International Journal of Agriculture and Biosciences 2025 xx(x): xx-xx.
https://doi.org/10.47278/journal.ijab/2025.054

RESEARCH ARTICLE
eISSN: 2306-3599; pISSN: 2305-6622

Behavioural Reactions and Physiological Responses of IPB-D1 Chickens under Acute Heat Stress

Jonathan Anugrah Lase [1,4], Rudi Afnan [2,*], Zakiah Wulandari [2], Sri Estuningsih [3], Tike Sartika [4], Sari Yanti Hayanti [4], Surya Surya [4], Tiurma Pasaribu [4] and Cece Sumantri [2]

[1]Graduate School of Animal Production and Technology, Faculty of Animal Science, IPB University, Bogor 16680, Indonesia
[2]Department of Animal Production and Technology, Faculty of Animal Science, IPB University, Bogor 16680, Indonesia
[3]Division of Veterinary Pathology, School of Veterinary and Biomedicine, IPB University, Bogor 16680, Indonesia
[4]Research Center for Animal Husbandry, Research Organization for Agriculture and Food, National Research and Innovation Agency (BRIN), Bogor 16915, Indonesia
*Corresponding author: rudi_afnan@apps.ipb.ac.id

ABSTRACT

This study examined the heat stress tolerance of IPB-D1 chickens through physiological responses, behaviour during heat stress tests, body surface temperature, rectal temperature, and changes in corticosterone and triiodothyronine hormone levels. The chickens were raised under two different rearing systems: the intensive system (P0) and the free-range system (P1). A total of 90 chickens were reared in each system for 12 weeks. Heat stress tests were conducted using random sampling, with 15 chickens representing each rearing system, intensive (P0) and free-range (P1). Data were analyzed using an independent T-test. The results showed that behavioural parameters, including the time to start panting, time to start rapid panting, and wing spreading, were significantly higher ($P<0.05$) in chickens raised in the free-range system. Furthermore, based on delta tests conducted before and after heat stress for each rearing system, body surface temperature in the comb, shank, and spur was significantly lower ($P<0.05$) in free-range chickens (P1) compared to intensive chickens (P0). Meanwhile, for rectal temperature and changes in corticosterone and triiodothyronine hormone concentrations, both free-range (P1) and intensive (P0) chickens showed similar results.

Keywords: Body temperature, Free-range, Heat stress, Hormones, IPB-D1 chickens.

Article History
Article # 25-022
Received: 20-Jan-25
Revised: 11-Mar-25
Accepted: 17-Mar-25
Online First: 11-Apr-25

INTRODUCTION

Global warming has led to increasingly significant and fluctuating environmental temperature rises, creating major challenges for living beings worldwide. High temperatures, accompanied by extreme weather patterns, not only affect natural ecosystems but also have serious impacts on the livestock sector, including poultry farming. Chickens, as warm-blooded animals, are highly susceptible to heat stress due to their limited thermoregulatory ability. Exposure to high environmental temperatures (acute) or prolonged heat stress (chronic) can affect their welfare, productivity, and health, such as reduced feed intake, decreased egg or meat production, and an increased risk of heat stress, which may lead to mortality (Goel, 2021). Various research findings on the effects of heat stress on growth, blood plasma biochemistry, behavioral changes, immunity, and stress hormone alterations in chickens have shown significant results (Vandana et al., 2021). This phenomenon is a crucial focus in modern livestock management to ensure sustainable production amidst increasingly evident climate change.

An increase in environmental temperature is perceived by chickens as heat stress. Under normal conditions, chickens possess homeostatic mechanisms to maintain body balance; however, during extreme heat conditions, their ability to sustain an ideal body temperature may be disrupted. This condition is referred to as heat stress (Apalowo et al., 2024). Heat stress leads to an increase in rectal temperature, prompting chickens to regulate their body temperature by dissipating heat through their beaks, a behaviour known as panting, as well as through their body surface. Currently, surface temperature measurements of an

Cite this Article as: Lase JA, Afnan R, Wulandari Z, Estuningsih S, Sartika T, Hayanti SY, Surya S, Pasaribu T and Sumantri C, 2025. Behavioural reactions and physiological responses of IPB-D1 chickens under acute heat stress. International Journal of Agriculture and Biosciences xx(x): xx-xx. https://doi.org/10.47278/journal.ijab/2025.054

A Publication of Unique Scientific Publishers

object can be conducted without direct contact using advanced tools such as infrared thermography (thermal imaging). This device has been utilized to detect heat stress and diseases in poultry (McCafferty, 2013; Solis et al., 2024). Research on the effects of high temperatures on physiological responses (rectal temperature, body surface temperature, and panting) in native chickens and commercial broilers has revealed that native chickens are more tolerant of environmental temperature changes.

One significant impact of heat stress is the alteration of hormonal profiles in chickens. The primary hormones involved in the response to heat stress include corticosterone, thyroid hormones and growth hormone. The detection of corticosterone and triiodothyronine hormones in chickens can serve as indicators of heat stress (Soleimani et al., 2011; Huang et al., 2024). Increased corticosterone levels due to heat stress can trigger metabolic changes that impact the immune response and production performance of poultry (Brown et al., 2021). On the other hand, triiodothyronine, which plays a role in energy metabolism regulation, is prone to decreased activity under high-temperature conditions, potentially leading to reduced growth and feed efficiency (Beckford et al., 2020). Hormonal changes due to heat stress not only affect the growth and productivity of chickens but also influence meat quality through changes in pH and oxidative reactions in the meat.

Furthermore, prolonged heat stress can lower chicken welfare, resulting in increased mortality rates and economic losses for farmers. Aryani et al. (2021) reported the effects of acute heat stress on KUB and Walik chickens, indicating changes in T3 and T4 hormone concentrations in each chicken haplotype, although statistically, there were no significant differences before and after heat stress within a specific time frame. Physiological indicators in chickens, including cortisol levels, body surface temperature, rectal temperature, heart rate, and other immune functions, as an objective picture of stress and health status in chickens (Neethirajan, 2024). Research on the impact of acute heat stress in IPB-D1 chickens, which are known for their resilience to Indonesia's tropical conditions, has not yet been conducted. This study aims to examine the physiological responses to acute heat stress in IPB-D1 chickens reared under different systems: intensive and free-range. The observed parameters include measurements of rectal temperature, body surface temperature (in the head, neck, body, and legs), behavioural patterns during acute heat stress challenge tests, as well as the concentrations of corticosterone and triiodothyronine hormones.

MATERIALS & METHODS

Ethical Approval

The animal care and protocols in the all stages of experiments were approved by the Animal Welfare Committee, Indonesian National Research and Innovation Agency (Approval number 110/KE.02/SK/01/2023).

