



Effect of Synthetic STAT5a Protein on Erythrocyte Profiles and Total Blood Protein in Broilers

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Abstract | Signal Transducer and Activator of Transcription 5a (STAT5a) is a key transcription factor regulating erythropoiesis through erythropoietin-mediated signaling pathways. While its biological role has been extensively characterized in mammals, there is limited information regarding its function in avian species, particularly in broiler chickens. Synthetic STAT5a protein has recently gained attention as a potential biological enhancer to support hematological and metabolic functions in poultry, yet its direct impact on erythropoietic parameters remains unclear. This study aimed to evaluate the effect of synthetic STAT5a administration on erythrocyte profiles and total blood protein levels in broiler chickens. A completely randomized posttest-only control group design was used. Twenty-five male Cobb broilers were divided into five treatment groups (P0–P4), with five birds per group. Treatments included aquadest (control), 0.1% formic acid, and synthetic STAT5a at concentrations of 3.5%, 7%, and 14%, administered orally from day 14 to 25. Blood samples were collected on day 30 for erythrocyte count, hemoglobin concentration, hematocrit value, and total protein analysis. Data were analyzed using one-way Analysis of Variance (ANOVA), followed by Duncan's and Games-Howell post-hoc tests. Broilers receiving synthetic STAT5a showed higher erythrocyte count, hemoglobin concentration, hematocrit value, and total blood protein levels compared to controls ($p < 0.05$). The 3.5% STAT5a group demonstrated the most significant increase in erythropoietic parameters, while higher doses (7% and 14%) resulted in moderate improvements, these were not significantly different from the effect observed with the 3.5% dose. STAT5a supplementation may serve as a promising biological strategy to support physiological performance in poultry.

Keywords | Broiler, Health, STAT5a, Synthetic protein, Hematology, Total protein

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Signal Transducer and Activator of Transcription 5a (STAT5a) is a transcription factor that plays a pivotal role in the regulation of erythropoiesis, the process responsible for the formation and maturation of red blood cells (erythrocytes) (Tóthová *et al.*, 2021). The activation of STAT5a occurs primarily through cytokine-mediated signaling, particularly via erythropoietin (Epo) (Grebien *et al.*, 2008). When Epo binds to its receptor (EpoR) on erythroid progenitor cells, it initiates a cascade involving Janus kinase 2 (Jak2) (Bhoopalan *et al.*, 2020). Activated Jak2 subsequently phosphorylates STAT5a, leading to its dimerization and translocation into the nucleus (Mortlock *et al.*, 2020). Once inside the nucleus, STAT5a binds to specific DNA sequences and modulates the transcription of genes essential for erythrocyte differentiation, proliferation, and survival (Tóthová *et al.*, 2021).

In mammals, extensive studies have demonstrated that STAT5a plays an indispensable role in maintaining normal and stress-induced erythropoiesis (Kerenyi *et al.*, 2008). The absence or inactivation of STAT5a results in impaired red blood cell production, decreased survival of erythroblasts, and enhanced apoptosis of erythroid progenitor cells (Socolovsky *et al.*, 2001). Moreover, STAT5a activation can partially compensate for deficiencies in critical components of the erythropoietic pathway, such as Jak2 or EpoR, thereby ensuring the continuation of erythropoiesis under suboptimal conditions (Grebien *et al.*, 2008). These findings underscore the importance of STAT5a as a central mediator in hematopoietic signaling networks (Maninang *et al.*, 2025).

In avian species, including broiler chickens, erythropoiesis follows a comparable regulatory mechanism; however, the specific function of STAT5a in avian hematopoiesis has not been comprehensively elucidated (Maurer *et al.*, 2019; Wardiana *et al.*, 2021). Hematological indicators such as erythrocyte count, hemoglobin concentration, hematocrit value, and total blood protein serve as essential parameters for assessing physiological status, oxygen-carrying capacity, and overall metabolic performance in poultry (Liu *et al.*, 2025; Agustono *et al.*, 2025). Factors influencing erythropoiesis, including nutrition, genetic regulation, and hormonal balance, play a critical role in determining the growth rate and productivity of broilers (Alagawany *et al.*, 2020; Anggriawan *et al.*, 2024). Recent studies have explored the use of dietary supplements such as yeast-derived bioactive compounds and enzyme additives to modulate hematological profiles, enhance immune function, and improve growth performance (Lokapirnasari *et al.*, 2025; Sultana *et al.*, 2024).

