

Multivariate Analyses of Physiological Traits and Thermal Response in Bali Cattle

James Wisnu Arya Tamar¹, Irida Novianti¹, and Veronica Margareta Ani Nurgartiningih^{1*}

¹Faculty of Animal Sciences, Universitas Brawijaya, Malang 65145, Indonesia

Abstract. Bali cattle are native to Indonesia and are highly adaptable to tropical environments. However, fluctuations in temperature and humidity can affect their physiological responses. This study aims to identify the main physiological traits that contribute to the adaptation of Bali cattle to hot tropical conditions in East Kalimantan. A total of sixty-three Bali cattle, including males, non-pregnant females, and pregnant females, were intensively raised and used in this study. Data were analysed using a descriptive approach, correlation test, one way Analyses of Variance (ANOVA), Least Significant Difference (LSD) test, and Principal Component Analyses (PCA). The parameters observed included body surface temperature at various anatomical sites, respiratory rate (RR), heart rate (HR), and rectal temperature. Descriptive analyses showed that respiratory rate had the highest coefficient of variation (CV = 26.15%), indicating high sensitivity to changes in temperature and humidity, while rectal temperature was the most stable (CV = 1.58%). The LSD test showed that bulls had the highest RR and HR values ($p < 0.05$; $p < 0.001$), while pregnant cows showed greater inter-individual physiological variation. Correlations between parameters showed a strong relationship between surface temperature and respiratory rate, but a weak relationship between body temperature and respiratory rate ($r < 0.2$). PCA analyses could identify two principal components explaining 55.2% of the total variation. PC1 represents the external body temperature component, while PC2 represents the internal physiological response (RR, HR). These results indicate that a combination of physiological factors and reproductive status influences Bali cattle adaptation to heat stress. These results provide a basis for developing precision selection strategies and environmental management in local cattle farming systems.

Keywords: Bali cattle, body temperature, environmental, physiological status.

1 Introduction

Bali cattle (*Bos javanicus*) are a local cattle germplasm that supports food security, the rural economy, and sustainable agricultural systems. The coat colour of Bali cattle is specific with dominant black colour (86.67%) in males and brick red (84.81%) in females

* Corresponding author: ym_ani@ub.ac.id

[1]. As a local livestock genetic resource with long adapted to extreme environmental conditions in Indonesia, Bali cattle have high reproductive efficiency and physiological adaptation to tropical climate temperature and humidity [2]. In addition, increasing ecological temperatures due to global climate change cause significant thermal stress on livestock, impacting physiological performance decline, metabolic efficiency, productivity, and disrupting the homeostatic system [3].

In tropical regions, Bali cattle are predominantly raised under managed production system, where heat stress represents a major environmental challenge affecting livestock productivity. Heat stress in livestock is a complex physiological condition characterized by adaptive responses such as increased respiration and heart rate, along with alteration of both core and surface body temperature [4]. Physiological parameters can serve as reliable indicators for accessing the level of livestock adaptation to environmental temperatures. Although numerous studies have been investigated heat stress in Bali cattle, most have primarily focused on single physiological indicators or relied on descriptive analyses [5]. Previous studies were conducted without exploring multivariate relationships, thereby providing limited insight into the physiological connections underlying thermal adaptation, particularly in relation to reproductive status under hot tropical conditions.

Principal Component Analyses (PCA) is a multivariate analytical technique used to identify patterns of physiological variability that cannot be detected through univariate analyses. PCA can reveal combinations of physiological variables that contribute most strongly to the adaptive response of livestock [6]. Furthermore, correlation analysis provides a quantitative assessment of the strength and direction of the relationship among physiological parameters, enabling the identification of the most influential primary indicators. This approach provides a more comprehensive understanding of the relationship between the physiology and heat adaptability in Bali cattle, while also highlighting the potential use of physiological traits as criteria or adaptive livestock selection under high environmental temperature.

