3 indicates that the bioactive compounds in CGSP were acceptable to the chicks up to a certain dosage level, but tolerance appeared to decrease at higher concentrations. According to Parakkasi (1999), feed intake is influenced by palatability and physical characteristics of the diet, as well as by management and health factors (Ferket and Gernat, 2006).

In the control group (P0), no active compound intake was observed, while P4 (Spirulina solution 2 g/L) showed a very low intake (0.00376 g/bird) due to the low Spirulina concentration, indicating that this method was less effective in achieving an adequate dosage. This contrasts with the findings of Ibrahim *et al.* (2018), who reported improved performance with Spirulina supplementation in solution form.

Table 1: Average amount of supplement provided and subsumed per Chick fed Cincau Gel-Spirulina plantensis supplement

Treatment	Provided	Consumed	unit of measurement
P0	0.00 ± 0.00^a	0.00 ± 0.00^a	g
P1	80.00 ± 0.00^a	3.73 ± 0.33^c	g
P2	80.00 ± 0.00^a	3.74 ± 0.07^c	g
P3	80.00 ± 0.00^a	3.19 ± 0.84^c	g
P4	40.00 ± 0.00^a	4.00 ± 0.00^b	ml

Note: Values within a row followed by different superscript letters differ significantly ($P < 0.05$); ns = not significant ($P > 0.05$).

Table 2: Average amount of supplement provided and subsumed per Chick fed Cincau Gel - Spirulina plantensis supplement

Treatment	Consumption Percentage (%)
P0	0.00^a
P1	93.25^c
P2	93.50^c
P3	77.98^c
P4	100.00^b

Note: Values within a row followed by different superscript letters differ significantly ($P < 0.05$); ns = not significant ($P > 0.05$).

Based on Table 2, In treatments P1, P2, and P3, active compound intake increased with higher Spirulina doses (25 g, 50 g, and 75 g, respectively), though the relationship was not linear. The increase from P2 to P3 was smaller, likely due to reduced palatability at higher Spirulina concentrations. The consumption percentages of P1 and P2 were high and not significantly different (93.25% vs. 93.5%), indicating that Spirulina up to 50 g was well accepted by chicks. However, consumption in P3 dropped markedly to 77.98%, suggesting that excessive Spirulina levels decreased palatability and feed intake.

Based on Table 3, the administration of Cincau Gel Spirulina (CGSP) had a significant effect ($P < 0.05$) on body weight loss of broiler chicks during transportation. Duncan's test indicated the lowest weight loss in treatment P2 and the highest in the control group (P0). Weight reduction in day-old chicks (DOC) generally occurs due to dehydration and yolk sac utilization as an energy source during fasting (Noy and Sklan, 1999).

Table 3: Average Body Weight (BW) and percentage of live weight loss of broiler chick supplemented with *Cincau-Spirulina plantesis gel (g/bird)* .

Treat-ment	Initial BW (g)	Final BW (g)	Weight Loss(g)	Weight Loss (%)
P0	44.05±0.09^{c}	41.55±0.34^{a}	2.49±0.36^{b}	5.66±0.82^{b}
P1	43.41±0.14^{a}	41.35±0.71^{a}	2.06±0.61^{ab}	4.74±1.42^{ab}
P2	43.59±0.20^{a}	41.83±0.43^{a}	1.76±0.50^{a}	4.04±1.13^{a}
P3	44.02±0.05^{bc}	41.86±0.28^{a}	2.16±0.26^{ab}	4.91±0.60^{ab}
P4	43.82±0.17^{b}	42.01±0.67^{a}	2.24±0.35^{ab}	4.13±1.26^{ab}

Note: Values within a row followed by different superscript letters differ significantly (P < 0.05); ns = not significant (P > 0.05). The experimental procedure and data collection flow are summarized in Figur 2.

