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Effect of the Nucleotide and Turmeric Extract Supplementation and different Cage Floors on the Blood Profile and Physiological Status of Broiler Chicken

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Bursa Fabricius Index

Respiratory Rate

Pulse Rate

Thermoregulation

Nucleotide

Tumeric extract

ABSTRACT

Climate change has been responsible for the high prevalence of heat stress (HS) among broiler chickens. In this research, efforts are made to curb the negative impact of HS on chickens by modifying the feed and cage floor. The blood profile and physiological responses of broiler chickens supplemented with nucleotide and turmeric powder and kept in different floor cages were recorded (litter, slatted, and combination of slat-litter). A total of 245 broiler day-old chicks (DOC) were randomly allotted to seven treatment groups of the combined supplementation of nucleotide and turmeric extract and different types of cage floor (litter, slate, combination of slat-litter) for 35-day maintenance. Each treatment was replicated five times. The supplementation of nucleotide and turmeric extract into feed and different types of cage floor did not significantly affect ($P>0.05$) body temperature, respiratory rate, pulse rate, lien index, PVC, TPP, heterophils, lymphocyte, and monocyte, but significantly affected ($P<0.05$) the erythrocyte level, hemoglobin, leukocyte, rectal temperature and the index of bursa fabricius of broilers. Results of this study concluded that the combined treatments of supplementing nucleotide and turmeric extract in feed and using slat-floored cages tend to reduce the comfort of broiler chickens.

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1 Introduction

The recent climate change has caused prevalent heat stress (HS) among broiler chickens. Temperature above 29^{o} C is hot for a chicken and can lead to heat stress. Heat stress (HS) occurs when the amount of heat produced by an animal surpasses its capacity to dissipate the heat to the surrounding environment. When the environmental temperature rises above the thermoneutral zone, birds typically reduce their physical activity and feed intake (FI) to limit heat production (HP), as well as increase their panting and water consumption to favour heat loss by evaporation Brugaletta et al. (2022).

In addition to causing evident changes in chicken behaviour, HS negatively acts upon metabolism and general homeostasis and impairs the functionality of the digestive system (Rostagno 2020). Birds reduce feed consumption and nutrient digestibility in a hot environment to limit metabolic heat production. High humidity makes it increasingly difficult for birds to cool themselves and evaporate water off their respiratory systems. Stress reduces feed intake and growth and impairs immune response and function, resulting in high disease susceptibility. Heat exposure causes several physiological impairments in birds, including oxidative stress, weight loss, immunosuppression, and dysregulated metabolism. Broiler chickens must live comfortably in a proper environment, known as the thermoneutral zone, with a temperature of 20-25°C and 50% humidity (Omomowo and Falayi 2021; Kpomasse et al. 2021). Indonesia has a humid tropical climate with temperatures between 28 - 38°C in the dry season and 25 - 29°C during the rainy season. Humidity during the dry season is around 40 -70%, while humidity during the rainy season is about 80 - 100%. In addition, areas that have a humid tropical climate will receive a lot of solar radiation (Mustamin et al. 2019).

In addition to heat stress, the prohibition of antibiotic growth promoters (AGP) has negatively affected the broiler industry. AGPs have been well documented as a widely used supplement to improve broilers' performance and food conversion rates because broilers are very susceptible to disease (Untari et al. 2021). AGP is offered to chickens from the age of 2-4 days to prevent infections that are responsible for broilers' poor immunity against disease, high mortality rate, slow growth rate, and declining physiological status and productivity (Manafi 2015; Ravindran and Reza Abdollahi 2021). Accordingly, there has been increasing development of natural compounds extracted from herbal plants as a source of antioxidants and as a substitute for AGP in broiler diets (Gharechopogh et al. 2021; Mnisi et al. 2022). Turmeric (*Curcuma longa*) is one of the most commonly used herbal plants, and it possesses immunomodulators, antiinflammation, and antioxidant properties (Irshad et al. 2018; Chanda and Ramachandra 2019). As a member of the Zingiberaceae family, turmeric contains an element known as curcumin. The distinctive bulb of curcumin [1,7-bis (4-hydroxy 3- methoxyphenyl)- 1,6-heptadiene-3,5-dione; diferulylmethane] allows the plant to retain its economic value (Salah et al. 2019). The use of turmeric as a feed additive has been reported to induce positive effects on the performance and immunity of broiler chickens, even during heat stress (Al-Jaleel 2012; Sugiharto 2020; Lagua and Ampode 2021). Despite this, studies have also reported that turmeric powder supplementing produces subpar effects compared to AGP on broilers (Nagar et al. 2021).