2 Materials and methods

2.1 Study Location and Animal Material

This research was conducted at the Cattle Livestock and Feed Unit of Api-Api Village, Waru District, North Penajam Paser Regency, East Kalimantan Province. The research was conducted from September to October 2025, with 63 Bali cattle selected by purposive sampling based on complete physiological data and health status. The sample consisted of three physiological groups: males ($n = 6$), non-pregnant females ($n = 30$), and pregnant females ($n = 27$). All cattle were kept in intensive pens, fed mainly natural grass and concentrates, and drinking water was provided *ad libitum*.

During the study, the average environmental temperature ranged from 26 to 30°C with a relative humidity of 70 to 85%. Physiological parameters measured as indicators of the Bali cattle's adaptive response to environmental conditions included respiratory rate, heart rate, body surface temperature (temples, forehead, back, and hips), and rectal temperature.

2.2 Physiological Measurement of Livestock

Physiological measurements are used to determine the adaptive response of Bali cattle to the farm's environmental conditions. The parameters measured include:

1. Eye Temperature (ET) was measured at the temple region using an infrared digital thermometer, positioned approximately 5 cm from the skin surface, while the animal is calm and stationary
2. Forehead Temperature (FT) was measured on the center of the forehead using a digital infrared thermometer, positioned approximately 5 cm from the skin surface, when the animal is calm and stationary
3. Dorsal Temperature (DT) was measured from the center of the back using the same infrared thermometer, held perpendicular to the body surface to ensure accurate temperature measurement
4. Rump Temperature (RT) was measured at the top of the buttocks (sacral area) using an infrared thermometer, indicates the surface temperature near the rump area
5. Respiration Rate (RR) was measured by counting the number of breaths taken by the animal within one minute, using a stopwatch for timing and a hand tally counter to record the number of breaths
6. Heart Rate (HR) was measured by palpating the coccygeal artery beneath the tail, approximately 10 cm from the anus, for one minute. Measurements were repeated three times using a stopwatch for timing, and a hand tally counter to record the number of beats
7. Rectal Temperature (RCT) was measured using a digital rectal thermometer, inserted approximately one-third of its length into the rectum for about one minute. Measurement were repeated three times until a stable reading was obtained and the indicator signal sounded.

All measurements were taken in the morning from 7 to 9 am and in the afternoon from 1 to 4 pm, and each measurement was repeated three times, with the average value used for further analyses. All cattle were handled according to animal welfare guidelines to minimize stress during data collection.

2.3 Statistical Analyses

Data analyses used a comprehensive multivariate approach with three complementary statistical models implemented in R Studio version 4.3.2. The data analyses used is described below:

2.3.1 Analyses of Variance (ANOVA) and Least Significant Difference (LSD)

LSD (Least Significant Difference) was used to compare the average between livestock groups (males, females, and pregnant females) for each physiological parameter that showed significant differences in the ANOVA results. The analyses used the R package, which provides the LSD test (*Agricolae*) function for further testing between groups.

Differences between groups are indicated by different superscript letters (a, b, c) at a significance level of $p < 0.01$.

2.3.2 Correlation Analyses

Correlation analyses was used to determine the relationship between physiological parameters of Bali cattle, including body surface temperature (temples, forehead, back, and hips), rectal temperature, respiratory rate, and heart rate. This method was chosen because it can demonstrate a linear relationship between variables [7]. The correlation coefficient (r) value was used to interpret the level of relationship, with criteria ranging from very weak to very strong. Correlation analyses was performed using the R package (*Ggally*), and the relationships between variables were visualised using a correlation matrix plot (*ggpairs*) to demonstrate the observed patterns.