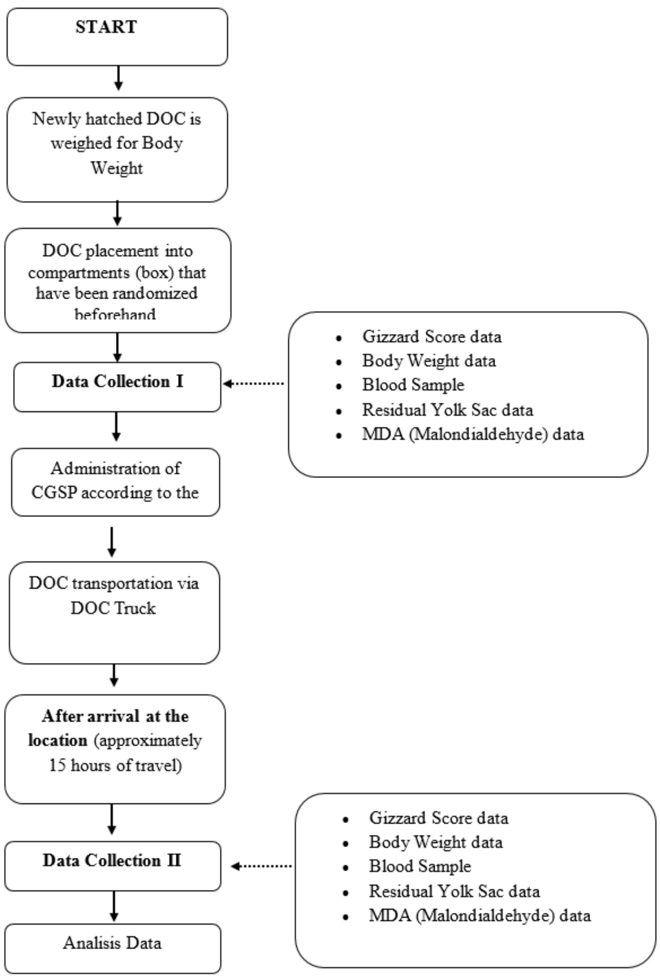

Figure 2: Research flow diagram.

The high weight loss observed in the control group was likely due to energy deficiency, causing muscle catabolism as an alternative energy source, particularly during prolonged transportation under suboptimal environmental conditions (Mitchell and Kettlewell, 1998). The highest feed residue observed in P3 (16.2 g) corresponded with relatively greater body weight loss (2.16 g), possibly due to reduced feed palatability. Conversely, P2 exhibited the lowest weight loss (1.76 g), indicating a beneficial effect of CGSP supplementation in maintaining body condition.

Table 4: Effect of Cincau–Spirulina platensis Gel Supplementation on Broiler Chick Quality (Pasgar Score).

Treatment	Before	After	Difference
P0	10.00±0.00	8.95±2.19a	1.05±2.19^{b}
P1	10.00±0.00	9.80±0.41b	0.20±0.41^{a}
P2	10.00±0.00	9.85±0.37b	0.15±0.37^{a}
P3	10.00±0.00	9.75±0.44b	0.25±0.44^{a}
P4	10.00±0.00	9.80±0.41b	0.20±0.41^{a}

Note: Values within a row followed by different superscript letters differ significantly (P < 0.05); ns = not significant (P > 0.05).

Table 5: Effect of Cincau–Spirulina Platensis (CGSP) Supplement Gel Administration on Cloacal Temperature (°C)

Treatment	Before	After
P0	40.25±0.53	41.08±0.18^{b}
P1	39.96±0.60	40.04±0.39^{a}
P2	39.95±0.12	40.01±0.29^{a}
P3	39.84±0.40	40.11±0.23^{a}
P4	39.94±0.12	40.03±0.08^{a}

Note: Values within a row followed by different superscript letters differ significantly (P < 0.05); ns = not significant (P > 0.05).

The percentage of body weight loss during 15 hours of transportation was highest in the control (5.66%) and lowest in P2 (4.04%). These findings are consistent with Putra *et al.* (2023), who reported that gel-based hydration supplements, such as Nutrichick Gel, effectively reduced weight loss during transportation in broiler chicks.

The effect of CGSP supplementation on chick quality based on Pasgar score is presented in Table 4. Evaluation of chick quality using the Pasgar Score (maximum score of 10) showed that all groups initially had uniform scores of 10. After treatment, the control group (P0) exhibited a significant decrease to 8.95 with high variability, whereas treatments P1–P4 remained stable around 9.75–9.80. The decline in the control group was attributed to stress, environmental factors, and limited access to feed and water during the pull-chick and transportation process, leading to severe dehydration due to plasma volume reduction and fluid loss (Randolph *et al.*, 2010). These conditions negatively affected the physical development, behavior, and cardiac function of the chicks.

Based on Table 5, The initial cloacal temperatures across all treatments (P0–P4) were homogeneous, ranging from 39.84 to 40.25 °C. After the transportation period, the control group (P0) exhibited a significant increase in cloacal temperature to 41.08±0.18 °C. In contrast, chicks receiving gel-based Cincau supplementation (P1–P4) maintained significantly lower cloacal temperatures (40.01–40.11°C).