Time and Location Research

This study began with the maintenance of male IPB-D1 chickens from October 2022 to February 2023. The maintenance was carried out at the Educational and Research Unit for Animal Husbandry (UP3J) of IPB University, located in Jonggol District, Bogor Regency, Indonesia. The chickens were reared under two different systems: the free-range system and the intensive system. The chickens were raised under two different systems: a free-range system and an intensive system, with a total of 90 chickens, 45 in each system. In the free-range system, the chickens were provided with an open roaming area while still having access to a primary shelter. In the intensive system, the chickens were kept solely in the main housing structure without access to a roaming area. Management practices and feeding protocols were identical for both groups, except that chickens in the free-range system had outdoor access to pasture from 8:00AM to 4:00PM, while those in the intensive system were kept indoors at all times. Chickens used for the acute heat stress challenge tests were sampled from each rearing system at 20 weeks of age.

Experimental Animal Procedure and Acute Heat Stress Challenge

This heat stress test study used 30 chickens selected through random sampling from each maintenance system group, consisting of 15 chickens from the free-range system and 15 chickens from the intensive system. Each chicken underwent an acute heat stress challenge at 35°C for 20 minutes using a heat chamber (Afnan, 2006; Aryani et al., 2021). The chamber was designed in a box shape with dimensions of 60cm (length) × 60cm (width) × 60cm (height), constructed from wooden boards coated with aluminium foil to prevent heat loss. The chamber was equipped with a heater, thermostat, blower, digital thermometer, and ventilation system. The floor of the chamber was lined with a wire grid, and aluminium foil was placed underneath to retain heat within the chamber. The chamber door was made of clear glass, allowing the chickens' behaviour inside the chamber to be monitored from outside. The acute heat stress challenge was conducted alternately for each chicken.

Observed Behavioural Patterns in Chickens during the Heat Stress Challenge

During the heat stress challenge in the heat chamber, an ethogram was observed to categorize six (6) behavioural patterns exhibited by the chickens during the test. The observed behavioural patterns during the heat stress challenge are presented in Table 1.

Body Surface Temperature

The body surface temperature of IPB-D1 chickens measured in this study included three areas: 1) The head area, which consists of the comb, beak, eyes, and wattle; 2) The body area, which includes the neck, back, chest, wings, and thighs; 3) The leg area, which includes the shank, spurs, and toes. The body surface temperature of IPB-D1 chickens was measured before and after the heat stress challenge test using an infrared thermography device, the FLIR A330 thermovision. Infrared thermography (IRT) is a technology used to detect and measure infrared radiation emitted by the surface of objects or organisms, such as animal bodies, including chickens bodies. The device works by capturing the heat radiation emitted by the object and converting it

Table 1: Types of behaviour observed in IPB-D1 chickens during the acute heat stress challenge test

Behavior category	Definition
Panting	Fast breath with mouth slightly or widely open (Iyasere et al., 2020; Kim et al., 2021)
Rapid panting	Fast breath with mouth slightly or widely open, intensity is faster than regular panting (Kim et al., 2021)
Wing lifting	A space can be seen between the chicken's wings and body, allowing more air movement between the body and wings (Kim et al., 2021; Ahmad et al., 2021)
Defecation	Chickens excrete in the test area (Iyasere et al. 2020)
Panic	In heat stress conditions, chickens show aggressive behavior, restlessness, peck at the walls and scratch at the baseboards (Mack et al., 2013; Kim et al., 2021)
Vocalization	Chickens made sounds and vocalize during the heat exposure test (Lee et al., 2015)

into visual images that display temperature variations on the object's surface (Solis et al., 2024). The temperature of the measured object is represented as colors in the thermogram, corresponding to the intensity of infrared radiation detected (Sanchez et al., 2024). Infrared thermography allows temperature measurement without direct contact with the object. Its advantages include the ability to measure and compare temperatures over a larger area, access hard-to-reach or hazardous locations, detect moving objects, measure objects in dark environments, diagnose diseases, perform non-destructive and contamination-free testing, and provide quick, accurate, and real-time results. However, the use of IRT is still limited due to its high cost (Solis et al., 2024). In this study, body surface temperatures were observed using IRT in the following areas: head, neck, body, and legs. The measurements were conducted with a FLIR A330 thermovision device (IRT) at a distance of 1–2 meters and an emissivity setting of 0.95 (Aryani et al., 2021).

Rectal Temperature

Rectal temperature in chickens reflects their internal body temperature. The rectal temperature of IPB-D1 chickens was measured both before and after the heat stress test. The data collection was performed by inserting a digital thermometer (Polygreen KD112) 1–2cm into the rectum for 5–10 seconds (Aryani et al., 2021).

Hormone Concentration

The hormones measured in this heat stress study were corticosterone and triiodothyronine. Hormone concentrations were measured both before and after the heat stress test. Blood samples were collected from the brachial vein located in the wing, using a 1cc insulin syringe and stored at approximately 3°C. The samples were then transported to the laboratory for analysis of corticosterone and triiodothyronine concentrations. Hormone analysis was conducted at the Agro-Industry and Biomedics Laboratory of the National Research and Innovation Agency (BRIN). The analysis employed the Enzyme-Linked Immunosorbent Assay (ELISA) method: Chicken Corticosterone, CORT (BZ-22055298-CPEB Bioenzy) for corticosterone, and Chicken T3 (BZ-22056498-CPEB Bioenzy) for triiodothyronine (Varun et al., 2021; Oluwagbenga et al., 2022).

Statistical Analysis

Behavioural data during heat stress were recorded while the heat stress test was conducted on the chickens. Meanwhile, corticosterone and triiodothyronine hormone concentrations, rectal temperature, and thermal surface temperatures (head, neck, body, and legs) were measured both before and after the heat stress test. All data were analyzed using the independent t-test (Steel & Torrie, 1993).

RESULTS

Behavior during Heat Stress Challenge Test

Data on the effects of acute heat stress on the behaviour of IPB-D1 chickens during the heat stress challenge test are presented in Table 2. The results showed that the rearing system caused different behavioural effects in response to heat stress in IPB-D1 chickens during the heat stress challenge test. Based on the T-test, significantly different behavioural parameters ($P<0.05$) appeared in the following: time to start panting, time to start rapid panting, spreading wings, and vocalization. Meanwhile, panic and feces excretion parameters did not show significant differences ($P>0.05$).

