Research Article

Egg Production and Physiological Assessment of Sentul Hens in Temperate and Lowland Regions of West Java, Indonesia

Andi Mushawwir[1]*, Lovita Adriani[1], Ronnie Permana[1], Eli Sahara[2]

[1]*Laboratory of Animal Physiology and Biochemistry, Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, Padjadjaran University, Jalan Ir. Soekarno Km. 21 Jatinangor, Sumedang, West Java, Indonesia;* [2]*Animal Science, Faculty of Agriculture, Sriwijaya University, Palembang, Indonesia.*

Abstract | The Sentul hen is a local Indonesian chicken that has been distributed across various regions in West Java. The location's topography significantly impacts the chickens' performance, as it is closely related to the environment's macro and microclimatic conditions. Low-lying areas tend to increase the risk of heat stress in chickens. In this study, 300 Sentul chickens were observed at different altitudes. The research sites at low elevations, located 150 meters above sea level, were in northern Majalengka and Cirebon, where the recorded environmental temperature was 30.5°C, and the humidity was 89%. In contrast, the sites at medium altitudes, ranging from 750 to 850 meters above sea level, were in Sumedang and South Subang, where the average temperature was 24°C, and the humidity was 65%. The study, which lasted for six months, aimed to assess the effects of heat stress on various stress markers, including malondialdehyde (MDA), cholesterol, total iron binding capacity (TIBC), and carbonic acid (H_2CO_3) levels. Additionally, it examined how heat stress affected the eggshell formation of Sentul chickens in the laying phase. Sixty Sentul chickens in the laying phase were used in this research, with two treatments: one group reared at medium altitude and the other at low elevation. The analysis revealed that the Sentul-laying chickens raised in the lowlands were more susceptible to heat stress, which negatively impacted the thickness of the eggshells they produced.

Keywords | Sentul chicken, Heat stress, Eggshell, Metabolism, Egg production, Altitude

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***Correspondence** | Andi Mushawwir, Laboratory of Animal Physiology and Biochemistry, Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, Padjadjaran University, Jalan Ir. Soekarno Km. 21 Jatinangor, Sumedang, West Java, Indonesia; **Email:** mushawwir@unpad.ac.id
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INTRODUCTION

Chickens are classified as *homeothermic* livestock, with specific characteristics of not having sweat glands, and almost all parts of the body are covered with feathers. This biological condition causes poultry in hot conditions to have difficulty dissipating body heat into the environment. The maintenance system set will significantly affect the performance of Sentul Chicken (Rahmania et al., 2022). The maintenance of Sentul Chickens at medium and low altitudes will impact these chickens, including physiological conditions. Physiological conditions in chickens can be determined based on blood plasma metabolite profiles (Kharazi *et al*., 2022). Every living thing has a physiological zone called homeostasis. Homeostasis will be disrupted if the chicken experiences heat stress.

High environmental temperatures can lead to heat stress (Aengwanich, 2007; Adriani and Mushawwir, 2020; Mushawwir *et al*., 2024). The mid and lowlands' environmental conditions vary significantly due to several factors, including temperature, humidity, and rainfall (El-Attrouny *et al*., 2021; Petrilla *et al*., 2022). These variations impact the health of Sentul chickens. When temperatures rise, chickens often pant to help evaporate body heat, significantly increasing their energy expenditure. This process consumes a lot of energy and stimulates the release of adrenocorticotropic hormone (ACTH). In turn, ACTH activates glucocorticoids and epinephrine, increasing heart rate and enhancing vascular dilation. Previous research has demonstrated that these physiological responses result in increased expression of heat shock proteins (HSP) and reduced feed intake, protein absorption, and vitellogenesis (the synthesis of egg precursors) in the liver. These findings highlight the detrimental effects of heat stress on the health and productivity of chickens. Continuous heat exposure causes the denaturation of essential proteins, such as enzymes and hormones, and decreases metabolism.

