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(RESEARCH ARTICLE)

The effect of synthetic protein (STAT) 5a administration on plasma albumin protein and blood cholesterol levels in broilers

Ratna Damayanti [1,*], M. Gandul Atik Yuliani [1], Kuncoro Puguh Santoso [1], Eduardus Bimo Aksono Herupradoto [1], Nanik Hidayatik [1], Arsinta Bunga Kurnia Gusti [2], Sugeng Muliono [2], Suzanita Utama [3], Pudji Srianto [3], Yeni Dhamayanti [4] and Supriyadi [5]

[1] *Veterinary Basic Medical Sciences Division, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.*
[2] *Graduate Students, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.*
[3] *Veterinary Reproduction Division, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.*
[4] *Veterinary Anatomy Division, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.*
[5] *Student in Master's Degree of Reproductive Biology, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.*

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Abstract

STAT5a is a key mediator in the Janus kinase (JAK)-STAT signaling pathway, which is activated by various cytokines and growth factors such as growth hormone (GH), insulin-like growth factors (IGFs), and prolactin. This study aims to investigate the effects of synthetic STAT5a administration on plasma albumin protein and blood cholesterol levels in broilers. This study was an experimental study using a complete randomized design with The Post-test Only Control Group Design which consisted of five experimental groups (treatments) using broiler chicken as a test animal. Chicken is treated as follows: (P0): Chicken is aquadest, (P1): Chicken is given formic acid 0.1%, (P2): Chicken is given a STAT 5a synthetic protein in the form of a 3.5% solution, (P3): Chicken is given a STAT 5a synthetic protein in the form of a 7% solution, (P4): Chicken is given the synthetic protein STAT 5a in the form of a 14% dose solution. Based on the study that has been done, it can be concluded thay the administration of Signal Transducers and Activators of Transcription (STAT) 5a synthetic protein affects the plasma albumin levels of broiler chickens. The administration of synthetic STAT 5a protein at a dose of 3.5% is the most effective in increasing the plasma albumin levels of broiler chickens. Introducing synthetic protein Signal Transducers and Activators Transcription (STAT) 5a to blood cholesterol levels in broiler chickens it can be concluded that synthetic protein STAT 5a can reduce blood cholesterol levels in broiler chickens.

Keywords: Chicken; Blood Cholesterol; Healthy; Plasma Albumin Protein; STAT-5a 5

1. Introduction

The poultry industry plays a crucial role in global food production, supplying high-quality protein sources for human consumption. In recent years, researchers have been exploring novel methods to improve growth performance, feed efficiency, and overall metabolic health in broilers. One promising approach involves the administration of synthetic proteins that can regulate metabolic pathways and enhance physiological functions. One such protein is Signal Transducer and Activator of Transcription 5a (STAT5a), a transcription factor that plays a vital role in cell proliferation, differentiation, and metabolism, particularly in protein and lipid homeostasis (Herrington et al., 2000; Yu et al., 2009).

STAT5a is a key mediator in the Janus kinase (JAK)-STAT signaling pathway, which is activated by various cytokines and growth factors such as growth hormone (GH), insulin-like growth factors (IGFs), and prolactin. These signaling

* Corresponding author: Ratna Damayanti

molecules are essential for metabolic regulation, immune response, and cellular growth (Gao et al., 2012). STAT5a is known to enhance protein synthesis by upregulating genes involved in amino acid metabolism, which can lead to improved plasma albumin levels and overall protein retention in animals (Cui et al., 2020). Given its role in metabolic regulation, STAT5a has been widely studied in mammals, particularly in relation to milk production in dairy cows and growth performance in livestock animals. However, its potential effects on poultry metabolism remain largely unexplored.

Albumin is the most abundant plasma protein in birds and mammals, synthesized primarily in the liver. It serves several vital physiological functions, including maintaining oncotic pressure, transporting fatty acids, hormones, and vitamins, and acting as an antioxidant (Peters, 1996). Plasma albumin concentration is often used as a biomarker for protein metabolism and overall health status in poultry (Musharaf & Latshaw, 1999). Higher albumin levels in broilers can indicate improved protein utilization and metabolic efficiency, which are desirable traits in commercial poultry production. Studies have shown that factors such as dietary protein levels, amino acid composition, and hormonal regulation influence plasma albumin concentrations (Maiorano et al., 2007). Given STAT5a's role in protein metabolism, its synthetic administration could potentially enhance albumin synthesis, thereby improving protein efficiency and growth performance in broilers. However, empirical studies evaluating this effect in poultry remain limited, necessitating further research to elucidate the underlying mechanisms.

