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Blood Profile and Carcass Production of Broiler Chickens Given Nucleotides and Turmeric Extract in Feed

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ABSTRACT

Broiler chickens are birds that grow very fast, but have low body resistance and are easily stressed. This study aimed to examine the effect of nucleotide and turmeric extract (*Curcuma longa* Linn) on blood profile (erythrocytes, hemoglobin and leukocytes) and carcass production (weight of carcass, weight of breast, thighs and wings) in broiler chickens. Data was collected using a completely randomized design with 7 treatments consisting of Control: basal feed + antibiotic Bacitracin Zinc 0.1 g/day; N₀: basal feed; N₁: basal feed + turmeric extract 600 mg/kg feed; N₂: basal feed + nucleotide 250 mg/kg feed; N₃: basal feed + nucleotide 250 mg/kg feed + turmeric extract 600 mg/kg feed; N₄: basal feed + nucleotide 500 mg/kg feed; N₅: basal feed + nucleotide 500 mg/kg feed + turmeric extract 600 mg/kg feed. The results showed that nucleotide supplementation and turmeric extract had no significant effect on weight of carcass, weight of breast, thighs and wings, hemoglobin, erythrocytes and leukocytes of broiler. The use of nucleotide and turmeric extract could not improve the blood profile of broiler.

Key words: Blood profile, Broilers, Carcass, Nucleotide, Turmeric extract

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Introduction

Indonesia has a wet tropical climate with an average temperature of up to 35°C with fluctuations of 29-36°C and an average humidity of 70-80%. This condition can cause broiler chickens to be exposed to heat stress because the comfortable temperature of broiler chickens is 20-25°C with 50-70% humidity (Putra *et al.*, 2018). Heat stress will trigger the formation of free radicals or reactive oxygen compounds, causing blood damage (erythrocytes, hemoglobin and leukocytes), impairs metabolism and the functionality of the digestive system and decreased broiler immunity (Nawaz *et al.*, 2021; Rostagno, 2020). Therefore, to prevent it, farmers often use antibiotics. The most common antibiotics added to broiler feed are tetracycline, sulfonamides, penicillin and bacitracin zinc (Thema *et al.*, 2019; Van Boeckel *et al.*, 2017). In addition to producing residues, antibiotics also cause bacterial resistance, therefore they are prohibited from being added to feed (Ronquillo and Hernandez, 2017). Even though we know that antibiotics are prohibited from being used and have a negative impact on livestock, humans and

the environment (Polianciuc *et al.*, 2020). Therefore, it is necessary to find alternative feed additives that can maintain the performance and physiology of chickens. One of these natural alternatives is supplementing the young animal diet with bioactive substances such as nucleotides (Mohamed *et al.*, 2020) and tumeric extract (Johannah *et al.*, 2018). Giving nucleotides mixed with turmeric extract (*Curcuma longa* Linn.), it is expected that broiler chickens can grow optimally and disease problems in the digestive organs can be minimized.

A nucleotide was the basic molecules of nucleic acids and play an important role in the storage and transfer of genetic information, cell division, and protein synthesis (Sanchez-Pozo and Gil, 2002). Nucleotide play an important role in the body's biological processes, such as the development of organs, tissues, cells of the intestinal mucosa and the early growth period requires a greater number of nucleotide which cannot be supplied by *de novo* synthesis alone. Furthermore, the salvage pathway, which harvests nucleobases from blood and diet, could support their demands (Gopi *et al.*, 2020). Heat stress results in insufficient *de novo* synthesis of

nucleotide, change chicken behavior and impair chicken performance (Brugaletta *et al.*, 2022). Therefore, nucleotides are often added to animals' diets in the form of yeast extracts (Mohamed *et al.*, 2020).

Besides nucleotide, this study also uses natural antibiotics sourced from herbs (phytobiotics) to improve poultry production. The use of phytobiotic is considered safer because it produces residue-free products, low toxicity, low cost and can improve broiler production (Houshmand *et al.*, 2012). Turmeric (*Curcuma longa* Linn.), is a phytobiotic that contains a specific bio-active compound called curcumin, a polyphenolic phytochemical with anti-microbial, anti-inflammatory, anti-cancer and antioxidant properties (Aggarwal and Harikumar, 2009; Al-Sultan, 2003). The use of turmeric for poultry feed had replaced antibiotics and shown beneficial effects on broiler health without any side effects (Dono, 2014).