Combining turmeric extract and nucleotide as a feed additive is expected to optimize the performance of broiler chickens. As the basic building blocks of nucleic acids (RNA and DNA), nucleotides consist of a sugar molecule (ribose in RNA or deoxyribose in DNA) bonded to a phosphate group and a nitrogen-containing base. The bases used in DNA are adenine (A), cytosine (C), guanine (G) and thymine (T). In RNA, the base uracil (U) replaces thymine. DNA and RNA molecules are polymers made up of long chains of nucleotides. Nucleotides can be synthesized in cells by de novo pathway from precursor amino acids, including glutamine, formate, glycine, and aspartic acid. Broiler chickens that experience heat stress cannot synthesize nucleotides in sufficient amounts, thus experiencing stunted cell growth, especially epithelial cells in the intestine (intestinal villi) and reduced metabolism, absorption, digestibility and performance, which all lead to less body weight (Aldiyanti et al. 2022).

Nucleotide is vital in energy metabolism, coenzyme formation, and cell defence mechanisms (Dawood et al. 2018). Previous research by Mohamed et al. (2020) has demonstrated that the supplementation of 0.1% nucleotide has improved performance and reduced *Clostridium perfringens* infection in broiler chickens. Meanwhile, 1.5% supplementation tends to boost growth and enhance the intestinal morphology of broiler chickens (Trairatapiwan et al. 2017). Furthermore, supplementing nucleotide to swine has been reported to improve immune response and the well-being of broiler chickens' digestive tracts, making nucleotide a potential alternative for AGP (Adedokun and Olojede 2019).

Tugiyanti et al. (2022) studied the effect of the combination of 0.5 g nucleotide and 0.6 g turmeric powder and reported the inability of this supplementation to significant improvement in the immune response of broiler chickens kept in battery cage, which was closely related to poultry welfare (Zhao et al. 2014; Mesa et al. 2017) The comfort of animals in the cage depends on the microclimate as well as the types of flooring inside the cage (Adler et al. 2020). Broiler farmers in Indonesia commonly use litter floor, slat floor, or slat-litter floor combinations.

Litter floor cage using husk is the most used type of floor in the broiler industry in Indonesia. Litter floor has some drawbacks,

such as poorly managed litter, which can cause respiratory disease and dermatitis on the feet and breasts (Çavuşoğlu et al. 2018). Meanwhile, keeping litter husk dry and unspoiled in the litter cage is difficult because broiler chickens drink water frequently (Petek et al. 2014). Wet litter will increase microorganism activities in fermenting organic materials, which further triggers heat release and negatively affects broilers' welfare, performance, and quality of carcass (De Jong et al. 2014; Petek et al. 2014; Saleh et al. 2021). Slatted floor systems resulted in higher body weights, reduced total feed consumption, lower feed conversion ratio, and less incidence of foot pad and hock joint deformations in broiler chickens (Eratalar 2021; Topal and Petek 2021). Although a slatted floor cage provides better air circulation, its design often makes broilers slip and bruise their feet and wings, deteriorating the carcass quality. Slatted-floor cages are mainly used in an open coop for chicken production (Heitmann et al. 2020). Considering that cage temperature is closely related to the cage floor, the production of nucleotides, and the health of digestive organs, this research aims to investigate the physiological response to heat stress of broiler chickens supplemented with nucleotide and turmeric powder and kept in different types of cage floors litter, slat, and slat-litter combination.

2 Materials and Methods

2.1 Birds and Experimental Design

A total of 245 broiler day-old chicks (DOC) were randomly allotted to seven treatment groups. Each group was replicated five times, and each cage unit contained seven DOC. The treatments were the combined supplementation of nucleotide and turmeric extract and the types of floors for poultry maintenance (litter, slat, and combination of slat-litter). The details of the formulated groups were as follows:

A: Basal feed (control) three floor types

B: Basal feed + nucleotide 0.5g/kg feed + turmeric powder 0.6g/kg feed+ litter floor

C: Basal feed + nucleotide 0.5g/kg feed + turmeric powder 0.6g/kg feed+ slatted floor