Higher temperatures make chickens raised at low altitudes more susceptible to heat stress. It is well known that the ambient temperature increases as altitude decreases from sea level. Both high and low ambient temperatures negatively impact weight gain (Tanuwiria *et al*., 2022a), feed conversion efficiency, health, and mortality rates (Mushawwir *et al*., 2011), as well as overall performance (Tanuwiria *et al*., 2022b; Kamil *et al*., 2020). Research has shown that extreme ambient temperatures can induce heat stress in chickens (Mushawwir *et al*., 2020; 2021a) and can lead to increased mortality (Wang *et al*., 2023; Zhan *et al*., 2017). Heat stress reduces performance, affecting body weight and egg production and increasing mortality rates. It disrupts metabolism (Hernawan *et al*., 2017; Kamil *et al*., 2020; Tanuwiria *et al*., 2023) and leads to the breakdown of fatty acids (lipid peroxides) due to increased free radical production (Mushawwir *et al*., 2021b; 2023; Firmansyah *et al*., 2024). The presence of free radicals can heighten lipid peroxidation (Nurfauziah *et al*., 2024) and result in the formation of malondialdehyde (MDA) in the blood (Firmansyah *et al*., 2024; Purwanti *et al*., 2024). Additionally, heat stress triggers a more significant loss of carbon dioxide (CO_2), which in turn reduces the concentration of carbonic acid (H_2CO_3) in blood plasma (Mushawwir *et al*., 2024; Manin *et al*., 2024). In Sentul chickens, heat stress also leads to lower cholesterol levels because it enhances gluconeogenesis, causing some cholesterol to be converted into glucose through this metabolic pathway.

Research on Sentul chickens reared at different altitudes is necessary. Valid and comprehensive data from this study can guide the selection of optimal rearing locations and improve management practices for Sentul chicken farming. This investigation will clearly illustrate how altitude affects the stress levels of Sentul chickens during the laying phase and its impact on the quality of the eggshells produced.

MATERIALS AND METHODS

Animal Samples and Methods

This study was conducted with two experimental treatments, each involving 300 samples of Sentul chickens. This sample size is representative of the entire population in both research locations, covering 45% of the total population. Observations were carried out in two areas at different altitudes. One group of Sentul chickens was reared at a medium altitude, while the other group was reared at a low altitude, with each group consisting of 150 chickens.

Location and Research Procedures

The experimental animals in this study comprised 300 Sentul chickens in the layer phase, aged 25 weeks. There were 150 Sentul chickens raised at medium altitudes and 150 raised at low altitudes.

The study sites at low altitudes, located 150 meters above sea level, were in northern Majalengka and Cirebon. During this study phase, the recorded environmental temperature, humidity, wind velocity, air pressure, and oxygen content were 30.5°C, 89%, 5.7 km/h, 1019 mbar, and 15 mg/L, respectively. The study sites at medium altitude, between 750 and 850 meters above sea level, were in Sumedang and South Subang, where the average temperature, humidity, wind velocity, air pressure, oxygen content were 24°C, 65%, 4.8 km/h, 1001 mbar and 11 mg/L respectively. Automatic detectors were installed at each observation site to record temperature, humidity, wind speed, pressure, and air oxygen content. The collection of physical environmental data was automated and conducted every week. Throughout the study, no significant fluctuations in the data were observed.

Blood samples were collected from the neck (jugular vein) using a 3 mL syringe. After collection, the blood was placed into a container with EDTA, shaken gently, and stored in a cooling box to prevent clotting before analysis. The analysis focused on measuring MDA, cholesterol, TIBC, H_2CO_3, CO_2 concentration, and shell thickness. All blood sample analyses were conducted using the spectrophotometric technique, following the protocols of the Rendox and Biolabo kits. The overall analytical procedure included reagents, standards, and blank solutions. Reagents were added to cuvettes containing 10 µL of plasma from standards and samples, each measuring 100 µL. Blank solutions were prepared without any samples. All cuvettes containing reagents, samples, and standards were measured at 450-500 nm wavelengths.

Data Analysis

The data analysis conducted in this study utilized an unpaired t-test (independent sample t-test). This analysis established a 5% error margin or a 95% confidence level.

RESULTS AND DISCUSSION

Altitude Impact on Performance

Based on the results of the analysis of variance (Figure 1), it shows that Sentul chickens in the layer phase reared in medium altitude areas produce higher egg production compared to Sentul chickens reared in low altitude areas (P<0.05). High ambient temperature hurts egg production. If chickens are exposed to environmental temperatures higher than thermoneutral, there is a direct change in the endocrine hormone activity of chickens (Kamil *et al.*, 2020; Tanuwiria *et al.*, 2022a).