Turmeric contains curcuminoids consisting of curcumin, desmethoxycurcumin, bisdemethoxycurcumin and tetrahydrocurcuminoids (Chattopadhyay *et al.*, 2004). *Curcuma longa* Linn are also known to enhance immune function, promote blood circulation, accelerate toxin elimination, and stimulate digestion (Dosoky and Setzer, 2018). The Curcuminoid are yellowish turmeric pigments that have anti-carcinogenic, anti-inflammatory properties, anti-oxidative, which can capable to inhibiting the generation of Reactive oxygen species (ROS) (Nishiyama *et al.*, 2005). The antioxidant effects of curcumin have been implicated in mechanisms of red cells damage, but also improve growth performances in broilers (Adegoke *et al.*, 2018; Pimson *et al.*, 2018).

Modern broilers are designed to be harvested in a short time, have a higher body mass and a higher metabolic rate (Borges *et al.*, 2003). Broilers are most susceptible to heat stress because their bodies are covered with hair and do not have sweat glands, so they cannot expel body heat (Ruff *et al.*, 2021). Broiler chickens exposed to heat stress will reduce their intake of feed and drink a lot to maintain homeothermia, thus affecting performance and low body size (Khosravinia, 2016).

According to Tugiyanti *et al.* (2016), blood is an important component to regulate the physiology of the body and as an indicator of poultry health. Leukocytes are part of the immune system against some infectious diseases, while erythrocytes determine physiology. Leukocytes are divided into agranulocytes consisting of lymphocytes and monocytes and granulocytes consisting of basophils, eosinophils, and heterophils. Lymphocytes, which are the most abundant leukocytes in chickens and their size varies from small to large as in mammals (Harahap, 2008). According to Yuniwanti (2015), erythrocytes have a function in gas exchange and oxygen distribution into cells and are used by cells for metabolic processes. According to Isroli

(2009), oxygen is an important component in the production of adenosine triphosphate (ATP), the energy for cells to metabolize. The process of forming new erythrocytes every day requires precursors to synthesize new cells, including iron, vitamins, and amino acids, where the process of cell formation is regulated by the hormone erythropoietin.

Dietary nucleotide supplementation improves the performance of broilers from seven to 20 days of age. However, from 21 to 35 days of age, supplementation of 0.3 g of purified nucleotide/kg of feed, independent of inflammatory challenge, does not contribute to improve performance of broilers (Kreuz *et al.*, 2020). Diets supplemented with nucleotides did not influence broiler performance or carcass yield at 42 days of age, and were not different from the feeds not containing any additive or with AGP. Therefore, the provision of nucleotide and turmeric extract as feed supplement are expected to maintain or improve performance and the blood profile of broilers.

Materials and Methods

Research material

The research used 168 head of DOC broiler with an average initial body weight of 36.25 ± 0.83 g. They were allocated randomly in 28 open cages with a size of $0.75 \times 0.75 \times 1$ m containing 6 birds each. The basal feed were consisted of corn, rice bran, soybean meal, fish flour, CaCO_3 , lysine, methionine. The nutrient content of basal feed was 19.33% protein, 3064 kcal/kg energy, 5.53% fat, 7.57% crude fiber and 15.90% ash content (Analysis result of Animal Feed Nutrition and Nutrition Laboratory, Faculty of Animal Science, University of Jenderal Sudirman, 2021) and 1.10% lysine and 0.50% methionine (manual calculation). The nucleotides used were BioNutrend produced by Wuhan Sunhy Biology Co. Ltd., China. Turmeric extract (*Curcuma longa* Linn.) used Herbana produced by PT Deltomed Laboratories. The antibiotic used in this research was Bacitracin Zinc produced by PT Qilu Pharmaceutical Co.Ltd.