Cholesterol is an essential lipid molecule involved in various physiological functions, including cell membrane stability, hormone synthesis, and vitamin D metabolism (Griffiths et al., 2018). In poultry, blood cholesterol levels serve as a key indicator of lipid metabolism and dietary fat absorption. Excessively high cholesterol levels in broilers may be associated with metabolic disorders, cardiovascular diseases, and decreased meat quality, which can negatively impact consumer preference and market value (Attia et al., 2017).

Previous research has demonstrated that dietary interventions, such as the inclusion of phytochemicals, probiotics, and genetic selection, can influence cholesterol metabolism in broilers (Rahimi et al., 2011). STAT5a, given its involvement in lipid regulatory pathways, may also play a role in cholesterol homeostasis. Studies in mammalian models suggest that STAT5a interacts with lipid metabolism-related genes, influencing hepatic cholesterol biosynthesis and lipid transport (Ho et al., 2016). However, its effects in avian species, particularly in broilers, remain largely unknown. Investigating the impact of synthetic STAT5a administration on cholesterol metabolism could provide valuable insights into potential strategies for optimizing lipid utilization and improving broiler health.

The commercial poultry industry continually seeks innovative solutions to improve feed efficiency, growth performance, and metabolic balance in broilers. The use of synthetic proteins such as STAT5a presents a novel approach to enhancing physiological functions and optimizing production efficiency. By modulating protein and lipid metabolism, STAT5a may contribute to improved nitrogen retention, enhanced muscle protein deposition, and balanced lipid profiles in broilers.

Furthermore, given the rising concerns regarding excessive fat deposition and associated health risks in poultry, understanding the role of STAT5a in cholesterol regulation could offer new avenues for reducing fat accumulation while maintaining optimal growth rates. This study aims to investigate the effects of synthetic STAT5a administration on plasma albumin protein and blood cholesterol levels in broilers. The findings could have significant implications for poultry nutrition, metabolic health, and overall production sustainability.

2. Material and methods

2.1. Research Design

This study was an experimental study using a complete randomized design with The Post-test Only Control Group Design which consisted of five experimental groups (treatments) using broiler chicken as a test animal. Environmental conditions, age, and sex of chickens are homogeneous, and sample treatment is performed randomly. Treatment is from the 14th to the 25th day.

2.2. Try Animals

The trial animal used is a day-old broiler chicken, a male Cobb strain derived from PT. Panca Patriot Prima Malang has a healthy condition and has been vaccinated against ND, IB Kill, and IBD, and has never been treated. The number of retries or numbers of animals per group (n) is calculated using Federer's formula (t) as the number of groups. From the

formula above, the number of trial animals per group is five. Overall, this study will use five samples in each treatment so that the total sample used is 25 chickens.

2.3. Samples and Number of Deuteronomies

The samples required in this study were 30 days old broiler chicken blood from each treatment and test totaling 25 samples. The blood sample required is whole blood or complete blood and is checked for plasma albumin protein and blood cholesterol levels.

2.4. The Place and Time of Research

This study was conducted from August to September 2023. Preparation of the synthetic protein STAT 5a is carried out at the Molecular Biology Laboratory of Airlangga University's Faculty of Veterinary Medicine. Animal maintenance is being carried out at the 4th floor of the Lecture Building with Campus B 25 of Airlangga University. The examination of blood samples was carried out at the Faculty of Medicine Faculty of Brawijaya University's Faal Science Laboratory.

2.5. Research Materials

The materials used in this study were experimental animals in the form of male broiler chickens DOC Cobb strain obtained from PT. Panca Patriot Prima. STAT 5a synthetic protein obtained from the research of Prof. Anwar Ma'ruf, drh., M.Kes. which was then produced by PT. Genetika Science Indonesia, chicken feed (BR 1 and BR 2), EDTA anticoagulant, 10% benzalkonium chloride, drinking water in the form of clean water, vitachick, distilled water, cotton, alcohol, disposable syringes, kits for examining blood cholesterol levels and newspapers/rice husks for the floor of the cage. On day 30, blood samples were collected from the brachial vein of the chickens, amounting to 3 mL. The blood samples were then centrifuged for 15 minutes at a speed of 3000 rpm to collect the blood plasma. Plasma albumin levels were examined using the sandwich ELISA method with the Chicken Albumin ELISA Kit from Medikbio.