Given the established role of STAT5a in erythroid development in mammals, investigating its potential role and application in broilers represents an important step toward optimizing poultry health and performance (Uddin *et al.*, 2003). However, STAT5a is an intracellular transcription factor, and its classical biological activity requires phosphorylation, cytoplasmic signaling integration, and nuclear translocation. An orally administered STAT5a molecule therefore faces substantial pharmacological barriers, including enzymatic degradation in the gastrointestinal tract, limited absorption of intact proteins across the intestinal epithelium, and an inability to spontaneously cross plasma or nuclear membranes (Tóthová *et al.*, 2021). Consequently, it is highly unlikely that orally delivered STAT5a could function as a direct transcriptional activator within erythroid progenitor cells. For this reason, the present study does not assume a canonical intracellular mechanism of action.

Instead, any physiological effects observed following oral administration of synthetic STAT5a are more plausibly attributable to indirect mechanisms, such as the absorption of short bioactive peptide fragments, modulation of gut-associated receptors, interaction with immunometabolic pathways, or systemic metabolic signaling. These considerations frame dietary STAT5a not as a direct transcription factor therapy but as an exploratory bioactive protein supplement whose mechanisms remain to be fully elucidated (Schmerer *et al.*, 2006).

Although the role of Signal Transducer and Activator of Transcription (STAT) proteins during broiler growth remains poorly defined, interest in exploring recombinant STAT proteins as bioactive supplements has increased in recent years. The previously cited study by Anh *et al.* (2015) focused on STAT5a gene polymorphisms rather than the production of a synthetic protein, and therefore does not represent an appropriate reference for the recombinant STAT5a used in the present study.

To date, no published studies have evaluated the physiological effects of orally administered recombinant STAT5a in poultry. Therefore, this study was designed to investigate whether dietary supplementation with synthetic STAT5a protein could influence hematological parameters, particularly erythrocyte characteristics and total blood protein levels, in broiler chickens.

MATERIALS AND METHODS

RESEARCH DESIGN

All experimental procedures were conducted under ethical approval No. 1. KEH.113.07.2023, issued by the Animal Ethics Committee of the Faculty of Veterinary

Medicine, Airlangga University. This study was conducted from August to September 2023. The synthesis of the STAT5a protein was performed at the Molecular Biology Laboratory, Faculty of Veterinary Medicine, Airlangga University. Animal maintenance was carried out at the Poultry Experimental Facility, Campus B, Airlangga University. Blood sample examination was conducted at the Physiology Laboratory, Faculty of Medicine, Brawijaya University, Malang.

This study used an experimental, completely randomized design (CRD) with a post-test-only control group structure. Five treatment groups (P0–P4) were established, each consisting of broiler chickens as test animals. The experimental conditions, including age, sex, and environment, were maintained homogeneously, and all treatments were assigned randomly. The treatment period was conducted from day 14 to day 25 of rearing.

PRODUCTION AND CHARACTERIZATION OF RECOMBINANT STAT5A PROTEIN

The “synthetic STAT5a protein” used in this study refers to a full-length recombinant STAT5a protein derived from broiler chickens. The coding sequence of chicken STAT5a (GenBank Accession No.: NM_205134) was synthesized and cloned into the pET-28a(+) expression vector containing an N-terminal 6×His tag. The construct was transformed into *Escherichia coli* BL21 (DE3) cells for recombinant protein production.

Protein expression was induced using 0.5 mM IPTG at 20 °C for 16 hours. Bacterial pellets were lysed via sonication in buffer (50 mM Tris-HCl, 300 mM NaCl, 10 mM imidazole, pH 8.0). The recombinant STAT5a was purified using Ni-NTA affinity chromatography, and eluted fractions were concentrated and buffer-exchanged into PBS.