2.3.3 Principal Component Analyses (PCA)

Principal Component Analyses (PCA) was used on Bali cattle physiological parameter data to identify the principal components explaining physiological variation between individuals. PCA analyses produced eigenvalues, percentage variance, and principal components (PCs) explaining the highest proportion of variance among variables. The analyses used the R package (*factoextra*) for visualisations, such as a scree plot to illustrate the proportion of variation in each component, and a PCA biplot to evaluate the relationships between physiological variables such as respiration, heart rate, rectal temperature, and body surface temperature, as well as individual distributions.

3 Results and discussion

The results of this study provide information on the physiological response of Balinese cattle to environmental conditions based on differences in the physiological status of cattle: males, females, and pregnant females. Statistical analyses include descriptive statistics, correlation analyses, Least Significant Difference (LSD), and Principal Component Analyses (PCA). The statistical results are used to identify the main physiological parameters influencing cattle adaptation and to see similarities or differences in physiological patterns and strategies in precision breeding selection [8].

3.1 Descriptive Statistics

Descriptive analyse provides information on the mean and variation of the observed physiological parameters of Bali cattle. The mean, standard deviation, minimum, and maximum values obtained reflect the general physiological response of the cattle to their rearing environment.

Table 1. Descriptive statistics of the overall population.

Variable	N	Mean	SD	Min	Max	SE	CV (%)
ET	64	35.70	1.05	31.90	37.80	0.13	2.95
FT	64	35.94	0.87	33.70	37.70	0.11	2.41
DT	64	35.68	1.01	32.00	37.00	0.13	2.82
RT	64	35.57	0.90	33.30	37.10	0.11	2.53
RR	64	34.75	9.09	22.50	72.00	1.14	26.15
HR	64	64.90	12.92	31.00	96.50	1.62	19.91
RCT	64	38.58	0.61	35.87	39.97	0.08	1.58

Note: ET (Eye Temperature), FT (Forehead Temperature), DT (Dorsal Temperature), RT (Rump Temperature), RR (Respiration Rate), HR (Heart Rate), RCT (Rectal Temperature), SD (standard deviation), N (number of observations), Min (minimum), Max (maximum), SE (standard error), CV (coefficient of variation).

Descriptive analyses (Table 1) showed that rectal temperature (RCT) has a mean value of $38.58 \pm 0.61^\circ\text{C}$, and the lowest coefficient of variation (CV = 1.58%), indicating that body temperature was the most stable and consistent physiological parameter between individuals. This suggests that Bali cattle can maintain their internal temperature well despite changes in environmental conditions. Conversely, respiration rate (RR) exhibited the highest coefficient of variation (CV = 26.15%), indicating significant differences in response between individuals to environmental stress, particularly ambient temperature and humidity. These results suggest that respiratory rate is the most sensitive parameter in responding to changes in environmental temperature.

Moderate variation in HR is indicated by CV = 19.91% which suggests that increased blood flow plays a role in heat dissipation [9]. Meanwhile, body surface temperatures, such as ET, FT, DT, and RT, have relatively low CV values (around 2–3%) and indicate stable external body temperature under normal physiological conditions. Overall, the order of physiological variations observed (RR > HR > ET/FT/DT/RT > RCT) indicates that respiratory rate is the most dynamic indicator of environmental conditions. At the same time, RCT is the most stable parameter. These findings indicate good thermal adaptation of Bali cattle to tropical environments, where the respiratory and cardiovascular systems are essential in maintaining body heat balance.

3.2 Analyses of the LSD of Bali Cattle

Table 2. Overview of the linear traits in the analyses of the LSD of Bali cattle

Variables	N	Linear traits (Mean ± SD)			
		Female	Female Pregnancy	Male	P-value

ET	64	35.54 ± 0.20	35.82 ± 0.20	35.88 ± 0.43	0.556
FT	64	35.92 ± 0.16	35.86 ± 0.16	36.45 ± 0.35	0.315
DT	64	35.90 ± 0.19	35.46 ± 0.19	35.73 ± 0.41	0.249
RT	64	35.50 ± 0.17	35.53 ± 0.17	36.05 ± 0.37	0.386
RR	64	32.02 ± 1.63 ^a	36.24 ± 1.63 ^a	40.75 ± 3.59 ^a	0.047
HR	64	67.71 ± 2.15 ^a	59.19 ± 2.15 ^b	78.92 ± 4.72 ^a	0.000
RCT	64	78.92 ± 4.72 ^b	38.84 ± 0.09 ^a	39.13 ± 0.21 ^a	0.000