2.6. A Research Tool

The equipment used in this study was a cage tool that included a cage for experimental animals, a place to feed and drink, an exhaust fan and a heating lamp. The equipment used for treatment and blood sampling included a gastric tube, syringe, needle, and EDTA tube. While the equipment used for blood cholesterol level examination included a centrifuge and a Pentra C200 chemistry analyzer. The sandwich ELISA method with the Chicken Albumin ELISA Kit from Medikbio.

2.7. Klirens Eticle

This study has been conducted an ethical qualification test with an ethical certificate number NO: 1.KEH.113.07.2023. A certificate of ethical conduct is attached to the Attachment.

2.8. Trial Animal Preparation

Pet breeding starts from a day to 30 days old chicks. 25 chickens are placed in a 1 m x 1 m x 50 cm postal cage that has been sprayed with disinfectant with 10% benzalkonium chloride using a hand sprayer, with a 40 watt light illumination. On the 10th day, chickens were randomly divided into five groups and moved into a 40 cm x 40 cm x 50 cm battery enclosure with 15 watts of light and one chicken per enclosure. The base of the cage is a newspaper. Chicken is given standard feed in the form of BR1 feed in the starter phase (ages 1 - 21 days) and BR2 feed in the finisher phase (22-35 days) twice a day at 08.00 WIB and 16.00 WIB. Drink clean water and add Vitachick administered adlibitum.

2.9. Treatment of Tried Animals

A day-old broiler chicken of the Cobb strain was adapted for 14 days. Chicken is randomly divided into five groups, namely groups P0, P1, P2, P3, and P4. In one group of chickens, each consists of five chickens. Chicken is treated as follows: (P0): Chicken is aquadest, (P1): Chicken is given formic acid 0.1%, (P2): Chicken is given a STAT 5a synthetic protein in the form of a 3.5% solution, (P3): Chicken is given a STAT 5a synthetic protein in the form of a 7% solution, (P4): Chicken is given the synthetic protein STAT 5a in the form of a 14% dose solution. Treatment is given from the 14th to the 25th day, then chicken is rested and harvested on the 30th day. All treatments are administered orally using probes in 0.5 mL per head per day.

2.10. Chicken Blood Sample Collection

On the 30th day, a sample of chicken blood was taken from each tail in all treatment groups, namely groups P0 to P4. The chicken blood sampling phase starts with satisfying the chicken for approximately 12 hours. In the next step, before blood sampling, the blood vessel area is sterilized using 70% alcohol-supplied cotton to prevent contamination. Blood

samples are taken through the brachial veins of 3 mL each and inserted into a 3 mL EDTA vacutainer tube. EDTA vacuum tubes containing chicken blood samples were placed in a cooler box for storage and the equipment used for blood cholesterol level examination included a centrifuge and a Pentra C200 chemical analyzer. Sandwich ELISA method with Chicken Albumin ELISA Kit from Medikbio.

2.11. Data Analysis

The data of the study results are blood profiles listed in them total leukocytes and differential leukocytes, the data are then statistically analyzed with One Way Analysis of Variance (ANOVA) and then continued with Duncan and Games-Howell's follow-up tests to find out the differences in each treatment group. The data analysis application used is Statistical Product for the Service Solutions (SPSS) 20.

3. Results and discussion

The data obtained from the measurement results of plasma albumin levels underwent normality and homogeneity tests. Subsequently, a One- Way ANOVA statistical test was conducted, revealing the influence of administering STAT 5a synthetic protein on plasma albumin levels in broiler chickens. Further, a Duncan post hoc test was performed, and the average and standard deviation of plasma albumin levels in broiler chickens which were given synthetic STAT 5a protein can be observed in Table 1.

Table 1 Plasma albumin levels (ng/mL; mean ± SD) in broiler which were given STAT 5a synthetic protein

Group (Treatment)	Plasma Albumin Levels (ng/mL) Mean ± SD
P0	$6780{,}40 \pm 1186{,}48^{a}$
P1	$9607{,}40 \pm 2713{,}20^{bc}$
P2	$11168{,}80 \pm 2299{,}20^{c}$
P3	$6962{,}40 \pm 374{,}18^{a}$
P4	$7975{,}80 \pm 972{,}24^{ab}$

Description: Different superscripts in the same column show significantly different (p<0,05); P0: were given distilled water; P1: were given 0.1% formic acid ; P2: were given STAT 5a synthetic protein at a dose of 3.5%; P3: were given STAT 5a synthetic protein at a dose of 7%; P4: were given STAT 5a synthetic protein at a dose of 14%; the treatments were administered orally at a volume of 0.5 mL and were repeated for 12 days from day 14 to day 25; sample: 5.