Table 2: The effect of acute heat stress on the behaviour of IPB-D1 chickens during the heat stress challenge test

Behavioral response	Treatment	Time (Seconds)
Time to start panting	P0	290.50±167.01b
	P1	525.78±239.49a
Time to start rapid panting	P0	465.00±251.81b
	P1	677.84±148.28a
Spreading wings	P0	445.27±157.86b
	P1	686.70±159.45a
Vocalization	P0	424.30±286.3a
	P1	107.50±45.96b
Panic	P0	612.50±213.18
	P1	425.75±376.13
Feces excretion	P0	555.43±189.28
	P1	470.34±229.67

Notes: P0: Intensive system; P1: Free-range system; Different alphabets (a, b) in the same behavioral response indicate a significant difference ($P<0.05$).

Body Surface Temperature

The results of measuring the body surface temperature of IPB-D1 chickens are presented in Table 3-5. The visualization of body surface temperature measurements in the head, body, and leg areas using infrared thermography (IRT) is presented in Fig. 1-3. The analysis of the body surface temperature of the head area, particularly the wattle, based on the delta (difference) before and after the heat stress test between intensively reared and free-range chickens, showed a significant difference ($P<0.05$). Furthermore, based on the T-test, the rearing system had a significant effect ($P<0.05$) on the body surface temperature of the comb, beak, and wattle before and after heat stress.

The analysis of body surface temperature in the body areas (neck, back, chest, wings, and thighs) based on the delta before and after heat stress testing between intensively reared and free-range chickens showed no significant difference ($P>0.05$) in all body area parameters. Furthermore,

based on the T-test, the rearing system had a significant effect on the surface temperature of the neck, back, chest, wings, and thighs both before and after heat stress.

Table 3: Body surface temperature of the head area of IPB-D1 chickens during the heat stress challenge test

Head part	Treatment	Head area temperature (°C)		Delta
		Before stress	After stress	
Comb	P0	39.46±1.13a,x	42.38±0.45a,y	2.92±0.98
	P1	38.39±1.67b,x	41.01±1.33b,y	2.62±1.45
Beak	P0	37.63±0.53a,x	40.38±0.66a,y	2.75±0.71
	P1	36.79±0.30b,x	39.10±1.36b,y	2.31±1.38
Wattle	P0	39.47±1.05x	42.74±0.42a,y	3.27±1.11a
	P1	39.44±1.29x	41.11±1.61b,y	1.67±1.59b

Notes: P0: Intensive system; P1: Free-range system; Different alphabets (a, b) in the same treatment indicate significant differences (P<0.05); Different alphabets (x,y) in the same row indicate significant differences (P<0.05).

Table 4: Body surface temperature of the body area of IPB-D1 chickens during the heat stress challenge test

Body part	Treatment	Head area temperature (°C)		Delta
		Before stress	After stress	
Neck	P0	33.47±0.36a,x	36.47±0.64a,y	3.00±0.66
	P1	32.11±1.44b,x	34.43±2.05b,y	2.33±1.28
Back	P0	34.39±0.98a,x	36.72±0.57a,y	2.33±0.96
	P1	31.80±1.65b,x	33.83±2.50b,y	2.03±1.64
Chest	P0	34.63±0.86a,x	36.03±0.27a,y	1.40±1.02
	P1	32.27±1.09b,x	34.18±1.79b,y	1.91±1.11
Wings	P0	34.63±1.06a,x	36.70±0.46a,y	2.07±0.93
	P1	32.30±0.94b,x	34.41±1.94b,y	2.11±1.52
Thighs	P0	34.88±0.46a,x	37.05±0.53a,y	2.17±0.53
	P1	32.11±1.05b,x	34.78±2.01b,y	2.67±1.12

Notes: P0: Intensive system; P1: Free-range system; Different alphabets (a, b) in the same treatment indicate significant differences (P<0.05); Different alphabets (x,y) in the same row indicate significant differences (P<0.05).

Table 5: Body surface temperature of the legs area of IPB-D1 chickens during the heat stress challenge test

Legs part	Treatment	Head area temperature (°C)		Delta
		Before stress	After stress	
Shank	P0	37.13±0.44x	41.74±0.73a,y	4.61±0.89a
	P1	37.56±1.42x	39.63±1.83b,y	2.07±1.79b
Spur	P0	37.05±1.15x	41.69±0.71a,y	4.63±0.88a
	P1	37.41±0.86x	40.01±1.44b,y	2.61±1.42b
Toes	P0	36.49±2.39x	40.26±0.89a,y	3.77±2.77
	P1	36.70±1.28x	39.03±1.48b,y	2.33±1.57

Notes: P0: Intensive system; P1: Free-range system; Different superscripts (a, b) in the same treatment indicate significant differences (P<0.05); Different alphabets (x,y) in the same row indicate significant differences (P<0.05).

The analysis of legs area surface temperature (shank, spur, and toes) based on the delta before and after heat stress testing between intensively reared and free-range chickens showed significant differences (P<0.05) in the shank and spur areas. Furthermore, based on the T-test, the rearing system significantly influenced the surface temperature parameters of the legs after experiencing heat stress.

Rectal Temperature

The results of rectal temperature measurements in IPB-D1 chickens subjected to heat stress challenge are presented in Table 6. Rectal temperature in chickens from each treatment group showed an increase after being exposed to heat stress, although no significant statistical difference was observed. Further analysis based on the delta before and after heat stress testing showed no significant difference (P>0.05) between intensively reared and free-range chickens. Additionally, based on the T-test, the rearing system significantly affected (P<0.05) the period before and after the heat stress challenge in chickens raised in the two different rearing systems.

Table 6: Rectal temperature of IPB-D1 chickens subjected to heat stress challenge test

Body part	Treatment	Before stress (°C)	After stress (°C)	Delta
Rektal	P0	41.94±0.35a	42.64±0.31a	0.70±0.02
	P1	41.47±0.11b	42.22±0.19b	0.74±0.06

Note: P0: intensive; P1: free-range; alphabets a,b in different treatments indicate significant differences (P<0.05).

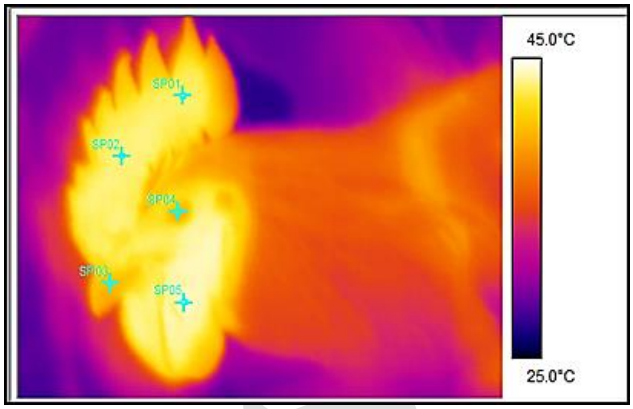

Fig. 1: Visualization of the head area temperature of chickens using the infrared thermography (IRT).