Data was collected using a completely randomized design (CRD) with 7 treatments consisting of Control: basal feed + antibiotic Bacitracin Zinc 0.1 g/day; N_0 : basal feed; N_1 : basal feed + turmeric extract 600 mg/kg feed; N_2 : basal feed + nucleotide 250 mg/kg feed; N_3 : basal feed + nucleotide 250 mg/kg feed + turmeric extract 600 mg/kg feed; N_4 : basal feed + nucleotide 500 mg/kg feed; N_5 : basal feed + nucleotide 500 mg/kg feed + turmeric extract 600 mg/kg feed. Each treatment was repeated 4 times and each cage unit was filled with 6 cages.

The variables measured were blood profile (the number of erythrocytes, hemoglobin, leukocytes and differential leukocytes) and carcass production (weight of carcass, breast, thighs, and wings). The data obtained were analyzed using analysis of variance.

Research procedure

Blood sampling was conducted at week 5th. A venous or capillary blood sample was taken from the brachial vein. Then, the blood was inserted into a tube containing an EDTA vacuum tube and homogenized. Wipe preparations were made from blood samples of such chickens. Observation of the picture of red blood and white blood differential was done with a complete hematological examination or *complete blood count* (CBC) and review of blood. Measurement of hematological values was carried out by looking at the profile of the blood, consisting of the number of erythrocytes, hemoglobin, leukocytes and differential leukocytes (Nengsih and Mustika, 2020).

Chickens were slaughtered when the chickens were 35 days old to measure weight of carcass, breast, thigh and wings. Carcass weight was measured by weighing the chicken after deducting the weight of blood, feathers, head, shank, internal organs except giblet. The weight of breast was measured by weighing the weight of the breast. The weight of thigh was measured by weighing of the right and left thighs, as well as for the wings (Tamzil and Indarsih, 2020).

Results and Discussion

Profile of bloods

The results of research on the use of turmeric extract and nucleotide in broiler chicken feed on the blood profile of broiler chickens are presented in Table 1.

The average levels of erythrocytes, hemoglobin, and leukocytes in this study was in the range of 2.19 to 2.65 μL , 7.28 to 7.88 g/dL, 8.7 to 11.4 $\times 10^3$ cells/ mm^3 , respectively. The results obtained were relatively similar to Sadarman (2013), normal erythrocytes of broiler chickens range from 2.5 to 3.2 $\times 10^6$ / mm^3 , normal hemoglobin levels of chicken range from 6.5 to 9.0 g/dL and according to Arfah (2015), leukocytes of broiler chickens range from 12 to 30 $\times 10^3$ /mL. The use of nucleotide and turmeric extract is considered as safe. This is because the number of erythrocytes, hemoglobin and leukocytes is within the normal range.

Lymphocytes are white blood cells that belong to the group of agranulocyte, while monocyte are differential white blood cells belonging to the group of agranulocytes formed in

the bone marrow and undergo maturation when they enter the circulation so that they become macrophages and enter the tissue. Average lymphocytes and monocyte found in this study were in the ranges of 59% to 71% and 5.25% to 7.75%, respectively. These results were relatively similar to those reported by Guyton (1997), that the normal lymphocyte count of broiler chickens was in the range of 24% to 84%. Eroschenko (2008) reported that the normal limit value of monocyte in the blood of broiler chickens is 3-10%.

The results of analysis of variance showed that the use of nucleotides and turmeric extract in the feed had no significant effect ($P>0.05$) on erythrocytes, hemoglobin, leukocytes, and leukocyte differential (lymphocyte and monocyte). This is because with a very fluctuating cage temperature ranging from 26 to 33^oC, the cage temperature conditions cause chickens to experience stress. This condition is not be able to meet the adequacy of nucleotide in their body for broiler chickens (Hakim *et al.*, 2021). In addition, it will cause damage to erythrocytes, this is because the chicken in heat stress conditions will trigger the formation of free radicals or reactive oxygen compounds. Reactive oxygen production accompanied by increased temperature will damage erythrocytes, so erythrocytes in the blood will decrease. Therefore, the use of nucleotide and turmeric extract is allegedly only able to normalize the adequacy of nucleotide in the body and prevent damage to hemoglobin, and leukocytes caused by ROS.