Note: ET, Eye Temperature; FT, Forehead Temperature; DT, Dorsal Temperature; RT, Rump Temperature; RR, Respiration Rate; HR, Heart Rate; RCT, Rectal Temperature; SD, standard deviation; N, number of observations; *** $p < 0.001$, very very significant; ** $p < 0.01$, very substantial; * $p < 0.05$, significant; NS, not significant; a,b,c Means in rows with different superscripts differ significantly.

The Least Significant Difference (LSD) test analyses showed that physiological status significantly affected several physiological parameters of Bali cattle (Table 2). In general, body surface temperatures (ET, FT, DT, and RT) did not show significant differences between groups, indicating that the thermoregulatory mechanisms of Bali cattle operate relatively stably in both females and pregnant females. This demonstrates the ability of Bali cattle to maintain body temperature balance despite physiological changes such as pregnancy and differences in hormonal activity between the sexes.

In contrast, RR and HR showed significant differences ($p < 0.05$ and $p < 0.001$), with the highest values found in bulls. The increased values in bulls are thought to be related to higher metabolic activity and physiological stress levels, primarily due to hormonal differences and physical activity levels. Meanwhile, pregnant cows tend to show lower respiration rates, heart rates, and rectal temperatures, which may be due to physiological adaptations during pregnancy to maintain growth, placental function, and energy needs.

The RCT parameters showed a highly significant difference ($p < 0.001$), with the highest mean value in non-pregnant cows. This indicates higher metabolic activity compared to pregnant cows and bulls, mainly due to the influence of the reproductive cycle, especially on higher metabolic activity due to the estrus cycle with increased estrogen and increased internal heat production [10].

These results suggest that hormonal conditions and reproductive status influence physiological differences between groups of Bali cattle more than external thermoregulatory capabilities. Stable body surface temperature, accompanied by significant variations in respiratory parameters and heart rate, indicates that the physiological system of Bali cattle can maintain a balanced adaptation to changes in internal conditions. These findings reinforce the adaptive characteristics of Bali cattle as a tropical germplasm with high tolerance to environmental variations and physiological status.

3.3 Correlation Test Analyses

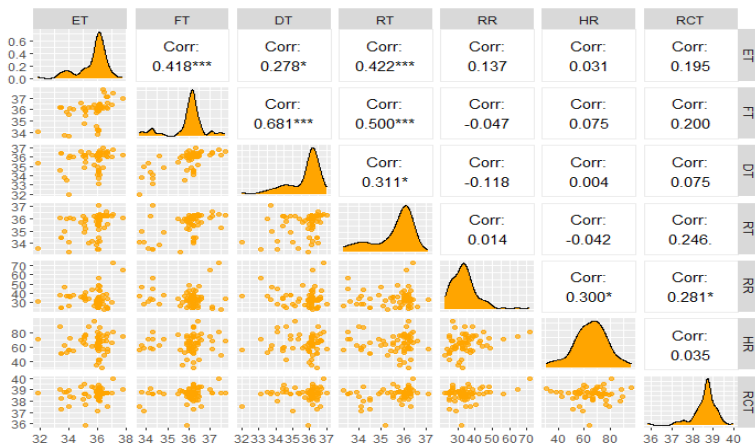


Fig. 1. Correlation matrix between the measured physiological variables of Bali cattle

Figure 1 shows the correlation matrix between the measured physiological variables of Bali cattle. In general, the correlation values obtained indicate varying relationships, both positive and weak, between parameters [11]. The strongest correlation between FT and DT was observed with an r value of 0.681 ($p < 0.001$), indicating a close thermal relationship between body parts exposed to the environment. A reasonably strong correlation was also found between ET and RT, with a value ($r = 0.422$; $p < 0.001$) indicating that both measurement locations are sensitive to changes in body surface temperature.