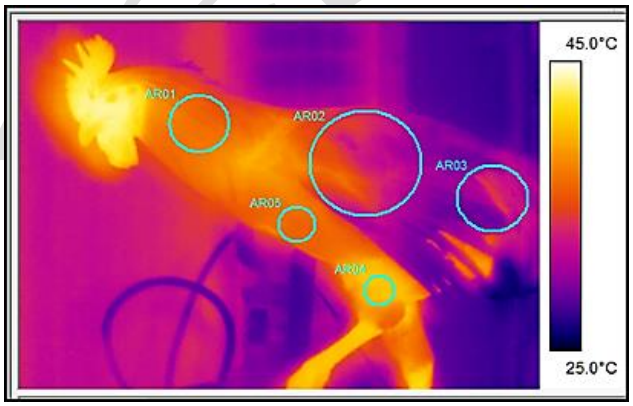

Fig. 2: Visualization of the body area temperature of chickens using the infrared thermography (IRT).

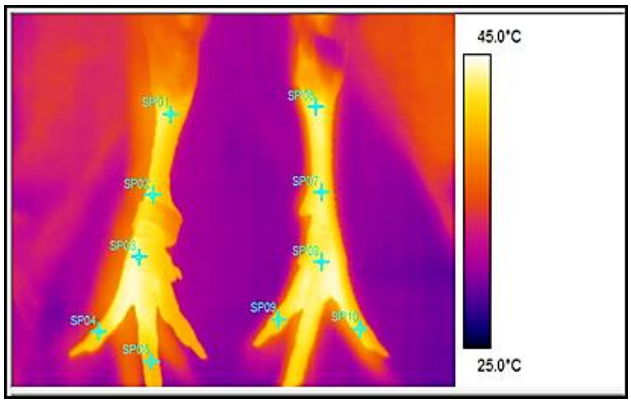

Fig. 3: Visualization of the leg area temperature of chickens using the infrared thermography (IRT).

Hormone Concentration

The results of corticosterone (CORT) and triiodothyronine (T3) hormone concentration measurements in IPB-D1 chickens subjected to heat stress

challenge are presented in Table 7. The analysis of cortisol hormone concentration, based on the delta values before and after heat stress testing, showed no significant difference (P>0.05) between free-range and intensively reared chickens. However, there was a significant difference (P<0.05) in cortisol concentrations before and after heat stress within each rearing system.

Table 7: Corticosterone and triiodothyronine hormone concentrations in IPB-D1 chickens subjected to heat stress challenge

Hormones	Treatment	Before stress	After stress	Delta
		Mean±SD	Mean±SD	Mean±SD
Corticosterone	P0	15.85±13.09x	34.33±16.49y	18.48±10.04
(ng/mL)	P1	12.05±5.67x	24.36±12.11y	12.31±7.42
Triiodothyrone	P0	222.90±144.12x	155.13±76.11y	-67.74±86.74
(pg/mL)	P1	302.27±169.86x	228.85±162.29y	-73.41±51.36

Note: P0: intensive; P1: free-range; Different superscripts (a, b) in the same column indicate significant differences; Different alphabets (x, y) in the same row indicate significant differences.

DISCUSSION

Observations of chicken behaviour can reflect the comfort of chickens in their environment, or as an indicator to assess the health and welfare status of the environment (Ferreira et al., 2021; Kiani, 2022). The time to start panting and rapid panting in this study was measured during heat stress (in seconds). Table 2 shows that the time to start panting and rapid panting between intensively reared chickens and free-range chickens differed significantly. The results revealed that the lowest time to start panting and rapid panting was observed in intensively reared chickens, while the highest time was recorded in free-range chickens. A higher panting time indicates that chickens are more tolerant to heat stress, whereas a lower panting time reflects lower tolerance or earlier signs of heat stress. Other studies have shown that Arabic chickens begin panting more quickly (4.05 minutes) compared to native chickens (4.68 minutes) (Tamzil et al., 2013). Another finding reported that layer chickens (22.33 seconds) experience panting earlier than native chickens (135 seconds) (Komalasari, 2014). Tamzil et al. (2013) reported that under acute heat stress conditions, panting occurred more quickly in broiler chickens (3.77 minutes), compared to Arabic and native chickens, which exhibited slower panting times, respectively (4.05 and 4.68 minutes).

Panting is the primary mechanism for releasing heat through evaporative cooling via the respiratory tract when environmental temperatures rise (Sesay, 2022). During panting, chickens appear to breathe in short, rapid bursts with their mouths open. The frequency of panting increases (rapid panting) as the environmental temperature rises (Mancinelli et al., 2023). Additionally, the behaviour of spreading wings, performed by chickens experiencing heat stress, is a physiological mechanism to regulate body temperature. This wing-spreading behaviour helps chickens release excess body heat when under heat stress (Mack et al., 2013; Kim et al., 2021). The free-range rearing system exposes chickens to direct sunlight daily in their environment, compared to chickens kept under intensive systems. Free-range chickens live outdoors and are frequently exposed to temperature fluctuations, unlike chickens raised in enclosed housing systems (Perez et al., 2023). This exposure allows chickens to better adapt to extreme weather conditions, including heat, enabling their thermoregulatory systems to withstand hot conditions longer before displaying signs of fatigue, such as panting and spreading wings. Therefore, environmental adaptability becomes a factor that causes the panting, rapid panting, and wing-spreading parameters to appear later in free-range chickens during the heat stress test compared to intensively reared chickens.

Vocalization behaviour in this study was measured during heat stress (in seconds). Table 2 shows that the vocalization behaviour of intensively reared and free-range chickens differed significantly. The results indicate that vocalization occurred more quickly in free-range chickens. Vocalization behaviour in chickens arises due to social behaviour and sensitivity to stress. Chickens produce sounds as a response to discomfort when exposed to stressors such as the heat test box. According to Papageorgiou et al. (2023), vocalization in chickens reflects their emotional state, particularly their comfort and distress. Chickens experiencing stress typically produce sounds that indicate discomfort, fear, or pain. Vocalization is a rapid response to express stress, confusion, or discomfort toward an unfamiliar environment (Neethirajan, 2024). Chickens placed in stressful conditions, such as heat stress, tend to vocalize as a natural social response to convey discomfort in such situations. On the other hand, chickens reared in intensive housing are accustomed to confined environments and tend to respond more slowly or less frequently to new stressors, such as the heat stress test box. Chickens' adaptation to a more static environment and limited social interactions causes them to react slower with vocalization compared to free-range chickens. Environmental stress often a causes chickens to make sounds and interact, reflecting their physiological condition under stress (Heuvel et al., 2022; Pereira et al., 2023).