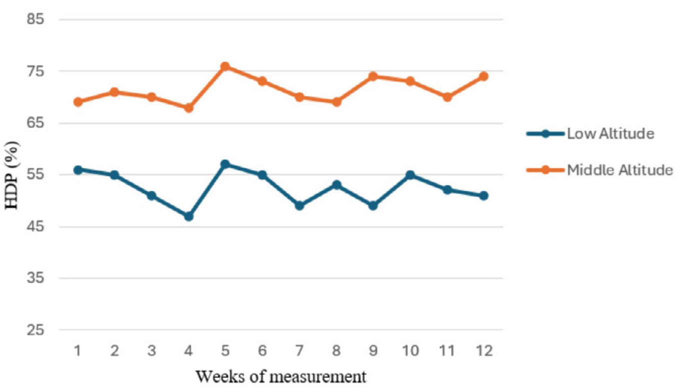

Figure 1: Impact of altitude on egg production.

The study results indicate that more livestock will experience heat stress as environmental temperatures rise. Heat stress leads to reduced feed consumption, which in turn disrupts the hormonal balance required for optimal reproductive performance in chickens. Consequently, this results in suboptimal egg production. Figure 1 shows that the average percentage of egg production in Sentul chickens during the laying phase differs significantly (P<0.05) between medium and lowland altitudes. In contrast, the average monthly HDP and egg cracking of Sentul chickens during the study can be seen in Table 1.

Chickens are homeothermic, and their ability to dissipate heat decreases as the ambient temperature increases. In addition to temperature, humidity also affects the perceived temperature of the chicken. Midland areas usually have lower temperatures and humidity, unlike lowland areas with high temperatures and humidity.

High temperatures and humidity create more stress for chickens than low temperatures and moisture levels. Heat stress has complex effects on metabolism, as the body's tissues work to regulate normal conditions in the chicken. Chickens lose heat through panting (Mushawwir *et al.*, 2021a,b). This panting requires energy for the muscles involved in respiration. In extremely high temperatures, chickens may breathe more rapidly and intensely, with respiratory rates increasing by up to ten times (Ahmed-Farid *et al.*, 2021).

Heat stress is a significant factor contributing to declines in performance. When an animal experiences heat stress, panting occurs, which leads to reduced levels of carbon dioxide (CO_2) in the blood. This results in an imbalanced acid-base condition known as blood alkalosis. As CO_2 levels decrease, the binding of oxygen to erythrocytes (oxyhemoglobin) is inhibited, lowering the oxygen available for nutrient metabolism. The impacts of heat stress include decreased egg production due to reduced nitrogen retention (Abouslezz *et al.*, 2022) and diminished protein and amino acid digestibility (Dudi *et al.*, 2023). The eggshell formation, primarily composed of calcium carbonate ($CaCO_3$), starts with the creation of carbonic acid (H_2CO_3), with CO_2 being a crucial precursor. Excessive panting, necessary for evaporative cooling, leads to a significant release of CO_2, which is a primary reason for the decline in egg production and an increase in shell cracking, as illustrated in Table 1, Figures 1 and 2.

Table 1: The average monthly HDP and egg cracking of Sentul chickens during the study at medium and low topography.

	Altitude	
Parameters	Medium Plains	Lowlands
HDP (%)	$71.3±2.14^a$	$49.5±1.75^b$
Egg cracking (%)	$3,4±0.18^a$	$6,1±0.42^b$

[a,b]Means followed by different notations in the same column indicate significant differences (P<0.05).

In addition, heat stress also inhibits the secretion of thyroid hormone (TRH) from the hypothalamus and thyroid-stimulating hormone (TSH) from the pituitary gland, thereby inhibiting the secretion of thyroxine, which results in growth inhibition. When CO_2 levels in the blood of heat-stressed chickens decrease, the blood becomes alkaline. This reduces the oxygen binding capacity of the blood (oxyhemoglobin), thereby disrupting metabolic processes. The subsequent impact is that the biological value of protein and nitrogen retention will decrease, resulting in reduced growth and productivity of Sentul chickens.

The increased body temperature of chickens will undoubtedly affect the release of hormones, such as FSH *(*follicle-stimulating hormone), LH (luteinizing hormone), and ovarian steroids. FSH and LH change with increasing temperature (Firmansyah *et al.*, 2024). FSH plays a role in follicle maturation, and LH produces mature eggs. The ovaries produce hormones during growth. FSH produced by the pituitary gland produces follicles in the ovary, and FSH plays the most crucial role in determining the number of eggs (Purwanti *et al.*, 2024; Selim *et al.*, 2021).