The number of erythrocytes indicates the ability of the chickens to transport oxygen for nutrient metabolism. The normal number of erythrocytes is an indicator that the chicken has stable metabolic system, so that erythrocytes can be produced in normal amounts. Hence, the nutrients needed in the formation of red blood cells, especially protein and vitamins are sufficient for the chicken to achieve an optimal condition for health. According to Ali *et al.* (2013), differences in the number of erythrocytes can be influenced by several factors including age, nation, temperature, environment, production level, and maintenance system.

The process of producing new erythrocytes daily requires precursors to synthesize new cells. The precursors such as iron, vitamins, amino acids, and the erythropoietin hormone stimulate

Table 1. Blood profile and differential leukocytes of broiler chickens that were treated nucleotide and tumeric extract in feed

Treatments	Blood profile of broiler			Diferensial leukosit	
	Eritrosit (μL)	Hemoglobin (g/dL)	Leukosit ($\times 10^3$ cell/ mm^3)	Limfosit (%)	Monosit (%)
Control (-)	2.19 \pm 0.12	7.28 \pm 0.15	9.10 \pm 1.70	71.00 \pm 6.50	6.00 \pm 3.60
N ₀	2.41 \pm 0.26	7.43 \pm 0.15	8.70 \pm 1.80	71.00 \pm 6.60	6.20 \pm 2.50
N ₁	2.34 \pm 0.26	7.35 \pm 0.17	9.50 \pm 2.20	59.00 \pm 4.30	7.75 \pm 3.90
N ₂	2.42 \pm 0.42	7.60 \pm 0.42	10.80 \pm 2.50	60.00 \pm 10.00	7.50 \pm 3.10
N ₃	2.58 \pm 0.11	7.53 \pm 0.29	9.60 \pm 1.60	69.00 \pm 11.00	5.75 \pm 3.60
N ₄	2.65 \pm 0.18	7.88 \pm 0.29	11.00 \pm 3.30	58.00 \pm 4.80	5.25 \pm 1.50
N ₅	2.65 \pm 0.29	7.60 \pm 0.42	11.4 \pm 1.90	64.00 \pm 10.00	5.25 \pm 3.20

Control: basal feed + antibiotic Bacitracin Zinc 0.1 g/day; N₀: basal feed; N₁: basal feed + turmeric extract 600 mg/kg feed; N₂: basal feed + nucleotide 250 mg/kg feed; N₃: basal feed + nucleotide 250 mg/kg feed + turmeric extract 600 mg/kg feed; N₄: basal feed + nucleotide 500 mg/kg feed; N₅: basal feed + nucleotide 500 mg/kg feed + turmeric extract 600 mg/kg feed.

the formation of erythrocytes by triggering the production of proerythroblasts from the hemopoietic cells in the bone marrow (Tugiyanti and Susanti, 2017). The active substance curcumin, which plays a role in helping the process of erythropoiesis does not increase the number of erythrocytes. Erythropoiesis (erythrocyte formation process) is driven by the need for O₂, erythropoietin hormone, and the availability of nutrients (Hanifa *et al.*, 2016). Giving turmeric water to water drinking of broiler chickens can maintain or improve the blood profile of broilers who are easily stressed and easy disease caused by viruses and bacteria (Khoirina *et al.*, 2017).

Hemoglobin is the most important part of erythrocytes because it is the third transporting oxygen to the body tissues. Hemoglobin is a complex organic compound consisting of four red porphyrins pigments (*heme*), where each pigment contains an iron atom plus globin which is a globular protein consisting of four chains of amino acids (Wientarsih, 2013). Therefore, the amount of hemoglobin determined in this study connects linearly to the number of erythrocytes, when the level of erythrocytes increase the amount of hemoglobin also will increase.