The results of the study showed that IPB-D1 chickens subjected to the heat stress challenge exhibited panic behaviour and feces excretion during the heat stress test. The behaviours of feces excretion and panic were generally observed earlier in free-range chickens, although the differences were not statistically significant. Chickens excreting feces after experiencing heat stress are part of the body's adaptation mechanism to thermal stress. This feces excretion is related to metabolic changes, as well as the regulation of body temperature and electrolytes (Iyasere et al., 2020). According to Mack et al. (2013), panic behaviour in chickens under stress is a natural response, characterized by restlessness, pecking, and even attacking other chickens.

The body surface temperature in this study was measured using the FLIR A330 thermovision device, covering the head, body, and leg areas before and after the chickens were exposed to heat stress. The heat stress treatment was conducted using a climate chamber at a temperature of 35°C for 20 minutes, with humidity levels of 55-60%. The significant difference in the wattle area between intensively reared and free-range chickens could be attributed to differences in thermoregulation between the two groups. Free-range chickens, accustomed to having

a larger space and engaging in more physical activities, help strengthen the body's natural thermoregulatory mechanisms, making them more effective in cooling the body, including reducing the temperature of the wattle, when facing heat stress. The wattle and comb are vascular structures with many blood vessels. Blood flow to the wattle and comb increases in chickens experiencing heat stress (Nascimento et al., 2014). Therefore, heat can be released from the blood to the environment through the wattle, which has a relatively large surface area (Pratama et al., 2016). The results obtained in this study are consistent with the report by Aryani et al. (2021), which stated that body areas of chickens with minimal feathers or featherless areas (comb, ears, wattle) have higher temperatures compared to other body areas covered with feathers (neck, back, wings, chest, thighs, and tail).

The results of body area temperature measurements in chickens from both rearing systems in this study may be attributed to the fact that the entire body area is covered with feathers, which function as insulators or natural protection against environmental influences (Pasayev et al., 2019; Rojas et al., 2021). Feathers in chickens serve as a physical barrier that plays a role in reducing heat loss or maintaining heat, so hairless animals are more sensitive to extreme temperatures and more vulnerable to sunlight exposure (Mutibvu et al., 2017). Feathers help minimize direct contact between the chicken's skin surface and hot air, thereby keeping the body surface temperature in these areas relatively stable. This protective function of feathers applies to both intensively reared and free-range chickens, resulting in no significant temperature differences between the two groups when subjected to heat stress. These findings align with Aryani et al. (2021), which states that the feathered body areas of chickens have higher temperatures compared to non-feathered areas.

The significant differences in the shank and spur areas between intensively reared and free-range chickens can be attributed to their adaptation to different environmental conditions. Free-range chickens are frequently exposed to fluctuating environmental temperatures, which enhances their adaptive capacity to heat stress, including maintaining more stable body temperatures in regions such as the shank and spur. In contrast, intensively reared chickens, accustomed to controlled environments, tend to have a less flexible thermoregulatory response, making their surface body temperature more prone to increase during heat stress (Mutibvu et al., 2017). Additionally, differences in physical activity levels also play a role. Free-range chickens have higher physical activity levels compared to intensively reared chickens. The higher physical activity in free-range chickens helps maintain thermoregulatory efficiency and better temperature adaptation through the skin surface (Mutibvu et al., 2017). The results obtained in this study were higher than those reported by Aryani et al. (2021), who stated that the overall leg area temperature of KUB and Walik chickens under heat stress testing ranged from 34.03 to 36.03°C.

Rectal temperature can reflect the body temperature of chickens. It is a manifestation of the effort to achieve a balance between the heat produced and the heat released (Aryani et al., 2021). Changes in rectal temperature are one of the effects of the thermoregulatory mechanisms used to maintain body temperature (Mutibvu et al., 2017; Aryani et al., 2021). Therefore, rectal temperature is often used as an indicator of a chicken's resistance to heat stress (Yehia et al., 2025). The significant difference in rectal temperature between intensively reared and free-range chickens before and after heat stress could be attributed to the thermoregulatory factors, as intensively reared chickens are more vulnerable to heat stress compared to free-range chickens. This results in an increased thermoregulatory response, which can be indicated by rectal temperature (Mutibvu et al., 2017). Aryani et al. (2021) reported that the rectal temperature of KUB and Walik chickens before heat stress ranged from 40.8 to 41.7°C, and after heat stress, it increased to 41.3 to 41.9°C. The results obtained in this study are consistent with Zuhri (2024), reported that the rectal temperature of IPB-D1 chickens reared in both intensive and free-range systems ranged from 41 to 42°C. Other studies, such as Soleimani et al. (2011), also reported that the rectal temperature of red junglefowl ranged from 41 to 42°C.

The rectal temperature obtained in this study remains within the normal range for poultry. The normal body temperature in poultry ranges from 40 to 42°C (Tao & Xin, 2003). On the other hand, heat stress can increase rectal temperature in village chickens and broiler chickens exposed to heat stress at 40°C for 0.5, 1, and 1.5 hours; as a result, the normal rectal temperature of village chickens increased from 41.18±0.27°C to 43.30±0.12°C, and the temperature of broiler chickens rose from 41.17±0.25°C to 43.28±0.13°C, while the rectal temperature of commercial chickens was found to be 43.71°C (Tamzil et al., 2013). According to Nawaz et al. (2021), in hot environments, chickens adjust their behaviour and physiological balance to lower their body temperature. They use various thermoregulatory mechanisms to protect themselves from the effects of high temperatures, depending on the duration of exposure. It is known that in laying hens, variations in normal temperature can be influenced by factors such as age, sex, environment, length of day and night, and the food consumed. Therefore, rectal temperature can be used as an indicator of a chicken's resistance to heat stress.