D: Basal feed + nucleotide 0.5g/kg feed + turmeric powder 0.6g/kg feed+ (slat-litter) floor combination

E: Basal feed + nucleotide 1g/kg feed + turmeric powder 1g/kg feed + litter floor

F: Basal feed + nucleotide 1g/kg feed + turmeric powder 1g/kg feed + raised cage

G: Basal feed + nucleotide 1g/kg feed + turmeric powder 1g/kg feed + (slat-litter) floor combination

2.2 Birds Diet and Husbandry

The basal diet used in this research for broiler chickens during the starter period was a commercial diet with formulation given in Table 1 containing 21% protein and 3,100 kcal/kg ME, while the basal diet at the finisher period had 19% protein and 2,900 kcal/kg ME.

Table 1 Feed formulation of the feed used in the experiment

Ingredients (%)	Starter diet (%)	Finisher diet (%)
Maize	53.6	62.5
Soya bean meal	35.6	29.2
Meat bone meal	5.0	5.0
Palm oil	3.1	1.2
Stone Grounded	0.64	0.4
Dicalcium phosphate (DCP)	0.44	0.5
Premix	1.6	1.3
Calculated Analysis		
Crude protein (%)	21	19
Crude Fibre (%)	2.57	2.6
Lysine	1.20	0.70
Methionine	0.71	0.50
Metabolizable energy (kcal/kg)	3100	2900

The nucleotides used were BioNutrend, produced by Wuhan Sunhy Biology Co. Ltd., China. The average nucleotide composition was 27.4% adenine, 22.6% guanine, 22.8% cytosine and 27.1% thymine, and the turmeric extract was Herbana brand produced by PT, Deltomed Laboratories. The broilers were maintained at 19-32℃ with a 292mm precipitation rate, 95 % humidity, and 10 km/h wind velocity. Broilers were supplemented with vitamins and vaccines at their appropriate age and kept in 30 units per cage, each measuring 1.5x1.5x1 m for 35 days. Feed and water were provided *ad libitum*.

2.3 Blood Profile Measurement

The blood sample was collected to measure erythrocyte, leukocytes, and leukocyte differential using a 3-ml pipette, EDTA vacutainer, *ice pack* and *cool box*. At the end of the maintenance period, venipuncture was conducted on the wing using the pipette and the blood was put into an EDTA vacutainer and shaken to prevent blood clotting, then put into an ice box prefilled with an ice pack. The erythrocyte count was performed by drawing the blood sample using an erythrocyte pipette up to 0.5 ml/µl and incorporating Hayem's solution up to 11 ml/µl. After that, the solution was homogenized, and then some drops were put into the improved Neubauer counting chamber sealed with cover glass. The erythrocyte was counted using a microscope with 100x magnification and a hand counter. The erythrocyte differential counts were conducted by placing a drop of blood onto the counting chamber, covered with a cover glass, and counting the number of leukocytes under the microscope.

The hemoglobin concentration was evaluated by matching acid hematin solution with a standard coloured solution found in Sahl's hemoglobin meter according to the methods described by Dein (1984). The Sahli (1902) method is based on converting hemoglobin to acid haematin (brown colour) and then visually matching its colour against a solid glass standard. Diluted hydrochloric acid was mixed into a graduated cylinder with 20µl of the blood sample, and distilled water was added until the colour of the diluted blood sample matched the glass standard. The blood sample's hemoglobin level determined the dilution, as Philippe (2009) described.

2.4 Physiological Response Measurement

Body temperature was measured on the broiler's back using an infrared thermometer (Codenoll digital infrared laser thermometer). The rectal temperature was measured by inserting a digital thermometer into the broiler's rectum (Suprayogi et al. 2017) to a depth of 1/3 of the rectum until the thermometer beeped. The respiratory rate was measured by calculating the breath or looking at the broiler's chest movement within 10 seconds. The pulse frequency was obtained by placing a stethoscope onto the broiler's left chest and counting the pulse for one minute (Hartono et al. 2002). Meanwhile, the ratio of bursa fabricius to the spleen was obtained after the broilers were sacrificed at the age of 35 days. The Bursa fabricius and spleen were weighed and divided by the broiler's body weight.

2.5 Statistical Analysis

This experimental research was conducted in a completely randomized design (CRD). All data were subjected to one-way ANOVA using the SPSS 25.00 (SPSS Ltd., Surrey, UK). Duncan's test would ensue when significant differences were observed across all variables measured at the probability of $P < 0.05$ for all treatment groups.