The marked rise in temperature observed in P0 aligns with previous findings indicating that transport stress elevates metabolic heat production and disrupts thermoregulatory mechanisms, particularly when ambient temperature exceeds the birds' thermoneutral zone (Van den Brand *et al*., 2010; Oloyo and Ojerinde, 2019). The mitigating effect of Cincau gel supplementation is presumably associated with its pharmacological properties; extracts from Cincau leaves have been reported to exhibit antipyretic effects capable of reducing body temperature in animal models (Umamah, 2010). Therefore, supplementation with Cincau gel may support the maintenance of thermal homeostasis in broiler chicks following transportation.

Based on Table 6, the average residual yolk weight ranged from 3.74 g to 4.78 g, aligning with Lourens *et al*. (2021), who reported residual yolk values of 2–3 g per chick after optimal incubation, though this may vary (1–10 g) depending on egg size, shell quality, and incubation conditions. Residual yolk should not exceed 10% of chick body weight at hatch.

Table 6: Avg weight of residual yolk.

Treatment	Before	After	Depreciation (%)
P0	4.78±1.20	1.20±0.45	72.24±14.65
P1	4.78±1.20	0.66±0.11	85.31±4.99
P2	4.10±0.90	0.68±0.08	82.40±5.85
P3	4.72±1.04	0.78±0.52	84.06±7.25
P4	3.74±0.78	1.10±0.51	71.64±7.50

Note: Results of Laboratory Analysis, Fish Nutrition Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University (2024). Values within a row followed by different superscript letters differ significantly ($P < 0.05$); ns = not significant ($P > 0.05$).

Early chick growth before exogenous feeding relies on nutrients absorbed from the yolk sac, which serves as the primary nutrient reserve and source of maternal immunoglobulins. Early feeding accelerates yolk utilization and gastrointestinal development, promoting higher early body weight (Noy and Sklan, 1998a; Sklan and Noy, 2000). In contrast, feed deprivation can delay yolk absorption and impair early performance due to limited gut stimulation (Noy and Sklan, 1998a, 2001). However, in the present study, fasting up to 36 hours post-hatch did not affect yolk sac retraction, indicating adequate nutrient utilization under controlled incubation conditions. Maintaining optimal temperature remains crucial for efficient yolk absorption and early growth (Lourens *et al*., 2021).

Table 7: Antioxidant Levels Based on C-Phycocyanin Concentration (CPC) (%), Inhibition Percentage (%), and Active Compound Intake (mg /chick).

Treatment	CPC	Inhibition	Active Compound Intake (mg/chick)
P0	0.00	0.00	0.00±0.00^{a}
P1	0.47	0.22	417.90±36.46^{c}
P2	0.56	0.87	411.68±7.60^{c}
P3	0.56	1.02	459.36±120.82^{c}
P4	0.72	3.20	188.00±0.00^{b}

Note: Results of Laboratory Analysis, Fish Nutrition Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University (2024). Values within a row followed by different superscript letters differ significantly ($P < 0.05$); ns = not significant ($P > 0.05$).

Based on Table 6, the highest antioxidant activity (DPPH inhibition assay) was observed in treatment P4 (3.2%), followed by P3 (1.05%), P2 (0.87%), and P1 (0.22%). Although Cincau alone exhibits weak antioxidant capacity compared to Spirulina (Khoiriyah and Amalia, 2014), treatment P3 demonstrated a relatively high antioxidant activity.

Table 8: Red blood cell profile, white blood cell differential, and heterophil-to-lymphocyte (H:L) ratio of broiler chicks supplemented with Cincau–Spirulina platensis gel.