The results of this study indicate that corticosterone levels increased in chickens from both rearing systems after being subjected to heat stress. In intensively reared chickens, cortisol levels increased from 15.85±13.09ng/mL before heat stress to 34.33±16.49ng/mL after heat stress. In free-range chickens, cortisol levels increased from 12.05±5.67ng/mL to 24.36±12.11ng/mL. These findings align with Kataria et al. (2008), who reported a significant increase in cortisol levels in the serum of male broiler chickens reared under different environmental temperatures: 13-16°C (low temperature), 24-27°C (moderate temperature), and 42-45°C (high temperature). Cortisol concentrations in male broilers reared under moderate temperature conditions (7.3±0.54ng/mL) increased to 13.1±0.78ng/mL at high temperatures. Similarly, Tamzil et al. (2013) observed an increase in cortisol levels in village chickens and Arabian chickens after heat

stress exposure. Cortisol levels in Arabian chickens increased from 1.30±0.58µg/dL to 4.62±0.26µg/dL, and in village chickens, from 1.68±0.53µg/dL to 5.11±0.26µg/dL. Furthermore, Aryani et al. (2021) reported that cortisol concentrations in KUB haplotype 2 chickens and Walik haplotype 1 chickens increased after acute heat stress. The results of this study are lower than those reported by Varun et al. (2021), where cortisol levels in several Indian native chicken breeds subjected to 39°C heat stress ranged from 17-19ng/dL.

On the other hand, the non-significant difference in cortisol hormone concentrations between free-range and intensively reared chickens in this study can be attributed to several factors, including physiological adaptation, heat stress duration, and genetic factors. From a physiological adaptation perspective, intensively reared chickens are accustomed to high stocking density and strict environmental control, while free-range chickens have adapted to more open environments and fluctuating environmental temperatures. This adaptation results in similar responses when both groups are exposed to the same extreme conditions, such as heat stress testing (Varun et al., 2021). The duration and intensity of heat stress exposure also influence cortisol hormone production (Oluwagbenga & Fraley, 2023). The length and intensity of heat stress play a significant role in hormonal responses (Varun et al., 2021). It is suspected that the duration and intensity of heat stress in this study were still tolerable for the chickens, leading to no significant differences between rearing systems. Additionally, the genetic factors of IPB-D1 chickens, which inherit the native traits of local chickens resistant to tropical conditions, provide a strong explanation for the absence of significant differences in cortisol hormone concentrations between chickens in the two rearing systems under heat stress (Sumantri & Darwati, 2017). Many studies report increased cortisol levels in chickens exposed to heat stress, making cortisol measurement an important index of stress in chickens (Kim et al., 2021; Farahani & Hosseinian, 2022).

The analysis of triiodothyronine (T3) hormone concentrations, based on delta values before and after the heat stress test, showed no significant difference ($P>0.05$) between free-range and intensively reared chickens. However, there was a significant difference ($P<0.05$) in T3 hormone concentrations before and after heat stress within each rearing system. Overall, the study results indicated that T3 hormone concentrations in chickens from both systems decreased after acute heat stress. Triiodothyronine (T3) is a hormone produced by the thyroid gland that regulates various physiological processes in the body, including energy metabolism, tissue and organ growth and development, maintenance of body temperature, and the stress response (Jiang et al., 2020; Oladokun et al., 2024). In intensively reared chickens, T3 concentrations decreased from 222.90±144.12pg/mL before heat stress to 155.13±76.11pg/mL after heat stress. Meanwhile, in free-range chickens, T3 levels declined from 302.27±169.86pg/mL to 228.85±162.29pg/mL. The results of this study align with Aryani et al. (2021), who reported a decrease in T3 hormone levels in KUB H1' chickens and Walik H1' chickens subjected to heat stress, from 3.96 to 3.69ng/mL and from 6.03 to 3.35ng/mL, respectively. The same was also reported by Skomorucha & Czajka (2024), stating that there was a decrease in triiodothyronine hormone levels in chickens exposed to heat exposure of 34°C and raised in a free-range system at the ages of 35 and 39 days, with reductions of 10.35 and 9.65 nmol/L, respectively. On the other hand, cortisol production increases during heat stress (Soleimani et al., 2011). Varun et al. (2021) also reported significant differences in T3 levels among Aseel, Naked Neck, Aseel Nandanam, and Naked Neck Nandanam chickens compared to control chickens exposed to heat stress for two hours over a 42-day period. Additionally, Abuoghaba et al. (2021) found that female quails exposed to heat stress at 39.5°C had significantly lower T3 levels ($P<0.05$) compared to quails not subjected to heat stress, with values of 65.67mg/L and 82.01mg/L, respectively.

The decrease in triiodothyronine (T3) hormone concentration after chickens experienced heat stress is caused by the thyroid gland's activity as a physiological response to reduce metabolic heat production (Aryani et al., 2021). The results of this study indicate that T3 concentrations decreased after both groups of chickens—intensive and free-range—were subjected to the same heat stress conditions. Moreover, the non-significant difference in T3 concentrations between chickens raised intensively and those raised in a free-range system may be attributed to similar thermoregulation limitations when responding to extreme heat stress. Both intensive and free-range chickens rely on thermoregulatory mechanisms, such as reducing metabolism and physical activity, to minimize internal heat production. Thermoregulation is a natural mechanism, which explains why T3 levels in chickens from both rearing systems can be similar after experiencing identical heat stress conditions (Beckford et al., 2020).

Conclusion

The IPB-D1 chickens raised in a free-range system are more heat-tolerant based on behavioural observations during heat stress. Meanwhile, based on heat dissipation through body surface, the intensive chickens release more heat in the head area, particularly in the comb, and in the leg area, specifically in the shank and spur. Based on rectal temperature measurements and hormone level changes, both free-range and intensive chickens show similar tolerance to heat stress.

Funding: IPB University and the National Research and Innovation Agency (BRIN), Indonesia, funded this research.

Acknowledgement: IPB University and the National Research and Innovation Agency (BRIN), Indonesia, funded and supported the study.

Conflict of Interest: The authors declare no conflict of interest.

Author's Contribution: JAL, RA, and CS: Conceptualization and designed the study. JAL, RA, CS, ZW, SE, and TS

executed the experiment and analyzed samples. JAL, SYH, SS, and TP tabulated and analyzed the data. JAL, RA, CS, ZW, SE, TS, SYH, SS, and TP prepared the manuscript, writing-review editing, and finishing the manuscript. All authors read and approved the final manuscript.

Data Availability: All the data is available in the article.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

Publisher's note: All claims stated in this article are exclusively those of the authors and do not necessarily represent the views of their affiliated organizations, the publisher, editors, or reviewers. Any product mentioned or evaluated in this article, or claimed by its manufacturer, is not guaranteed or endorsed by the publisher or editors.

REFERENCES

Abuoghaba, A.A., Ali, F., Ismail, I.I., & Saleh, M. (2021). Impact of acute short-term high thermal stress during early embryogenesis on hatchability, physiological body reaction, and ovarian follicles development of quails. *Poultry Science,* 100(2), 1213-1220. https://doi.org/10.1016/j.psj.2020.11.019

Afnan, R. (2006). Aspects of Adaption of Slow Growing Broilers and Broiler Parents to Heat Stress. Gottingen (GER): Cuvillier Verlag Gottingen.