3 Results and Discussion

3.1 Blood profile

The effects of nucleotide, turmeric powder, and floor types on broilers' blood profile (erythrocyte, hemoglobin, PCV, Total plasma proteins, leukocytes, and leukocyte differential) are presented in Table 2. The values for the red series and hematimetric indexes were calculated for RBC (2.20±0.06 - 2.93±0.07 ×106/µL), PCV (21.33±2.31-28.33±1.5%), Hb (5.91±0.25-9.93±0.67 g/dL), TPP (3.53±0.12-3.80±0.20fL). The values of white series were WBC (8.52±1.00 - 14.25±3.92/µL), heterophils (59.67±16.80- 77.33±6.43 %), monocytes (2.67±1.53-4.33±0.58 %), lymphocytes (16.67±6.66- 31.00±9.85 %). Blood profile data in this study were within the normal range (Aldiyanti et al., 2022).

Tugiyanti et al. (2022) and Aldiyanti et al. (2022) stated that blood is an essential component for the physiological regulation of the body and an indicator of poultry health. While leukocytes are part of the immune system against some infectious diseases, erythrocytes determine physiology. Leukocytes are divided into agranulocytes (lymphocytes and monocytes) and granulocytes (basophils, eosinophils, and heterophils). Lymphocytes are the most abundant leukocytes in chickens, and their size varies from small to large, as in mammals (Colin et al. 2015). According to Yuniwarti (2015), erythrocytes function in gas exchange and oxygen distribution into cells and are used by cells for metabolic processes. Oxygen is essential in producing adenosine triphosphate (ATP), the energy for cells to metabolize (Salin et al. 2015). The process of forming new erythrocytes daily requires precursors to synthesize new cells, including iron, vitamins, and amino acids, while the hormone erythropoietin regulates the cell formation process.

Supplementing nucleotide and turmeric extract to broiler chickens kept in cages with different types of floors resulted in a non-significantly different effect (P>0.05) on the erythrocyte,

Table 2 The effect of feed treatment on the blood profile of broiler chickens

Parameter	Treatment							
	A	B	C	D	E	F	G	Sig.
Erythrocyte (10^6/µl)	2.75±0.15^{c}	2.93±0.07^{c}	2.77±0.23^{c}	2.67±0.36^{bc}	2.20±0.06^{a}	2.35±0.03^{ab}	2.40±0.07^{ab}	0.003
HB (g/dL)	9.6±0.36^{d}	8.75±0.37^{c}	9.93±0.67^{d}	7.87±0.64^{b}	7.33±0.23^{b}	5.91±0.25^{a}	6.13±0.03^{a}	0.006
PCV (%)	24.33±3.21	28.33±1.53	27.33±4.93	24.67±5.03	21.33±2.31	22.00±2.00	23.67±2.51	0.159
TPP (g/dL)	3.80±0.20	3.67±0.12	3.73±0.23	3.67±0.23	3.67±0.12	3.53±0.12	3.67±0.12	0.597
Leukocyte (/µl)	14.25±3.92^{b}	11.70±0.11^{b}	8.52±1.00^{a}	14.06±4.83^{b}	13.68±5.02^{b}	12.73±0.04^{b}	11.12±0.02^{b}	0.007
Heterophils (%)	68.33±1.68	73.67±8.39	59.67±16.80	64.67±10.26	65.33±8.08	77.33±6.43	70.25±10.23	0.427
Lymphocyte (%)	19.33±8.02	30.00±8.72	31.00±9.85	23.00±10.15	28.67±10.21	16.67±6.66	21.33±10.21	0.319
Monocyte (%)	4.33±0.58	4.33±0.58	3.67±0.58	3.00±1.73	4.00±1.73	2.67±1.53	3.67±1.73	0.488