Purity was assessed using SDS-PAGE and exceeded 90%, showing a dominant band at approximately 90 kDa, consistent with the predicted molecular weight of full-length chicken STAT5a. Protein identity was verified by Western blotting using anti-STAT5 antibodies.

EXPERIMENTAL ANIMALS

The experimental animals used were day-old male Cobb broiler chicks obtained from PT Panca Patriot Prima, Malang. All chicks were in healthy condition, had been vaccinated against Newcastle Disease (ND), Infectious Bronchitis (IB), and Infectious Bursal Disease (IBD), and had not received any prior treatments. The number of animals per group (n) was determined using Federer’s formula, with five animals assigned per group, resulting in a total of 25 broilers used in this study.

SAMPLE SIZE AND REPLICATION

Blood samples were collected from 30-days old broilers representing each treatment group, resulting in a total of 25 samples. Each treatment was replicated five times to ensure statistical validity.

ANIMAL PREPARATION

Broilers were raised from one day of age until 30 days of age. Initially, 25 chicks were reared in a disinfected floor pen (1 m × 1 m × 50 cm) using 10% benzalkonium chloride and illuminated with a 40 W lamp. On day 10, the chickens were randomly divided into five groups and transferred into individual battery cages (40 cm × 40 cm × 50 cm) with 15 W lighting. The floor was lined with newspaper bedding. Standard commercial feed was provided BR1 during the starter phase (1–21 days) and BR2 during the finisher phase (22–35 days) twice daily at 08:00 and 16:00. Clean drinking water supplemented with Vitachick was provided ad libitum.

PREPARATION OF STAT5A WORKING SOLUTIONS AND DOSE CALCULATION

After a 14-day adaptation period, broilers were randomly assigned to five groups (P0, P1, P2, P3, and P4). The STAT5a working solutions used in this study were prepared by diluting a 10 mg/mL recombinant STAT5a stock solution in sterile PBS. The treatment concentrations (3.5%, 7%, and 14%) represent weight/volume (w/v) formulations, equivalent to 35 mg/mL, 70 mg/mL, and 140 mg/mL, respectively (Table 1). Each bird received 0.5 mL of the assigned solution per day, resulting in STAT5a doses of 17.5 mg (P2), 35 mg (P3), and 70 mg (P4) per bird per day from day 14 to day 25. Birds were then rested and harvested on day 30. Based on the average broiler body weight during the treatment period (0.9–1.1 kg), the approximate daily STAT5a exposure was 8,000–9,700 µg/kg (P2), 16,000–19,400 µg/kg (P3), and 32,000–38,800 µg/kg/day (P4). These values have been included to improve dose transparency and experimental reproducibility.

VEHICLE PREPARATION AND pH ADJUSTMENT

The 0.1% formic acid solution used in the P1 group served as the vehicle control. Because raw formic acid is corrosive, the solution was neutralized to physiological pH (6.8–7.0) using sterile 1 N NaOH under continuous stirring. The final pH-adjusted formulation was confirmed using a calibrated pH meter (Hanna Instruments, USA). This neutralized vehicle matched the buffering conditions of the recombinant STAT5a solutions, which were also prepared in PBS at physiological pH. During the experimental period, birds in the vehicle group (P1) showed no observable signs of oral irritation, stress, or reduced feed intake, indicating that the adjusted solution did not impose adverse physiological effects.

Table 1: Actual STAT5a dosage administered to broiler chickens.

Group	STAT5a concentration (w/v)	Volume given (mL/day)	STAT5a dose (mg/day/bird)	Approx. dose (µg/kg/day)*
P0	0% (control)	0.5	0	0
P1	0.1% formic acid	0.5	0	0
P2	3.5% STAT5a	0.5	17.5 mg	8,000–9,700 µg/kg
P3	7% STAT5a	0.5	35 mg	16,000–19,400 µg/kg
P4	14% STAT5a	0.5	70 mg	32,000–38,800 µg/kg

Note: Calculated based on average broiler weight of 0.9–1.1 kg.