Furthermore, ET has a significant positive correlation with FT ($r = 0.418$; $p < 0.001$), indicating that an increase at other points follows an increase in temperature at one point of body surface measurement. In contrast, the correlation between RR rate and body temperature parameters tends to be low ($r < 0.2$), indicating that changes do not entirely influence inter-individual respiratory variations in body surface temperature, but may be influenced by other factors such as humidity, physical activity, stress levels, and hormonal conditions.

Another correlation between HR and RT ($r = 0.300$; $p < 0.05$) indicates that increased cardiac activity is slightly associated with increased temperature in the area as a physiological response to mild heat stress. However, RCT only shows a weak correlation with most other variables ($r < 0.3$), indicating a stable response to internal body temperature compared to surface temperature.

3.4 Combined Plot PCA

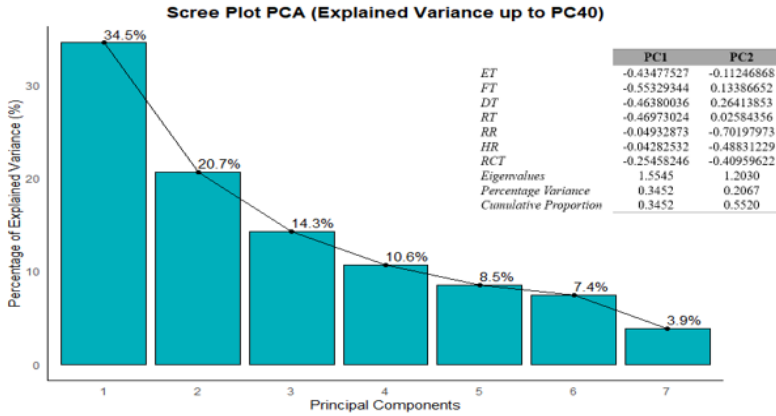


Fig. 2. Scree Plot PCA

Principal component analyses (PCA) identify patterns of relationships between physiological variables and determines the principal components that explain the most significant proportion of data variation. The results showed that PC1 had an eigenvalue of 1.55, explaining the most significant amount of data variation, while PC2 had an eigenvalue of 1.20, contributing additional variation to the data. Based on the analyses results, the first two principal components (PC1 and PC2) explain 55.2% of the total data variation, contributing 34.5% and 20.7%, respectively (Figure 2). This indicates that most of the information contained in the seven physiological parameters can be summarized in two principal axes.

The first principal component (PC1) shows a high-loading on-body surface temperature, especially at (DT = -0.46), (RT = -0.47), and (FT = -0.55). This indicates that PC1 can describe the magnitude of external body temperature related to the thermoregulatory mechanism of Bali cattle in environmental conditions. In the first component (PC1), the variables with the most significant contributions are ET, FT, DT, and RT, with relatively high-negative loading values. This indicates that PC1 reflects the body temperature component or thermal profile [12].

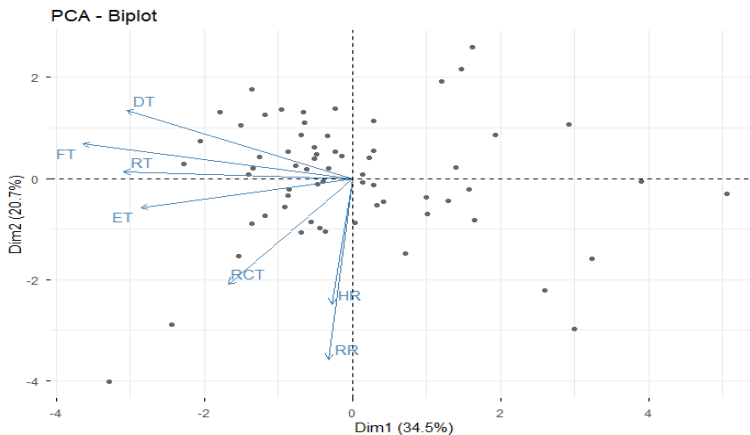


Fig. 3. PCA Biplots combined plot (PC1 and PC2).