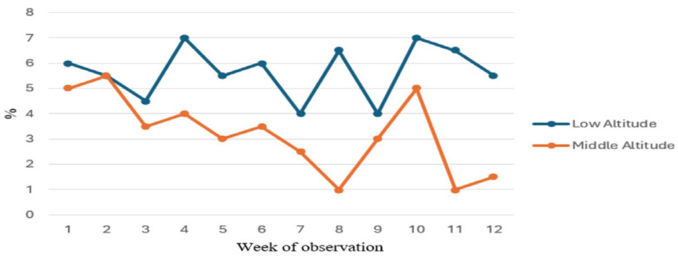

Figure 2: Altitude impact on % egg cracking.

Figure 2 and Table 1 show that Sentul chickens in the layer phase reared in lowland areas produce a higher percentage of egg cracks than Sentul chickens reared in lowland areas (P<0.05). Chickens living in lowland areas with lower temperatures tend to experience lower heat stress, which can positively impact eggshell quality. The research results by Adriani *et al.* (2021; 2024) stated that the thickness of egg shells in different topographies ranged from 0.38 mm to 0.47 mm. The highest to lowest average eggshell thickness was obtained from lowlands (0.38 mm), mediumlands (0.42 mm), and highlands (0.47 mm). Feeding with high levels of calcium and other nutrients is instrumental in forming strong egg shells (Muller *et al.*, 2022). However, heat stress may cause hens in hot areas to eat less, affecting calcium absorption and, ultimately, shell quality.

Shell weight and thickness are variables that determine shell quality. Eggshells mostly form from calcium carbonate ($CaCO_3$) sourced from feed and bone marrow. Research that has been reported by Selim *et al.* (2021) and Setiawan *et al.* (2024) showed that 35-75% of calcium for eggshell formation is obtained from feed, but if calcium from the feed is insufficient for mineralization, calcium from bone marrow is used.

Calcium obtained from bone marrow is limited. Therefore, when the temperature rises, and feed consumption decreases, the calcium required for shell formation will decrease, resulting in thinner and softer shells. Thinner shells will lead to a higher percentage of shell cracks. Therefore, topography has an authentic influence on the thickness of the shell. The same research results have been reported by Mushawwir *et al.* (2021b; 2023) that the poor quality of egg shells at high temperatures (>32°C) is also caused by low feed consumption in chickens, and at high temperatures, feed consumption is reduced so that fewer nutrients are retained lower.

The blood plasma profile of Sentul chickens in the layer phase reared at medium and low altitudes can be seen in Table 2 below,The effect of rearing altitude on the blood plasma profile in the Sentul chicken layer phase is shown in Table 2.

Table 2: Blood plasma profile in layer phase sentul chickens at medium and low topography.

Parameters	Topography	
	Medium Plains	Lowlands
MDA (nM)	1.26±1.15[b]	3.61±1.31[a]
Cholesterol (mg/dL)	186.94±2.77[b]	113.74±3.26[a]
TIBC (mg/dL)	274.93±4.29[b]	229.03±2.42[a]

[a,b]Means followed by different notations in the same column indicate significant differences (P<0.05).

MDA (Malondialdehyde)

The study results in Table 3 show that the average MDA (malondialdehyde) levels in Sentul chickens in the layer phase reared in the lowlands are higher than those of Sentul chickens in the layer phase reared in the mediumlands. Based on the results obtained, it can be interpreted that the MDA profile is higher in the lowlands; this can be interpreted due to differences in temperature and humidity values at each altitude. High environmental stress causes the activation of catabolic and anabolic pathways simultaneously in the framework of homeostasis (Aengwanich *et al.*, 2007; Wang *et al.*, 2023) to maintain normal physiological conditions and energy supply.

The increased environmental temperature and heat stress experienced by chickens can also cause an increase in the level of free radicals in the cells.

The concentration of free radicals is the main trigger for the formation of MDA. High concentrations of free radicals result in the reaction of free radicals with fats, proteins, and cellular nucleic acids, causing localized damage and dysfunction of specific organs, so the level of damage to cells or body tissues due to free radical activity can be determined by measuring blood plasma MDA levels. Fat is a biomolecule that is susceptible to free radical attack. Animal cell membrane components contain a significant source of Poly Unsaturated Fatty Acid (PUFA) (Zhan *et al.*, 2017; Selim *et al.*, 2021; Muller *et al.*, 2022; Mushawwir *et al.*, 2024). These PUFAs are biomolecules easily damaged by oxidizing materials; the process is called fat peroxidation. The ongoing oxidative stress caused by high ambient temperatures harms health and production (Mushawwir *et al.*, 2020; Firmansyah *et al.*, 2024). When free radical activity exceeds antioxidant levels, it leads to oxidative stress, disrupting animal physiological balance. This disruption negatively impacts their health (Mushawwir *et al.*, 2021a) and productivity (Rahmania *et al.*, 2022; Muhammad *et al.*, 2023). One of the mechanisms behind this effect is tissue damage and cell death, which occur due to free radicals through lipid peroxidation. This process is marked by an increase in malondialdehyde (MDA) levels. As a result of tissue damage, the rate of egg synthesis decreases.