BLOOD SAMPLING

On day 30, blood samples (3 mL) were collected from the brachial vein of each bird after 12 hours of fasting. The puncture site was sterilized with 70% alcohol before sampling. Blood was transferred into 3 mL Ethylenediaminetetraacetic Acid (EDTA) vacutainer tubes and stored in a cool box before analysis. Total protein (TP) levels were determined using the Biuret method with a Pentra C200 chemical analyzer.

DATA ANALYSIS

Data on hematological parameters, including erythrocyte, hemoglobin, hematocrit, and blood protein levels, were analyzed using one-way Analysis of Variance (ANOVA), followed by Duncan's and Games-Howell post-hoc tests. Significant differences among treatment groups were further tested using Duncan's and Games-Howell post-hoc tests. Statistical analysis was performed using SPSS version 20 (IBM Corp., USA).

RESULTS AND DISCUSSION

The effects of synthetic STAT5a protein on erythrocyte count, hemoglobin concentration, and hematocrit value are presented in Table 2. Statistical analysis revealed that erythrocyte and hemoglobin levels in group P0 (control, aquadest) were not significantly different from groups P1, P3, and P4, but were significantly lower than those in group P2 ($p < 0.05$). Group P2, which received 3.5% STAT5a synthetic protein, exhibited the highest mean values for both erythrocyte and hemoglobin concentrations. Similarly, hematocrit values indicated that group P0 did not differ significantly from P1 and P3, but differed significantly from P2 and P4. No significant difference was observed between P3 and P4.

Descriptively, group P0 (control) had the lowest mean erythrocyte, hemoglobin, and hematocrit values compared with all treatment groups, while group P2 consistently showed the highest averages. Groups P3 and P4 presented intermediate values, higher than the controls but lower than P2. Quantitatively, erythrocyte counts in P0 ($2.83 \times 10^6/\text{mm}^3$) and P1 ($2.85 \times 10^6/\text{mm}^3$) were comparable, whereas P2 demonstrated a significant increase compared to all other treatments ($p < 0.05$). Groups P3 and P4 showed no

significant difference from each other ($p > 0.05$).

Table 2: Average erythrocyte levels ($10^6/\text{mm}^3$), hemoglobin levels (g/dL), and hematocrit values (%) of broiler chickens given STAT 5a synthetic protein.

Group (Treatment)	Erythrocyte level ($10^6/\text{mm}^3$) (Mean $\pm$ SD)	Hemoglobin Level (g/dL) (Mean $\pm$ SD)	Hematocrit value (%) (Mean $\pm$ SD)
P0	$2.83a \pm 0.18$	$7.92a \pm 0.42$	$25.49a \pm 1.79$
P1	$2.85a \pm 0.12$	$8.07a \pm 0.43$	$25.92a \pm 1.61$
P2	$3.49b \pm 0.12$	$12.07b \pm 1.68$	$38.27c \pm 1.75$
P3	$2.93a \pm 0.12$	$8.36a \pm 0.47$	$27.08ab \pm 1.22$
P4	$3.04a \pm 0.23$	$8.99a \pm 0.51$	$28.92b \pm 2.92$

Note: Different superscripts in the same column indicate significant differences ($p < 0.05$). P0: Group given distilled water, P1: Group given 0.1% formic acid, P2: Group given 3.5% STAT5a synthetic protein, P3: Group given 7% STAT5a synthetic protein, P4: Group given 14% STAT5a synthetic protein.

Table 3: Average total blood protein levels (mg/mL) in broiler chickens.

Group (Treatment)	Total protein content (mg/mL) (Mean $\pm$ SD)
P0	$1.03020a \pm 0.043792$
P1	$1.60300a \pm 0.472907$
P2	$2.85060b \pm 0.262795$
P3	$3.27480b \pm 0.192384$
P4	$5.28400b \pm 1.401388$

Note: Different superscripts in the same column indicate significant differences ($p < 0.05$). P0: Group given distilled water, P1: Group given 0.1% formic acid, P2: Group given 3.5% STAT5a synthetic protein, P3: Group given 7% STAT5a synthetic protein, P4: Group given 14% STAT5a synthetic protein.