Data are mean of five replicates; ± Standard Error of mean; A: Basal Feed (control); B: nucleotide 0.5 g + turmeric powder 0.6 g + litter floor; C: nucleotide 0.5 g + turmeric powder 0.6 g + slatted floor; D: nucleotide 0.5 g + turmeric powder 0.6 g + (latted+litter) floor combination; E: nucleotide 1 g + turmeric powder 1 g + litter floor; F: nucleotide 1 g + turmeric powder 1 g + slatted floor; G: nucleotide 1 g + turmeric powder 1 g + (slat-litter) floor combination; Values without common superscripts letters in row differ significantly at LSD P<0.05

hemoglobin, and PCV levels, as presented in Table 2. Turmeric extract helps the nucleotide trigger the hypothalamus for inhibiting heat stress, facilitating the erythropoiesis process and preventing the delay of hemoglobin synthesis (Sugiharto et al. 2011). According to Hafez et al. (2022), the antioxidant properties of curcumin reduce free radicals and improve immune performance and haematology. The red blood cell count in the present research was 2.20 and 2.35 x10^6/dl in the E and F groups, respectively, but the hemoglobin level was slightly under 10.26-10.71 g/dL as reported by Daudu et al. (2020). Meanwhile, Ifelayo et al. 2020) reported PCV slightly lower than 30.83-32.33%.

Supplementing nucleotide and turmeric extract to broiler chickens kept in cages with different types of floors resulted in a non-significant difference (P>0.05) in the levels of erythrocyte, hemoglobin, and PCV (Table 2). Different types of cage floors generate different temperatures inside the cage, but this did not affect broiler chickens' levels of erythrocyte, hemoglobin and PCV. Turmeric extract helps the nucleotide trigger the hypothalamus for inhibiting heat stress, using the erythropoiesis process and preventing declining hemoglobin synthesis (Zhang et al. 2015; Balakrishnan et al. 2023). According to Hafez et al. (2022) curcumin's antioxidant properties reduce free radicals and improvchickens' immune performance and haematology. The red blood cell count in the present research was 2.42-2.84 x10^6/dl, but the hemoglobin level was slightly under 10.26-10.71 g/dL, as Daudu et al. (2020) reported. Meanwhile, Kafi et al. (2017) reported PCV slightly lower than 31.50-32.50%.

Total protein plasma (TPP), leukocytes, and leukocyte differential (lymphocyte and monocyte) are important indicators in broiler chickens to evaluate their health status and protein metabolism regarding the activities of some body organs like the liver and kidneys. The phagocytosis process by leukocytes will protect the body from diseases (Rosales and Uribe-Querol 2017). The total plasma protein and leukocyte levels in broiler chickens depend on some conditions, including stress, physiological activities, nutrition, and age. Previous study reported the range of total plasma protein (TPP) was 4.17±0.05-4.27±0.05 g/dL (Ifelayo et al. 2020), leukocytes was 11.18-14,78 x10^9/L (Makeri et al. 2017), leukocyte differential (lymphocyte) was 58.00±4.80-71.00-6.60%, and monocyte was 5.25± 1.50-7.75-3.90% (Aldiyanti et al. 2022). In this research, TPP, leukocytes and leukocyte differential were not significantly different (P>0,05) from these studies. The absence of increasing TPP or declining leukocytes and leukocytes differential and non-existent variation in temperature across different types of cage floor demonstrated that broiler chickens were healthy and not suffering from bacterial infection (Salam et al. 2013; Saputro et al. 2013). Different cage temperatures because of different types of floor did not cause protein aggregation and imbalance in the overall protein homeostasis in the cells.

3.2 Physiological Status

The thermal comfort (TC) zone for chickens is characterized by a range of environmental temperatures within which chickens have minimal and nearly constant energy expenditure for maintaining body temperature (Chang et al. 2018). The thermoregulatory system adjusts physiological responses to increase or decrease body heat loss. Outside of the TC zone is a situation characterized by heat or cold stress where birds adjust their metabolism to compensate for their energy balance (Liu et al. 2021; Belkhanchi et al. 2023).

Body temperature and rectal temperature are the indicators of comfort in broiler chickens. According to Skomorucha and