Ahmad, S., Mahmud, A., Hussain, J., Javed, K., Usman, M., Waqas, M., & Zaid, M. (2021). Behavioural assessment of three chicken genotypes under free-range, semi-*intensive*, and intensive housing systems. *Ankara Universitesi Veteriner Fakultesi Dergisi,* 68, 365-372. https://doi.org/10.33988/auvfd.791155

Apalowo, O.O., Ekunseitan, D.A., & Fasina, Y.O. (2024). Impact of heat stress on broiler chicken production. *Poultry,* 3, 107-128. https://doi.org/10.3390/poultry3020010

Aryani, A., Solihin, D.D., Sumantri, C, Afnan, R., & Sartika, T. (2021). Physiological response of kub (kampung unggul balitbangtan) and walik chickens with different hsp70 gene haplotype exposed to acute heat stress. *Indonesian Journal of Agriculture Science,* 26(2), 276-283. https://doi.org/10.18343/jipi.26.2.276

Beckford, R.C., Ellestad, L.E., Weglarz, M.P., Farley, L., Brady, K., Angel, R., Liu, H.C., & Porter, T.E. (2020). Effects of heat stress on performance, blood chemistry, and hypothalamic and pituitary mRNA expression in broiler chickens. *Poultry Science,* 99(12), 6317-6325. https://doi.org/10.1016/j.psj.2020.09.05

Farahani, M.M., & Hosseinian, S.A. (2022). Effects of dietary stinging nettle (urtica dioica) on hormone stress and selected serum biochemical parameters of broilers subjected to chronic heat stress. *Veterinary Medicine and Science, 8, 660-667.* https://doi.org/10.1002/vms3.721

Ferreira, V.H., Guesdon, V., & Calandreau, L. (2021). How can the research on chicken cognition improve chicken welfare: a perspective review. *World's Poultry Science Journal,* 77(3), 679-698. https://doi.org/10.1080/00439339.2021.1924920

Goel, A. (2021). Heat stress management in poultry. *Journal of Animal Physiology and Animal Nutrition,* 105, 1136-1145. https://doi.org/10.1111/jpn.13496

Heuvel, H.V., Youssef, A., Grat, L.M., & Neethirajan, S. (2022). Quantifying the effect of an acute stressor in laying hens using thermographic imaging and vocalisations. *BioRxiv*, 2, 1-23. https://doi.org/10.1101/2022.07.31.502171

Huang, Y., Cai, H., Han, Y., & Yang, P. (2024). Mechanisms of heat stress on neuroendocrine and organ damageandnutritional measures of prevention and treatment in poultry. *Biology*, 13(11), 1-31. https://doi.org/10.3390/biology13110926

Brown, C.L., Zaystoff, S.J., Montina, T., & Inglis, G.D. (2021). Corticosterone-mediated physiological stress alters liver, kidney, and breast muscle metabolomic profiles in chickens. *Animals*, 11(11), 3056. https://doi.org/10.3390/ani11113056

Iyasere, O.S., Bateson, M., Beard, A., & Guy, J.H. (2020). Provision of additional cup drinkers mildly alleviated moderate heat stress conditions in broiler chickens. *Journal of Applied Animal Welfare Science*, 24(2), 188-199. https://doi.org/10.1080/10888705.2020.1846534

Jiang, S., Mohammed, A.A., Jacobs, J.A., Cramer, T.A., & Cheng, H.W. (2020). Effect of synbiotics on thyroid hormones, intestinal histomorphology, and heat shock protein 70 expression in broiler chickens reared under cyclic heat stress. *Poultry Science,* 99(1), 142-150. http://dx.doi.org/10.3382/ps/pez571

Kataria, N., Kataria, A.K., & Gahlot, A.K. (2008). Ambient temperature associated variations in serum hormones and interrelated analytes of broiler chickens in arid tract. *Slovenian Veterinary Research,* 45(4), 127-134.

Kiani, A. (2022). Effects of group sizing on behavior, welfare, and productivity of poultry. *Journal of World's Poultry Research*, 12(1). 52-58. https://dx.doi.org/10.36380/jwpr.2022.7

Kim, D.H., Lee, Y.K., Lee, S.D., Kim, S.H., & Lee, K.W. (2021). Physiological and behavioral responses of laying hens exposed to long-term high temperature. *Journal of Thermal Biology*, 99, 103017. https://doi.org/10.1016/j.jtherbio.2021.103017

Komalasari, (2014). Effect of high ambient temperature on physiological responses, blood profil and productive performance of two different chicken breeds. IPB Univ. pp: 1-39.

Lee, J., Noh, B., Jang, S., Park, D., Chung, Y., & Chang, H.H. (2015). Stress detection and classification of laying hens by sounds analysis. *Asian Australas Journal Animal Science,* 28(4), 592-598. http://dx.doi.org/10.5713/ajas.14.0654

Mack, L.A., Gant, J.N., Dennis, R.L., & Cheng, H.W. (2013). Genetic variations alter production and behavioral responses following heat stress in 2 strains of laying hens. *Poultry Science*, 92(2), 285-294. http://dx.doi.org/10.3382/ps.2012-02589

Mancinelli, A.C., Baldi, G., Soglia, F., Mattioli, S., Sirri, F., Petracci, M., Castellini, C., & Zampiga, M. (2023). Impact of chronic heat stress on behavior, oxidative status and meat quality traits of fast-growing broiler chickens. *Frontiers Physiologic*, 14, 1242094. https://doi.org/10.3389/fphys.2023.1242094

McCafferty, D.J. (2013). Applications of thermal imaging in avian science. *The International Journal of Avian Science,* 2013(155), 4-15. https://doi.org/10.1111/ibi.12010

Mutibvu, T., Chimonyo, M., & Halimani, T.E. (2017). Physiological responses of slow-growing chickens under diurnally cycling temperature in a hot environment. *Brazilian Journal of Poultry Science*, 19(04), 567-575. http://dx.doi.org/10.1590/1806-9061-2017-0485

Nascimento, S.T., Da Silva, I.J.O., Maia, A.S.C., De Castro, A.C., & Vieira, F.M.C. (2014). Mean surface temperature prediction models for broiler chickens—a study of sensible heat flow. *International Journal of Biometeorology,* 58, 195-201. http://dx.doi.org/10.1007/s00484-013-0702-7