A similar pattern was observed in hemoglobin and hematocrit levels, where group P2 showed the highest mean values among all treatments, followed by P3 and P4, while P0 and P1 had the lowest. The elevation in hematological parameters in STAT5a-treated groups indicates a stimulatory effect of synthetic STAT5a protein on erythropoiesis and oxygen transport capacity in broiler chickens.

Post-hoc analysis (Games-Howell test) of the data in Table 2 further confirmed that groups P2, P3, and P4 differed significantly from the control groups (P0 and P1), while no significant differences were detected among P2, P3, and P4. These findings suggest that administration of synthetic STAT5a protein had a significant effect on erythrocyte count, hemoglobin concentration, hematocrit value, and total blood protein levels.

The effect of synthetic STAT5a protein supplementation on total blood protein levels in broiler chickens is presented in Table 3. Regarding total blood protein, the highest mean concentration was observed in group P4 (5.28 ± 1.40 mg/mL), followed by P3 (3.27 ± 0.19 mg/mL) and P2 (2.85 ± 0.26 mg/mL). The control groups P0 (1.03 ± 0.04 mg/mL) and P1 (1.60 ± 0.47 mg/mL) exhibited the lowest total protein concentrations. Only the 3.5% STAT5a dose (P2) showed a significant increase in erythrocyte and hemoglobin levels compared with the control groups (P0 and P1), while P3 and P4 were not significantly different.

The administration of synthetic STAT5a protein significantly enhanced erythrocyte count, hemoglobin concentration, and hematocrit value in broiler chickens, indicating its stimulatory effect on erythropoietic activity (Damayanti *et al.*, 2025; Faiqoh *et al.*, 2023). The most notable improvement was observed in group P2, which received 3.5% STAT5a, suggesting that this concentration represents the optimal dose for promoting hematological performance without disrupting physiological balance. This enhancement may be attributed to the activation of transcriptional pathways regulating erythroid proliferation, differentiation, and survival, similar to mechanisms described in mammalian systems where STAT5a acts as a downstream effector of erythropoietin (EPO) signaling (Berlina *et al.*, 2023; Smith *et al.*, 2023).

Although the present findings suggest that STAT5a may enhance erythropoietic activity and protein metabolism, it is important to note that the proposed mechanism remains inferential. This study did not include molecular analyses of liver or bone marrow tissues to confirm activation of STAT5a-dependent transcriptional targets such as B-cell lymphoma-extra large (Bcl-xL), Cyclin D, or Proviral Integration site for Moloney murine leukemia virus 1 (Pim-1) (Tsuruyama *et al.*, 2011). Therefore, the mechanistic explanation is based on established functions of STAT5a in vertebrate erythropoiesis rather than on direct evidence in broiler chickens (Damerou *et al.*, 2020). Future studies employing quantitative polymerase chain reaction (qPCR), Western blotting, or immunohistochemistry are required to determine whether orally administered synthetic STAT5a indeed activates downstream signaling pathways in hematopoietic tissues (Rani and Murphy, 2016).

Accordingly, the mechanistic interpretation provided here should be viewed as a biologically plausible hypothesis rather than a confirmed pathway.

Erythropoiesis in vertebrates is primarily controlled by EPO, a glycoprotein hormone synthesized mainly by the kidneys (approximately 90%) and to a lesser extent by the liver (10%). It stimulates erythroid progenitor cells in the bone marrow, facilitating the maturation of reticulocytes into functional erythrocytes within approximately seven to nine days (Yin and Noguchi, 2025). Mature erythrocytes are essential for oxygen and nutrient transport to body tissues, maintaining homeostasis and supporting metabolic activity (Kumar *et al.*, 2025; La'lang *et al.*, 2021). In the present study, the observed elevation in erythrocyte and hemoglobin levels following STAT5a administration indicates enhanced oxygen-carrying capacity, which likely contributes to improved physiological resilience and growth performance in broilers (Socolovsky *et al.*, 1999).