Sosnówka-Czajka (2017), chickens' average body and rectal temperature is 41-42°C and 41.4-41.9°C, respectively. In this study, broiler chickens' body and rectal temperatures were lower than those of previous studies (Table 3). Moe et al. (2017) stated that chickens' body temperature would drop by 2°C after one-minute handling. Furthermore, the results of the analysis of variance of this study showed that supplementing nucleotide and turmeric extract into feed and different types of floor used in chicken cages did not significantly affect (P>0,05) the body temperature but had a significant effect (P<0,05) on the rectal temperature of broiler chickens. This is because rectal temperatures are considered the peripheral temperature, the most similar to the core body temperature, which is easily affected by the environmental temperature. This study recorded the temperature of the litter floor was 28.9-30.3°C, the slatted floor was 27.8-30.2°C, and the combined slat-litter floor was 28.7-30.2°C. The litter floor, slatted floor, and the combination of slat-litter floor combination resulted in different microclimate temperatures inside the cage (Li et al. 2017). When the environmental temperature is high, the rectal temperature will increase. In other words, rectal temperature manifests a thermoregulation mechanism in chickens to balance the generated heat and emitted heat to maintain the ideal body temperature. In addition, the supplementation of nucleotide and turmeric extract can improve the performance of digestive tracts and help support the proper functions of the thermoregulation system and physiological response in broiler chickens (Taylor et al. 2014; Trairatapiwan et al. 2017). To maintain the ideal body temperature, chickens either increase or decrease heat loss (Taylor et al. 2014).

The thermoregulation system in broiler chickens is related to the molecular functions of broiler chickens' hormone and nerve system(Ruuskanen et al. 2021). Thyroid hormones (THs, triiodothyronine, T3 and thyroxine, T4) are the most important hormones in regulating thermogenesis (Sawicka-Gutaj et al. (2022). Chickens that suffer from heat stress will experience a declining synthesis of nucleotide that results in low production and release of thyroid hormones; consequently, chickens have a high body temperature (Bruno et al. 2011; Balakrishnan et al. 2023). The supplementation of nucleotide and turmeric extract would improve the comfort of broiler chickens. Broiler chickens living within the comfortable thermal zone have a constant heat production and balanced heat loss to maintain the ideal body temperature (Chang et al. 2018).

In addition to body and rectal temperatures, panting is a commonly used physiological response by a distressed chicken (Iftitah et al. 2022). When a broiler chicken is panting, it will draw heavy and rapid breaths with an open mouth, which causes water loss through evaporation that will help limit heat stress due to high temperature or vigorous physical activities (Kang et al. 2020). Respiratory rate (RR) and pulse rate (PR) are the physiological responses that can be utilized to evaluate the impact of the thermal environment on the thermoregulation status in broiler chickens. RR and PR in this study were within the normal range (Table 3.), which, as reported in the previous study, was 19.90 breath/minute, and the pulse rate was 67.41 ± 7.22 beats/min (Ijadunola et al. 2020; Bello et al. 2022). The analysis of variance showed that the supplementation of nucleotide and turmeric extract into feed and the use of different types of cage floor did not significantly affect (P>0,05) RR and PR. It demonstrated that both supplementation and different floors successfully inhibited heat stress in broiler chickens, so RR and PR did not increase and were relatively similar across treatments. The constant RR and PR showed that broiler chickens' thermoregulation system (Hypothalamus, hypophyses, autonomic nervous system) usually functions (Nawaz et al. 2021).

The lymphatic organs in poultry's immune system consist of the primary and secondary lymphoids. Bursa fabricius is the primary organ, and the spleen is the secondary lymphatic organ (Toivanen

Table 3 The effect of treatments on the physiological status and percentage of bursa fabricius and spleen

	Feed treatment							
Parameter	A	B	C	D	E	F	G	Sig.
Body temperature	37.49±1.18	38.05±0.53	38.25±1.28	37.80±1.58	36.85±1.36	38.02±0.99	37.78±1.28	0.618
Rectal temperature	40.75±0.86^{b}	39.80±0.15^{a}	39.98±0.18^{a}	40.28±0.38^{ab}	40.01±0.12^{a}	39.98±0.14^{a}	40.06±0.03^{a}	0.047
Respiratory rate (times/minute)	27.25±3.93	28.40±2.86	27.45±3.64	28.30±4.24	26.50±4.33	28.40±3.42	27.80±3.00	0.062
Pulse (times/minute)	69.75±4.27	69.48±4.06	70.04±7.65	84.95±11.44	70.78±7.93	73.69±8.84	72.45±6.89	0.074
%Bursa Fabrisius	2.26±0.02^{a}	2.36±0.04^{bc}	2.38±0.03^{c}	2.39±0.03^{c}	2.33±0.02^{b}	2.38±0.03^{c}	2.34±0.03^{b}	0.000
%Spleen	0.15±0.04	0.11±0.02	0.15±0.01	0.14±0.02	0.14±0.01	0.13±0.01	0.14±0.01	0.083

Data are mean of five replicates; ± Standard Error of mean; A : basal feed (control); B: nucleotide 0.5 g + turmeric powder 0.6 g + litter floor; C: nucleotide 0.5 g + turmeric powder 0.6 g + slatted floor; D: nucleotide 0.5 g + turmeric powder 0.6 g + (latted+litter) floor combination; E: nucleotide 1 g + turmeric powder 1 g + litter floor; F: nucleotide 1 g + turmeric powder 1 g + slatted floor; G: nucleotide 1 g + turmeric powder 1 g + (slat-litter) floor combination.