Nawaz, A.H., Amoah, K., Leng, Q.Y., Zheng, J.H., Zhang, W.L., & Zhang, L. (2021). Poultry response to heat stress: its physiological, metabolic, and genetic implications on meat production and quality including strategies to improve broiler production in a warming world. http://dx.doi.org/10.3389/fvets.2021.699081

Neethirajan, S. (2024). Vocalization patterns in laying hens-an analysis of stress-induced audio responses. *Agriculture*, 14, 1-30. https://doi.org/10.1101/2023.12.26.573338

Oladokun, S., & Adewole, D. (2024). Research Note: Non-invasive urate sphere corticosterone metabolite as a novel biomarker for heat stress in poultry. *Poultry Science*, 103(3), 103439. https://doi.org/10.1016/j.psj.2024.103439

Oluwagbenga, E.M., Tetel, V., Schober, J., & Fraley, G.S. (2022). Chronic heat stress part 1: Decrease in egg quality, increase in cortisol levels in egg albumen and reduction in fertility of breeder pekin ducks. https://doi.org/10.3389/fphys.2022.1019741

Oluwagbenga, E.M., & Fraley, G.S. (2023). Heat stress and poultry production: a comprehensive review. *Poultry Science*, 102(12), 103141. https://doi.org/10.1016/j.psj.2023.103141

Papageorgiou, M., Goliomytis, M., Tzamaloukas, O., Miltiadou, D., & Simitzis, P. (2023). Positive welfare indicators and their association with sustainable management systems in poultry. *Sustainability,* 15(14), 1-27. https://doi.org/10.3390/su151410890

Pasayev, N., Kotacepe, S., & Maras, N., (2019). Investigation of sound absorption properties of nonwoven webs produced from chicken feather fibers. *Journal Industrial Textiles*, 48(10), 1616-1635. https://doi.org/10.1177/1528083718766843

Perez, M.M., Franco, L.S., Castro, C.A., Ricalde, R.H., Safwat, A.M., & Hernandez, Y.G. (2023). Effect of indoor free-range raising systems on growth performance of male Rhode Island Red chickens. *Tropical and Subtropical Agroecosystems*, 26(2023), 1-7.

http://doi.org/10.56369/tsaes.4312

Pereira, E., Nääs, I.A., Ivale, A.H., Garcia, R.G., Lima, N.D.S., & Pereira D.F. (2023). Energy assessment from broiler chicks' vocalization might help improve welfare and production. *Animals*, 13(1), 1-10. https://doi.org/10.3390/ani13010015

Pratama, T.A., Yani, A., & Afnan, R. (2016). The influence of M-CLOVE and conventional transportation with sex againts the broiler physiological response. *Journal of Animal Production and Processing Technology*, 4(1), 204-211.

Rojas, D.M., Titto, C.G., Geraldo, A.M., Burnes, J.M., Gomez, J., Avalos, I.H., Casas, A., Domingues, A., Jose, N., Bertoni, A., Reyes, B., & Pereira, A.M. (2021). Efficacy and function of feathers, hair, and glabrous skin in the thermoregulation strategies of domestic animals. *Animals*, 11(12), 1-23. https://doi.org/10.3390/ani11123472

Sanchez, R.C., Castañeda, F.E., Olvera, D.A., Ortega, M.E., Sanchez, V.M., Ramirez, E.S., Hernandez, E.P., Flores, I.M., & Hernandez, E. (2024). Systematic review and meta-analysis of thermal stress assessment in poultry using infrared thermography in specific body areas. *Animals*, 14(22), 3171. 1-17. https://doi.org/10.3390/ani14223171

Sesay, A.R. (2022). Impact of heat stress on chicken performance, welfare, and probable mitigation strategies. *International Journal of Environment and Climate Change*, 12(11). 3120-3133. https://doi.org/10.9734/IJECC/2022/v12i111360

Skomorucha, I., & Czajka, E.S. (2024). Effect of rearing technology on production performance, selected and blood parameters and welfare levels of broiler chickens during the summer production cycle. *Annual Animal Science*, 24(3), 939-948. https://doi.org/10.2478/aoas-2024-0009

Soleimani, A.F., Zulkifli, I., Omar, A.R, & Rahas, A.R. (2011). Physiological responses of 3 chicken breeds to acute heat stress. *Poultry Science*, 90(7), 1435–1440. https://doi.org/10.3382/ps.2011-01381

Solis, I.L., Boreli, F.P., Sousa, R.V., Martello, L.S., & Pereira, D.F. (2024). Using thermal signature to evaluate heat stress levels in laying hens with a machine-learning-based classifier. *Animals*, 14(13), 1-12. https://doi.org/10.3390/ani14131996

Steel, R.G., & Torrie, J.H. (1993). Principles and procedures of statistics - A biometric approach. 2nd ed. New York: McGraw-Hill Book Co.

Sumantri, C., & Darwati, S. (2017). The latest development IPB-D1 superior chicken research. Proceedings of the National Seminar on the Livestock Industry (Pp: 3-7). Bogor: IPB University. ISBN: 978-602-96530-5-2

Tamzil, M.H., Noor, R.R., Hardjosworo, P.S., Manalu, W., and Sumantri, C. (2013). Acute heat stress responses of three lines of chickens with different heat shock protein (hsp)-70 genotypes. *International Journal of Poultry Science*, 12(5), 264-272.

Tao, X., & Xin, H. (2003). Acute synergistic effects of air temperature, humidity, and velocity on homeostasis of market–size broilers. *Engineering Sustainable Future*, 46(2), 491-497. https://doi.org/10.13031/2013.12971

Vandana, G.D., Seijan, V., Lees, A.M., Pragna, P., Silpa, M.V., & Maloney, S.K. (2021). Heat stress and poultry production: impact and amelioration. *International Journal of Biometeorology,* 65(2021), 163–179. https://doi.org/10.1007/s00484-020-02023-7

Varun, A., Omprakash, A.V., Kumanan, K., Vairamuthu, S., & Karthikeyan, N. (2021). Thyroid hormones and Corticosterone investigation under heat stress in native chicken. *Indian Journal of Animal Science*, 91(1), 28-30. https://doi.org/10.56093/ijans.v91i1.113269

Yehia, M., Askri, A., Achour, A., Prus, J.M., Ouellet, V., & Alnahhas, N. (2025). Rectal temperature and heat transfer dynamics in the eye, face, and breast of broiler chickens exposed to moderate heat stress. 104(2), 104748. https://doi.org/10.1016/j.psj.2024.104748

Zuhri, N.I. (2024). Physiological response and production performance of intensively and free-range IPB-D1 chicken maintained. Dissertation IPB (pp: 1-37). Bogor: IPB University