Cholesterol

The results in Table 2 show that the cholesterol levels of Sentul chicken obtained are still within normal limits. Normal cholesterol levels in chickens are between 125-200 mg/dl. The analysis results in Table 3 show that the blood cholesterol levels of Sentul chickens reared in the medium plains are higher than those of Sentul chickens reared in the lowlands. When chickens are exposed to heat stress, a common adaptation is panting to release body heat. This condition causes the need for additional energy to lay hens. Stress stimulates the hypothalamus to secrete the hormone CRH, which is then forwarded to the anterior pituitary to secrete ACTH. ACTH will be sent to the adrenal cortex and regulate glucocorticoid hormones (Mushawwir et al., 2024; Muhammad *et al*., 2023).

Table 3: Carbon dioxide, blood plasma carbonic acid, and egg shell thickness profiles in layer phase sentul chickens at medium and low topography.

Parameters	Topography	
	Medium Plains	Lowlands
Plasma CO_2	1546.53±42.51[a]	6643.51±27.38[b]
H_2CO_3 (U/L)	672.33±4.04[b]	286.49±6.35[a]
Thickness of Shell (mm)	0.36±0.02[a]	0.24±0.03[b]

[a,b] Means followed by different notations in the same column indicate significant differences (P<0.05).

Glucocorticoids can increase liver glucose production by increasing the rate of gluconeogenesis (Kharazi *et al*., 2022). It is known that gluconeogenesis is the formation of glucose from non-carbohydrate compounds; according to Rahmania *et al*. (2022), the primary substrates are glucogenic amino acids, lactate, glycerol, and propionate.

Cholesterol needs to be involved first in the Krebs cycle by turning into acyl-CoA, then into citrate, succinyl-CoA, fumarate, oxaloacetate, pyruvate, phosphoenolpyruvate, triose-P, glucose-6-P, glucose-3-P and finally into glucose (Mushawwir *et al*., 2011). According to Selim *et al*. (2021), glucose will then enter the glycolysis and Krebs cycle to produce ATP as energy for panting. The increased need for ATP causes the gluconeogenesis cycle to grow and continue to be maintained, causing blood cholesterol concentrations to decrease.

This condition is one of the leading causes of cholesterol concentrations in chickens reared at moderate altitudes higher than those reared at low altitudes, cholesterol being one of the energy precursors during gluconeogenesis. The higher the rate of gluconeogenesis, the greater the concentration of cholesterol involved, causing a decrease in plasma cholesterol levels (Tanuwiria *et al*., 2022a,b; Abouelezz *et al*., 2022; Adriani *et al*., 2024).

Total Iron Binding Capacity (TIBC)

Total Iron Binding Capacity (TIBC) measures the total capacity of transferrin in the blood to bind iron. Transferrin is a protein formed in the helpful liver as an iron carrier for hemoglobin synthesis or used by body cells (Adriani *et al*., 2021). Iron is a carrier of oxygen from the lungs to the rest of the body, a carrier of electrons in cells, and an integrated part of various enzyme reactions in body tissues.

Table 2 indicates that the Total Iron-Binding Capacity (TIBC) value in chickens raised at moderate altitudes is higher. Specifically, the TIBC level in these chickens is 429 mg/dl (Rahmania *et al*., 2022). Most transition metals, including iron, are bound to specific proteins that inhibit the catalysis of metal-dependent free radical reactions (Mushawwir *et al*., 2021a; 2021b). Iron bound by transferrin can be shielded from being released by natural free radicals generated by the mitochondria (Rahmania *et al*., 2022). An increase in transferrin production enhances iron binding, thereby raising TIBC levels. Consequently, when malondialdehyde (MDA) levels increase, TIBC levels tend to decrease.