Parameters	P0	P1	P2	P3	P4
Eritrosit (Butir/mm^3)	3.36±0.54^{b}	3.05±0.84^{ab}	2.91±0.41^{ab}	2.91±0.36^{ab}	1.78±0.40^{a}
Leukosit (Butir/mm^3)	21.25±4.35^{ab}	15.55±2.39^{a}	19.88±6.88^{a}	22.05±4.11^{ab}	13.68±2.23^{a}
Hemoglobin (g%)	11.72±1.83^{a}	13.64±4.46^{a}	11.36±1.13^{a}	10.92±1.63^{a}	8.72±2.47^{a}
Hematokrit (%)	37.00±11.38^{a}	30.20±3.38^{a}	31.60±11.50^{a}	37.20±4.87^{a}	22.40±13.01^{a}
Limfosit (%)	49.14±2.07^{a}	48.88±1.79^{a}	50.52±1.09^{a}	50.21±1.60^{a}	49.30±2.35^{a}
Heterofil (%)	37.74±1.51^{a}	37.05±2.51^{a}	35.55±1.09^{a}	37.23±1.64^{a}	37.48±2.50^{a}
Heterofil /Limfosit (H/L) (%)	0.77±0.05^{a}	0.76±0.08^{a}	0.70±0.03^{a}	0.74±0.05^{a}	0.77±0.09^{a}
Eosinofil (%)	7.78±1.21^{a}	8.19±0.64^{a}	8.48±0.77^{a}	7.50±0.90^{a}	8.03±1.09^{a}
Monocyte (%)^{s}	4.57±0.87^{a}	5.10±0.59^{a}	4.69±0.65^{a}	4.30±0.92^{a}	4.40±0.83^{a}
Basophils (%)	0.76±0.02^{a}	0.77±0.03^{a}	0.76±0.02^{a}	0.77±0.02^{a}	0.77±0.03^{a}

Note: Results of the Laboratory of Meat and Work Animal Nutrition, Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, IPB University (2024). Values within the same row followed by different superscript letters indicate significant differences ($P < 0.05$); ns = not significantly different ($P > 0.05$).

Table 9: Mean Malondialdehyde (MDA) Content Across Treatments (mikromol/g)

Treatment	Before	After
P0	3.79+3.28	7.87+2.37 [b]
P1	3.79+ 3.28	4.76+1.55[b]
P2	3.79+ 3.28	4.93+1.57[a]
P3	3.79+ 3.28	7.26+1.20[b]
P4	3.79+ 3.28	2.72+3.51[a]

Note: Results of Laboratory Analysis, Fish Nutrition Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University (2024). Values within a row followed by different superscript letters differ significantly ($P < 0.05$); ns = not significant ($P > 0.05$).

Based on Table 7, The percentage of C-phycocyanin (C-PC) was positively correlated with DPPH inhibition, indicating that higher C-PC content was associated with greater antioxidant activity (Latief *et al*., 2013). However, P3 exhibited slightly lower C-PC levels than P2 but showed higher inhibition, suggesting that additional factors such as compound purity and analytical methods may have influenced the results.

Interestingly, active compound consumption showed an inverse relationship with antioxidant efficiency. Treatment P4, which had the lowest active compound intake (188 mg), exhibited the highest antioxidant activity (3.2%). This finding aligns with previous reports that phycocyanin bioavailability is higher in liquid form (85–90%) compared to gel form (40–50%) (Karkos *et al*., 2011; Proietti *et al*., 2014).

Hematologi profile

Based on Table 8, The administration of Cincau leaf and *Spirulina platensis* in gel form showed no significant effect (P>0.05) on erythrocyte count, which remained within the normal range of 2.0–3.9 $\times 10^6/mm^3$ (Smith and Mangkoewidjojo, 1987; Samour, 2015). The observed erythrocyte stability indicates that CGSP supplementation is physiologically safe. Differences among treatments may reflect residual energy reserves supporting erythropoiesis, as erythrocyte synthesis depends on amino acids, vitamins, iron, and erythropoietin (Hoffbrand and Petit, 1996). Erythrocyte count correlates with oxygen transport capacity and ATP synthesis essential for cellular metabolism (Frandson, 1986).

Leukocyte counts ranged from 13.68–27.05 $\times 10^3/mm^3$, remaining within the physiological range (Swenson, 1984; Feldman *et al*., 1995). Treatment P4 exhibited the highest leukocyte level, likely due to the immunostimulatory components of Spirulina, such as phycocyanin and bioactive proteins. Conversely, P1–P3 showed lower or stable values, suggesting a moderating effect of Cincau on immune activation. Transient leukocyte reductions in P1–P2 may relate to mild stress or adaptation to the new supplement (Damayanti *et al*., 2022).

Hemoglobin concentrations (10.92–13.94 g/dL) were unaffected (P>0.05) and within normal limits (7–13 g/dL; Wahyudi, 2021; Darmawan, 2022). Decreases in hemoglobin are often associated with transport stress and reduced nutrient intake (Parwati *et al*., 2018).