P0 group which were given 0.5 mL of distilled water, had an average plasma albumin level of 6780.40 ± 1186.48 ng/mL, indicating the lowest plasma albumin levels. P1 group which were given 0.5 mL of 0.1% formic acid, had an average plasma albumin level of 9607.40 ± 2713.20 ng/mL, showing a significant difference compared to P0 group. Formic acid supplementation is known to enhance nutrient digestibility (Garcia et al., 2007; Khan and Iqbal, 2016). pH changes in the chicken digestive tract due to formic acid impact the activity of digestive enzymes. A decrease in pH increases pepsin enzyme activity, enhancing protein proteolysis and digestion of protein and amino acids (Samanta et al., 2010). However, increased digestibility does not certainly improved chicken performance (Kopecký et al., 2012; Khan and Iqbal, 2016) or the blood plasma metabolic profile of chickens (Hernández et al., 2006). P2 group, which were given STAT 5a synthetic protein at a dose of 3.5%, had an average plasma albumin level of 11168.80 ± 2299.20 ng/mL. P3 group which were given STAT 5a synthetic protein at a dose of 7%, had an average plasma albumin level of 6962.40 ± 374.18 ng/mL, and P4 group which were given STAT 5a synthetic protein at a dose of 14%, had an average plasma albumin level of 7975.80 ± 972.24 ng/mL. These three treatment groups showed an increase in albumin levels compared to P0 group, which only given distilled water. GH mediated by STAT 5a protein stimulates albumin production (Rothschild et al., 1988; Kaneko et al., 1997), indicating that the administration of STAT 5a synthetic protein increases plasma albumin levels. Moreover, the absence of hypoalbuminemia conditions which indicate malnutrition or disorders of the liver, kidneys, and digestive tract (Kaneko et al., 1997), suggests that the administration of STAT 5a synthetic protein in broiler chickens is safe and does not disrupt the physiological functions of the chicken body.

The average plasma albumin levels of P2, P3, and P4 groups increased by 64.72%, 2.68%, and 17.63%, respectively, compared to P0 group. The increase in the average plasma albumin levels of P3 and P4 groups, though not as significant as in P2 group, is presumed to be due to an increase in the rate of albumin degradation. Although the albumin degradation process is not precisely known, it is closely related to the mass of albumin (Rothschild et al., 1976).

Research by Rothschild et al. (1964) showed that when albumin is injected, there is an increase in the rate of albumin degradation and albuminuria to prevent excessive colloid accumulation. Experimental research on hyperalbuminemia conditions also mentioned an increase in the rate of albumin degradation (Rothschild et al., 1976). The increase in the degradation rate occurs when plasma albumin levels are too high conversely, the degradation rate decreases when albumin levels are low, such as in hypoalbuminemia conditions (Weigand, 1977; Rothschild et al., 1988). P2 group showed a higher average plasma albumin level by 16.25% compared to P1 group, although not significantly different. The use of 0.1% formic acid as a solvent for STAT 5a synthetic protein is believed to maximize the role of STAT 5a synthetic protein in promoting growth. However, the use of 0.1% formic acid alone may not have an impact on chicken performance (Hernández et al., 2006; Kopecký *et al*., 2012; Khan and Iqbal, 2016). Meanwhile, the lower average plasma albumin levels in P3 and P4 groups compared to P1 group are closely related to the albumin degradation process.

The results of the blood cholesterol levels of male broiler chickens for 25 male broiler chickens given STAT 5a synthetic protein as experimental animals are stated in table 2.

Table 2 Blood cholesterol levels (mg/dL; ± SD) of plasma of male broiler chickens fed with synthetic protein STAT 5a

Group (Treatment)	Blood Cholesterol Level (mg/dL) (Mean Rank ± SD)
P0	158,97^{c} ± 2,72 mg/dL
P1	139,19^{c} ± 16,53 mg/dL
P2	102,11^{b} ± 11,66 mg/dL
P3	85,72^{b} ± 6,63mg/dL
P4	65,49^{a} ± 2,07 mg/dL

Description: Different superscripts (abc) in the same column indicate significant differences ($p<0.05$). P0: chickens were given distilled water; P1: chickens were given 0.1% formic acid; P2: chickens were given STAT 5a synthetic protein at a dose of 3.5%; P3: chickens were given STAT 5a synthetic protein at a dose of 7%; P4: chickens were given STAT 5a synthetic protein at a dose of 14%; treatments were given orally using a tube in a volume of 0.5 mL per head per day for 12 days (days 14–25); replications: 5.