Observations of the impact of heat stress on CO_2 release about shell quality are presented in Table 3. In Table 3, it is generally shown that CO_2 and H_2CO_3 concentrations, as well as shell thickness, appear to be lower in Sentul chickens at lower altitudes compared to those at higher altitudes (P<0.05).

Carbonic Acid (H_2CO_3)

A balance between the input and output of hydrogen ions from the body is required to achieve a state of homeostasis. The respiratory tract can adjust the blood pH within a few minutes by exhaling CO_2 from the body. The level of carbonic acid in the blood is controlled by the expulsion of CO_2 through the lungs. The respiratory system contributes to the acid and alkaline balance in the body by regulating the carbonic acid level in the blood. When the level of CO_2 in the blood increases, the excess CO_2 will react with water to form carbonic acid, thus lowering the pH of the blood.

Elevated temperatures, especially at lower altitudes, lead to an increased respiratory rate, which causes the body to release more carbon dioxide (CO_2). This increase in CO_2 results in a reduction of carbonic acid (H_2CO3) in the blood, as H_2CO_3 is formed from CO_2 and water (H_2O). Consequently, the decrease in carbonic acid raises the pH level of the blood (Table 3). High blood plasma acidity, can cause respiratory alkalosis, which reduces the activity of carbonic anhydrase, an essential enzyme for eggshell formation.

According to Zhan *et al.* (2017), respiratory alkalosis occurs when blood becomes alkaline due to rapid and deep breathing (hyperventilation), leading to low levels of carbon dioxide in the blood (Mushawwir *et al.*, 2023; Kharazi *et al.*, 2022). Therefore, Sentul chickens raised at medium altitudes have higher levels of carbonic acid in the laying phase than those raised at low altitudes. Furthermore, laying hens that experience heat stress tend to produce thinner and weaker eggshells due to the ionization of calcium diffusion, which reduces the secretion of calcium and carbonate.

Thickness of the Shell

The results of previous studies reported average eggshell thickness ranges from 0.33-0.35 mm (Kamil *et al.*, 2020). The results of this study showed that the shell thickness of eggs produced by Sentul chickens reared in the lowland (0.24 mm) and those reared in the mediumland (0.36 mm), both of which are significantly different (Table 3). These results indicate that the quality of the egg shells produced by Sentul chickens reared in the lowlands appears thinner than the standard shell thickness. It is known that environmental temperature affects the thickness of the shell. The temperature required by laying hens is $21C^0$ 25 with 50-60% humidity (Aengwanich, 2007; Abouelezz *et al.*, 2022). The research location in the medium, showed an average ambient temperature of 24^0 C while in the lowlands,> 31 C. ^{0}

Heat stress activates homeostatic mechanisms facilitated by the interaction of various tissues and biochemical processes (Aengwanich, 2007). As ambient temperatures rise, respiration frequency increases, leading to more excellent heat release (Mushawwir *et al.*, 2021b; Dudi *et al.*, 2023; Purwanti *et al.*, 2024). In lowland areas, the average temperature tends to be higher, resulting in increased heat loss through respiration. In poultry, heat loss through exhalation is significantly more detrimental than loss through evaporation. Consequently, the greater the amount of CO_2 released, the fewer precursors are available for shell formation, which results in a decline in shell quality (Muhammad *et al.*, 2023; Mushawwir *et al.*, 2023).

CONCLUSIONS AND RECOMMENDATIONS

The results of this study indicated that Sentul chickens raised in lowland areas were at a higher risk of heat stress, which negatively affected their shell quality and physiological-biochemical status. In contrast, Sentul chickens at medium altitudes exhibited no signs of anxiety, maintained average shell quality, and showed no physiological stress. The use of extensive rearing techniques in lowland areas, without accounting for environmental temperature exposure, may contribute to their lower performance. Future researchers could explore nutrigenomic feed interventions to mitigate the adverse effects of environmental heat and its impact on gene expression.

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NOVELTY STATEMENT

Studies on Sentul chickens have been widely reported with a focus on work on performance improvement through ration engineering presented with controlled rearing conditions, especially cage microclimate. Sentul chicken cultivation by most farmers does not use cages, which allows them to control environmental factors. Traditional rearing is an important issue because the topography of West Java is mostly lowland, so ecological temperature threatens chicken performance. This study demonstrated a valid assessment of the impact of rearing location on the physiological condition and performance of these Sentul chickens.

AUTHOR'S CONTRIBUTIONS

The research and writing of this article have been undertaken by the authors with equal contribution and equity.

Conflict of Interest

The authors have declared no conflict of interest.

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