Leukocyte differentials revealed no significant treatment effects (P>0.05). Lymphocyte percentages (48.88–50.52%) were within the normal range (Scanes, 2015) and may be supported by flavonoid-mediated immune enhancement (Islami *et al*., 2022). Neutrophil counts (35.55–37.74%) were also normal (Hendro *et al*., 2013), indicating an absence of infection. Eosinophil values (7.28–8.48%) showed mild elevations in P1–P2, potentially reflecting minor immune or allergic responses (Suriansyah *et al*., 2016).

Monocyte levels remained normal (4.3–5.1%) with no significant variation, consistent with their role in phagocytosis and immune regulation (Frandson, 1992; Lengkong *et al*., 2015). Basophil counts (0.00–0.77%) were within physiological limits (Hodges, 1977; Jain, 1993), functioning as histamine mediators during tissue repair (Ganong, 1996).

The heterophil-to-lymphocyte (H:L) ratio ranged from 0.70 to 0.77 across treatments, indicating no significant stress response. Ratios below 1 suggest that all chicks maintained stable physiological conditions and adapted well post-transportation (Aengwanich, 2007; Ulupi *et al*., 2018).

The results showed that the control group (P0) exhibited the highest MDA concentration (7.87 nmol/mL), indicating a natural level of oxidative stress without antioxidant intervention. Treatment P3 (7.26 nmol/mL) did not differ significantly from the control, suggesting limited efficacy in suppressing lipid peroxidation. Treatments P1 (4.76 nmol/mL) and P2 (4.93 nmol/mL) demonstrated comparably lower MDA values; statistically, P2 belonged to group "a" (significantly lower than P0/P3), while P1 remained in group b. This variation may be attributed to differences in dose response or individual physiological variability.

Treatment P4 recorded the lowest mean MDA level (2.72±3.51 nmol/mL), though its high standard deviation suggests heterogeneous responses among chicks, possibly due to inconsistencies in supplement intake or environmental factors. Despite this variability, the overall reduction in MDA in P4 supports the potential antioxidant activity of Spirulina extract. According to Kalantar *et al*.

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(2019), antioxidants serve as a primary defense against free radicals, yet newly hatched chicks typically possess limited endogenous antioxidant reserves. This aligns with laboratory findings (Appendix), which revealed an antioxidant activity of 3.2% in Spirulina solution.

In the P4 group, increased feed intake likely enhanced the absorption of active antioxidant compounds, contributing to reduced lipid peroxidation. However, the absence of standardized dosing across individuals may have led to uneven antioxidant bioavailability, where some chicks received optimal effective doses while others did not. Furthermore, intrinsic variability in genetic background, metabolic capacity, and health status may have influenced nutrient assimilation and oxidative responses, collectively explaining the low mean but high variation in MDA values observed.

CONCLUSIONS

Based on the results of this study, The administration of Spirulina-Enriched Grass Jelly (CGSP) significantly improved the physiological responses and hematological status of broiler chicks (DOC). The P2 treatment-grass jelly supplemented with 50 g of *Spirulina platensis* had a positive effect on the physiological responses of broiler chicks by preventing body weight loss, maintaining pasgar scores, reducing the heterophil-to-lymphocyte (H/L) ratio, and minimizing malondialdehyde (MDA) levels.

The spirulina solution (P4) was more effective in reducing MDA levels (2.72 mikromol/g). However, the formulation of grass jelly supplemented with 50 g of *Spirulina platensis* (P2) demonstrated superior efficacy in preventing body weight loss and maintaining the quality of broiler chicks, making it more suitable for routine application.

RECOMMENDATIONS

Based on the findings of this study, it is recommended to conduct further investigations on the performance of broiler chicks supplemented with Spirulina-Enriched Grass Jelly (CGSP), including the measurement of corticosterone hormone levels as an additional indicator of stress.

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NOVELTY STATEMENT

This study represents the first research in Indonesia to utilize Cincau (Grass Jelly) leaves in combination with Spirulina platensis as a functional supplement for improving the quality of day-old broiler chicks (DOC).

AUTHOR'S CONTRIBUTIONS

All authors did the manuscript together and the contribution is distributed equally.

Ethical consideration

All experimental procedures were approved by the Ethics Committee for Animal Care and Use, National Research and Innovation Agency (BRIN), Indonesia (Approval No. 201/KE.02/SK/08/2024).

Generative AI and AI-assisted technology statement

In preparing this manuscript, the authors used ChatGPT (OpenAI) to assist in language editing and improving the readability of the manuscript. Following the use of this tool, the authors reviewed and edited the content as necessary and take full responsibility for the accuracy and integrity of the published article.

Conflict of interest

The authors have declared no conflict of interest.

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