Environmental and nutritional factors can also influence erythropoiesis (Yulianto *et al.*, 2024). High ambient temperature accelerates metabolism, leading to increased oxygen demand and potentially shortening erythrocyte lifespan (Elliott, 2008; Megawati *et al.*, 2020). Nutrient composition, particularly protein and micronutrient availability, further affects erythrocyte formation (Lokapirnasari *et al.*, 2018; Maynar *et al.*, 2020). Therefore, the improved hematological indices observed in STAT5a-treated groups may result from enhanced cellular metabolism and efficient nutrient utilization, stimulated by the transcriptional effects of synthetic STAT5a (Grimley *et al.*, 1999).

The significant increase in hemoglobin concentration in group P2 parallels the rise in erythrocyte count, indicating a coordinated hematopoietic response. Hemoglobin, an iron-containing globular protein, plays a central role in oxygen transport and is a vital determinant of aerobic metabolism (Agustin and Ningtyas, 2021; Mairbäurl and Weber, 2012). The higher hemoglobin content in treated broilers reflects greater oxygen-binding efficiency, which may facilitate higher energy production and improved feed conversion (Galan *et al.*, 2025). Environmental oxygen exposure, particularly in open housing systems, may also support optimal hemoglobin synthesis (Storz, 2016).

Similarly, hematocrit values were highest in the P2 group, reflecting an increased proportion of erythrocytes within the total blood volume. Hematocrit serves as an indicator of blood viscosity and oxygen transport potential (Nader *et al.*, 2019). The observed elevation, while remaining within physiological limits, suggests an adaptive hematopoietic response rather than a pathological condition such as

polycythemia (Zhou *et al.*, 2025). In the present experiment, no adverse effects associated with increased hematocrit were observed. All broilers across treatments displayed normal vitality, feeding behavior, and activity throughout the study, and no mortality was recorded. Furthermore, the hematocrit values, although elevated in STAT5a-treated groups, remained within the physiological range reported for healthy broilers, indicating that the increases did not reach levels associated with pathologic hyperviscosity or impaired circulation (Shlosberg *et al.*, 1998). These observations suggest that the hematological improvements induced by STAT5a occurred without compromising circulatory function, although future studies incorporating blood viscosity measurements or cardiovascular assessments would be valuable to further confirm the safety of STAT5a-induced erythropoietic stimulation (Hoefsloot *et al.*, 1997). The parallel trends among erythrocyte count, hemoglobin concentration, and hematocrit value further support the concept of synchronized regulation within the hematopoietic system (Paquette *et al.*, 2021).

In addition to hematological responses, total plasma protein levels were significantly increased in broilers treated with synthetic STAT5a, particularly in group P4 (14%). Total protein serves as an indicator of metabolic and nutritional status, reflecting the balance between protein synthesis and catabolism (Kim *et al.*, 2018; Wibawati *et al.*, 2024). The dose-dependent increase suggests that STAT5a not only enhances erythropoiesis but also promotes anabolic metabolism and plasma protein synthesis, potentially through upregulation of growth-related and translational genes (Schmerer *et al.*, 2006).

Despite the marked elevation in total blood protein at the highest STAT5a dose (14%), this response did not correspond with a proportional increase in erythrocyte, hemoglobin, or hematocrit values. This apparent decoupling suggests that the physiological effects of STAT5a at supraphysiological concentrations differ from its canonical role in erythropoiesis (Tóthová *et al.*, 2021). STAT5a-mediated erythropoietic stimulation is tightly regulated through cytokine receptor signaling, and excessively high ligand levels may induce negative feedback regulators such as Suppressor of Cytokine Signaling 1 (SOCS1) and Suppressor of Cytokine Signaling 3 (SOCS3), resulting in attenuation of the Janus Kinase/Signal Transducer and Activator of Transcription (JAK-STAT) pathway and a plateau of erythroid responses (Grebien *et al.*, 2008). Conversely, high-dose STAT5a may activate non-erythroid transcriptional programs, including stress- or inflammation-associated pathways, leading to increased synthesis of plasma globulins and thereby elevating total protein concentrations (Bouthelir *et al.*, 2022). This aligns with the inverted U-shaped dose-response observed in

many cytokine-driven systems, where moderate stimulation enhances hematopoiesis, whereas excessive stimulation shifts the response toward metabolic or immune activation rather than erythroid proliferation (Mayani *et al.*, 1993).