The normal number of erythrocytes and hemoglobin content indicate that nucleotides and turmeric extract do not contain toxic substances that can cause lysis of erythrocyte cells or interfere with the process of formation of red blood cells (Napirah *et al.*, 2013). Normal levels of hemoglobin in each treatment can be used as an indicator of the adequacy of oxygen transported throughout the tissues for metabolic processes

Leukocyte is one of the blood plasma suspensions that function as the body's defense system from bacterial, viral, and pathogenic attacks through the mechanism of antibody formation which is currently widely used as one of the determining indicators of animal health. The animal's health status can be recognized through the number of white blood cells that have attacking agents to fight bacteria (Pristiwanti *et al.*, 2017).

Livestock infected with bacteria will suffer health problems indicated by the increased number of leukocytes. In addition, the increase in leukocytes is also caused by environmental stress which ultimately disrupts the physiological process of becoming abnormal and affecting the hormonal balance in the chicken body (Pristiwanti *et al.*, 2017). White blood cells and differentiation are

one indicator that is generally used to indicate the health status of livestock broiler chickens (Sugiharto, 2014). According to Suriansyah *et al.* (2016) studying on the broiler, each broiler sometimes have differences in leukocyte counts, which are generally differences caused by several factors including physiological activity, age, nutrition, stress and others. The number of leukocytes deviated from normal conditions has a relationship with the health condition of broiler chickens. Lymphocytes are one type of white blood cells, and their function can increase the immune system and fight the the germs of disease that enter the body (Yosi and Sandi, 2014). Yalcinkaya *et al.* (2008) also reported that lymphocytes play a role in responding to antigens by forming antibodies. Excessive stress in chickens can increase the production of excess cortisol hormone in the body. Excessive production of the hormone cortisol can cause immunosuppression, which is characterized by shrinking lymphoid organs to make lymphocyte decline. This is in accordance with the opinion of Puvadolpirod and Thaxton (2000) which states that factors that can affect the number of lymphocytes is heat stress or environmental stress, because heat stress results in reduced weight of lymphoid organs thymus and fabrisius bursa which has an impact on the decrease in the number of lymphocytes. Davis *et al.* (2008) explained that high temperature environment will be trigger high secretion of corticosteroid hormone. High levels of these hormones in the blood, can inhibit the formation of lymphocytes. According to Ma'rifah *et al.* (2020) the content of curcumin in turmeric extract which functions as an immunomodulatory can stimulate the formation of lymphocytes, so that more lymphocytes will be produced. According to Agustanti (2014) Kurcumin in turmeric can activate T and B lymphocyte cells. At a time when livestock were kept with a very fluctuating cage temperature, the administration of nucleotides and turmeric extract produced normal lymphocyte levels.

Monocytes are the second line of defense against infection, while a decrease in monocytes below the normal range can be caused by livestock experiencing stress (Harahap, 2014). The level of monocytes in the study was included in the normal category, although the chickens were kept at fluctuating temperatures. This is because curcumin is an antioxidant in turmeric extract. Antioxidant compounds can protect cells

Table 2. Production of carcass of broiler chickens which were treated nucleotide and tumeric extract in feed

Treatments	Weight of carcass (g)	Weight of breast (g)	Weight of thigh (g)	Weight of wing (g)
Control (-)	543.00±150.27	141.75±21.20	157.25±22.85	64.75±11.76
N ₀	548.50±116.99	160.50±26.64	146.50±17.79	59.75±10.40
N ₁	668.25±108.28	195.75±25.20	204.25±29.10	77.50±7.59
N ₂	655.25±143.46	186.00±21.66	187.50±43.71	79.75±12.34
N ₃	660.50±92.07	188.50±26.19	186.25±28.76	75.75±7.50
N ₄	604.25±107.71	168.00±36.41	179.75±28.93	69.75±11.81
N ₅	658.00±53.35	182.00±27.41	196.50±16.34	75.50±3.42

Control: basal feed + antibiotic Bacitracin Zinc 0.1 g/day; N₀: basal feed; N₁: basal feed + turmeric extract 600 mg/kg feed; N₂: basal feed + nucleotide 250 mg/kg feed; N₃: basal feed + nucleotide 250 mg/kg feed + turmeric extract 600 mg/kg feed; N₄: basal feed + nucleotide 500 mg/kg feed; N₅: basal feed + nucleotide 500 mg/kg feed + turmeric extract 600 mg/kg feed.

from the harmful effects caused by reactive oxygen free radicals. Antioxidants are electron-giving compounds (electron donors) to dampen the negative impact of ROS (reactive oxygen species) (Fahrurrozi *et al.*, 2014).