The results of blood cholesterol level examination in broiler chickens treated with distilled water, formic acid, and STAT 5a synthetic protein with several different doses showed differences in each treatment group. Administration of STAT 5a synthetic protein in treatment groups P2, P3 and P4 was proven to reduce blood cholesterol levels when compared to treatment groups P0 and P1.

Treatment groups P0 and P1 were not significantly different because the two treatment groups, namely the administration of distilled water for P0 and the administration of formic acid for P1, did not have a significant effect on blood cholesterol levels. Treatment groups P2 and P3 were not significantly different because the dose of STAT 5a synthetic protein between the two treatments was not too far apart, namely P2 as much as 3.5% and P3 as much as 7%, so the difference between them was not too significant. In treatment group P4, it was significantly different from all treatment groups, this can be stated that P4 with a dose of STAT 5a synthetic protein as much as 14% is an effective dose in this study.

Treatment group P0, with the provision of 0.5 mL of distilled water, with an average blood cholesterol level of 158.97 ± 2.72 mg/dL, is the treatment group with the highest blood cholesterol level compared to other treatment groups. This shows that distilled water does not affect blood cholesterol levels. Treatment group P1, which is the provision of 0.1% formic acid solution 0.5 mL, with an average blood cholesterol level of 139.19 ± 16.53 mg/dL, showed a decrease in blood cholesterol levels when compared to treatment group P0, but not as much as in treatment groups P2, P3 and P4. This shows that formic acid does not have a significant effect on blood cholesterol levels. Research conducted by Hernández et al. (2006), found no difference in weight gain and feed consumption in chickens when formic acid at a dose of 5,000 or 10,000 mg/kg was added to the feed. The calculation of biochemical parameters in plasma such as glucose, creatinine, cholesterol, triglycerides, calcium and phosphorus, which were analyzed at the end of the study also did not provide a significant effect. García et al. (2007) wrote that formic acid did not increase body weight significantly, but could increase the feed conversion ratio (FCR).

Treatment groups P2, P3, and P4, namely the administration of STAT 5a synthetic protein with doses of 3.5%, 7%, and 14% as much as 0.5 mL, showed a significant decrease in blood cholesterol levels. Treatment group P2 with an average blood cholesterol level of 102.11 ± 11.66 mg / dL, followed by treatment group P3 which had an even lower average blood cholesterol level, namely 85.72 ± 6.63 mg / dL, and treatment group P4 which had the lowest average blood

cholesterol level, namely 65.49 ± 2.07 mg / dL. The decrease in blood cholesterol levels in broiler chickens in treatments P2, P3, and P4 was due to the working mechanism of STAT 5a synthetic protein. The higher the concentration of STAT 5a synthetic protein, the greater its ability to lower blood cholesterol levels in broiler chickens. GH is known to be negatively correlated with adiposity. Individuals with acromegaly, characterized by GH hypersecretion, experience decreased body fat (Kaji et al., 2001). Restricting the time and amount of feeding in chickens has been shown to produce chickens with low fat content (Ma'ruf, 2004). The right restriction of time and amount of feeding will increase GH secretion, thereby increasing the metabolic effects on all body tissues (Ma'ruf, 2004).

The metabolic effects of GH related to fat are the transport of fatty acids from fat tissue and the use of fatty acids as an energy source. GH will cause metabolic effects when the GH receptor binds and activates tyrosine kinase. The binding of GH to the receptor will activate Janus Kinase 2 (JAK 2) and form an attachment site for the STAT protein. Activation of STAT 5a, first requires binding between cytokines and hormones, including GH. The STAT 5a protein can only be activated when there is GH attached to the receptor. Administration of synthetic STAT 5a protein will directly form a janus kinase complex and replace the STAT 5a protein so that there is no need for STAT 5a activation by GH attachment to its receptor, which will then cause a growth effect.

4. Conclusion

Based on the study that has been done, it can be concluded thay the administration of Signal Transducers and Activators of Transcription (STAT) 5a synthetic protein affects the plasma albumin levels of broiler chickens. The administration of synthetic STAT 5a protein at a dose of 3.5% is the most effective in increasing the plasma albumin levels of broiler chickens. Introducing synthetic protein Signal Transducers and Activators Transcription (STAT) 5a to blood cholesterol levels in broiler chickens it can be concluded that synthetic protein STAT 5a can reduce blood cholesterol levels in broiler chickens.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors declare that they have no conflict of interest.

Statement of ethical approval

This study protocol was approved by the Animal Ethical Committee of Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia (Number: 1.KEH.113.07.2023).

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