1998; Berthault et al. 2018). The index of bursa fabricius (BFI) and lien index (LI) of broiler chickens in this study were 2.26-2.39% and 0.11±-0.15% (Table 3). BFI and LI in this study were higher than those of Hakim et al. (2021), reporting that BFI and LI in broiler chickens supplemented with nucleotide were only 0.044-0.047% and 0.112-0.146%, respectively. The result of the analysis of variance showed that the supplementation of nucleotide and turmeric extract and different types of floors did not significantly affect (P>0,05) LI, but this combination significantly affected (P<0,05) the BFI of broiler chickens. This is because the average temperatures of the litter floor, slatted floor, and slat-litter floor combination were different, impacting the index of bursa fabricius differently (Kusnadi 2009). These results follow the findings of Hirakawa et al. (2020) that high environmental temperature can cause the weight loss of several lymphatic organs such as bursa fabricius, spleen, and thymus, and therefore, less lymphocyte production. The average temperature of litter floor cage in the morning, afternoon and night was 23.98±2.38℃, 31.8±3.47℃, and 21.84±1.23℃ respectively. While the average temperature of the slatted floor in the morning, afternoon, and night was 22.98±2.81℃, 30.14±2.88℃, and 20.32±1.67℃, respectively, the slat-litter floor was 22.76±1.94℃, 30.3±2.32℃, and 21.03±2.68℃, respectively. The excessive reactive oxygen species (ROS) resulting from heat stress (HS) has imposed unwanted effects on the immune balance of organs immune systems (Hirakawa et al. 2020; Liu et al. 2021). Bursa Fabricius is the central organ of the immune system in broiler chickens that can produce lymphocyte B and antibodies specific to complete the immune-specific response and play important roles in maintaining the immune system of poultry (Liu et al. 2021). Nucleotide and turmeric extract keep the BFI high, thus producing a high level of lymphocytes. As a result, the antibody produced by the lymphocyte (like gamma globulin) is relatively high. Curcumin inhibits the dysfunction of liver mitochondria and damaged mtDNA and stimulates the thioredoxin mitochondria system in broiler chickens, which undergo heat stress (Zhang et al. 2018).

The spleen is the biggest peripheral lymphatic organ in chickens, which play an important role in the antibacterial and antiviral immune response against antigen obtained by the chickens. The development of peripheral lymphatic organs is closely related to maintaining immune function (Liao and Padera 2013; Zhang et al. 2019). The effects of the treatment were not different on LI because the chickens were in healthy conditions during the observation. The role of the spleen in the defence system is related to the immunology response against antigen that can reach blood circulation to defend against the invasion of organisms or toxins before they spread. Furthermore, the spleen is the organ where antibody-producing cells are maturing. In addition to being a defence system against microorganisms, the spleen is the central location where macrophage degrades old erythrocyte cells and reacts against the antigens carried in the bloodstream while performing immunological filtration to blood (Lewis et al. 2019).

Conclusions

The supplementation of nucleotide and turmeric extract into feed, and different types of cage floor did not significantly affect (P>0.05) body temperature, respiratory rate, pulse rate, lien index, PVC, TPP, and heterophils, lymphocyte, monocyte, but significantly affected (P<0.05) erythrocyte level, hemoglobin, leukocyte, the rectal temperature and the index of bursa fabricius of broilers. The combined supplementation of nucleotide and turmeric extract in feed and the types of cage floor tend to reduce the comfort of broiler chickens.

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Author contributions

ET, I., and R. contributed to designing the research model data analysis and wrote the paper. D. M. S., S. H. and T. L. W. contributed to enrich the discussion.

Conflict of interest

The authors state no conflict of interest.

Data availability statement

The data sets generated during the current study are available from the corresponding author on reasonable request

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