Carcass production

The carcass weight of broiler chickens in this study was low. It ranged from 459.42±21.76 to 501.70±30.34 g. Research by Resnawati *et al.* (2002) produced a broiler carcass weight of 680.00 to 710.80 g/head. The results of the analysis of the variance showed that the effect of the combination of nucleotide and turmeric extract treatment had no significant effect ($P>0.05$) on carcass weight, although there was an increasing trend in the treatment given nucleotides and turmeric extract when compared to the treatment without nucleotides supplementation and turmeric extract or those given antibiotics. It is suspected that the administration of turmeric extract combined with nucleotide caused the feed consumption of chicken, the body weight and carcass weight were low, so that between treatments the carcass weight was relatively the same. Swastike (2012) stated that the addition of turmeric can reduce feed consumption and body weight. This is due to the bitter taste caused by natural phenolic compounds such as curcuminoids, sesquiterpenoids, and the presence of essential oils. There are 3 components in curcuminoids, consisting of curcumin (94%), demethoxycurcumin (6%), and bisdemethoxycurcumin (0.3%). Meanwhile, the sesquiterpenoid compounds consist of arturmerone, curlone, bisacumol, zingiberene, curcumene, germacrone, curcuminol, bsabolene. Curcuminoids have a yellow color effect on the turmeric rhizome, while turmerone, arturmerone and zingiberene contained in sesquiterpenoid compounds give turmeric a distinctive aroma and taste (Kumar *et al.*, 2017). In addition to the effect of treatment, the chicken carcass is influenced by the health condition of the chicken, the condition of the feathers, the size of the chest and physical composition, the condition of the back and the condition of the wings and fatness (Nematbakhsh *et al.*, 2021).

The breast weights of broiler chickens in this study ranged from 141.75±21.20 to 188.50±26.19 g and the thigh weight of broiler chicken was 146.50±17.79 to 204.25±29.10 g. The weight of broiler chicken breast in this study was relatively the same as that of Budiarta *et al.* (2020). The breast weight was 158.23 to 188.30g, but the thigh weight (206.70±252.40 g vs 146.50±17.79 to 204.25±29.10 g) was lowered. The results of the analysis of variance showed that the effect of a combination of nucleotide and turmeric extract treatment had no significant effect ($P>0.05$) on the weight of the chest and thighs. It was because the effect of turmeric, which results in low feed consumption and the provision of nucleotide to chickens reared in open cages, is partly used to meet nucleotide

needs and the remaining part is used for muscle growth in the chest and thighs. Turmeric extract has a bitter taste, but when it reaches the digestive organs it will function effectively (Thavorn *et al.*, 2014; Kwiecien *et al.*, 2019), so that the ability of the intestines and the digestive process can also be maximized (Cas and Ghidoni, 2019; Kwiecien *et al.*, 2019). The breast and thigh weights of chickens which were given nucleotide and turmeric extract in feed were higher than the basal diet and antibiotics (Table 2). During the growth of broiler chickens, nutrient deposition is mostly deposited in the chest and thighs (Mehri *et al.*, 2016; Attia *et al.*, 2017).

Wing carcass weight in this study ranged from 59.75±10.40 to 79.75±12.34 g. The wing weight is lower than the wing weight in the research by Weimer *et al.* (2022) in the amount of 196-246 g. The results of the analysis of variance showed that the effect of the combination of nucleotide and turmeric extract had no significant effect ($P>0.05$) on wing weight. This is because the administration of nucleotide and turmeric extract affects the overall growth of chickens including the wings. According to the statement of Sakomura *et al.* (2011), the growth of the wings is in line with the growth of other body parts. Wings in broiler chickens are one of the body parts that are often used to maintain balance during activities (Rahayu *et al.*, 2019).

Conclusions

The provision of nucleotide and turmeric extract in the feed has not been able to improve the blood profile and carcass production of broiler chickens.

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