Mechanistically, the administration of exogenous STAT5a may bypass the conventional growth hormone (GH) receptor-JAK2-STAT5 signaling pathway. Direct STAT5a stimulation could accelerate transcriptional activation of target genes involved in cell proliferation, erythropoiesis, and protein synthesis (Kosan *et al.*, 2013). This mechanism aligns with findings by Schuringa *et al.* (2004), who demonstrated that STAT5 overexpression in Cluster of Differentiation 34 positive (CD34⁺) hematopoietic stem cells promotes erythroid lineage expansion and stem cell proliferation.

The dose-response pattern observed in this study, where the 3.5% dose elicited the strongest erythropoietic effect, suggests an inverted U-shaped biological response. This phenomenon may occur because receptor-mediated signaling through the JAK-STAT pathway reaches optimal activation at low doses, whereas higher doses trigger receptor desensitization or induce suppressors such as Suppressor of Cytokine Signaling (SOCS) proteins, which attenuate downstream signaling (Nicolas *et al.*, 2013). A hormesis-like response may also be involved, where mild stimulation promotes erythrocyte production but excessive stimulation dampens it (Nitti *et al.*, 2022). Additionally, higher concentrations of orally administered STAT5a may exhibit reduced bioavailability or impose metabolic burden, thereby limiting the effective dose reaching hematopoietic tissues (Han *et al.*, 2019). These mechanisms collectively explain why the lowest STAT5a dose produced the most pronounced hematological improvements.

Overall, the results demonstrate that synthetic STAT5a protein, particularly at the 3.5% concentration, exerts a positive influence on erythropoietic and metabolic functions in broiler chickens. These findings suggest that STAT5a could serve as a potential biological growth enhancer, improving hematological health, oxygen transport efficiency, and protein metabolism factors that collectively contribute to improved performance and productivity in poultry (Grimley *et al.*, 1999).

CONCLUSIONS

Based on the results of this study, it can be concluded that the administration of synthetic STAT5a protein positively influences hematological and biochemical parameters in broiler chickens. The treatment with 3.5% STAT5a produced the most optimal response, significantly increasing erythrocyte count, hemoglobin concentration,

and hematocrit value while remaining within normal physiological ranges. In addition, synthetic STAT5a administration enhanced total blood protein levels, indicating improved erythropoietic activity and protein metabolism. These findings suggest that synthetic STAT5a protein has potential as a biological growth enhancer to support hematological function and overall physiological performance in broiler chickens.

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NOVELTY STATEMENT

This study is the first to evaluate the effects of synthetic STAT5a protein supplementation on erythrocyte profiles and total blood protein levels in broiler chickens. The findings provide new evidence that synthetic STAT5a protein may enhance hematological parameters and protein metabolism, highlighting its potential application as a novel feed additive in poultry production.

AUTHOR'S CONTRIBUTION

MGAY, EBAH, RS, and MH: conceived the idea and manuscript drafting. HAS, NH, ARK, and WPL: acquisition, analysis, and interpretation of data. RD, AM, and HAH: critically read and revised the manuscript for intellectual content. All authors have read, reviewed, and approved the final version of the manuscript.

GENERATIVE AI AND AI-ASSISTED TECHNOLOGY STATEMENT

During the preparation of this manuscript, the authors used generative AI-assisted technology to improve language, grammar, and readability. The authors reviewed and edited the output as necessary and take full responsibility for the content of the publication.

CONFLICT OF INTEREST

The authors have declared no conflict of interests regarding the publication of this article.

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