In the biplot visualisation, the direction of the arrow indicates the relationship between variables, while the length of the arrow indicates how strongly the variable influences the principal component. Variables with similar arrow directions (ET, FT, DT, and RT) indicate a strong positive relationship, while opposite arrow directions indicate a negative relationship. These results show that the body surface temperature variables form one closely related group, while RR and HR form another group representing physiological activity. This finding is in line with the results of research [13] that the first principal components, PC1 and PC2, in the physiological variables of Girolando cattle can explain 85% of the total variation, where an increase in environmental temperature is positively correlated with an increase in rectal temperature and respiratory rate. The PCA biplot based on the fixed effect, namely sex, is presented as follows:

3.4.1 PCA Biplots by Sex

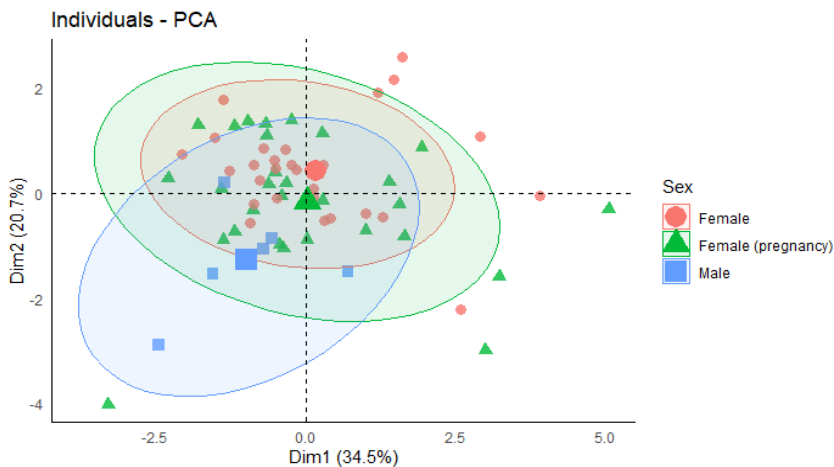


Fig. 4. PCA biplots by sex

The results of the PCA analyses of individuals based on sex and physiological status of Bali cattle (Figure 4) show a distribution pattern for groups of males, non-pregnant females, and pregnant females that form overlapping distribution ellipses, but with different directions and area sizes.

The biplot results show that the pregnant female group has the largest ellipse, indicating high physiological diversity within the group. The area of the ellipse shows that the physiological responses of pregnant females vary between individuals, especially in the variables RT, RR, ET, and FT. These results indicate a diversity of physiological adaptations from some individuals who can maintain better temperature and heart rate stability. At the same time, other groups show increased physiological activity as a form of compensation for metabolic stress. Variation explains that there are differences in pregnancy levels (gestation age). During pregnancy, significant hormonal changes occur,

such as increased progesterone and estrogen, which can affect thermoregulation and blood circulation mechanisms, resulting in physiological improvements compared to male individuals and non-pregnant females [14]. The area of the ellipse in this group illustrates a more diverse physiological adaptation to the heat and metabolic loads due to pregnancy.

Meanwhile, the male group showed the highest physiological response to environmental factors, indicated by significantly higher RR and HR values compared to other groups ($p < 0.05$ and $p < 0.001$). It formed a narrower ellipse shifted to the negative direction on the PC1 axis. This indicates that males have a more homogeneous physiological response with uniform rectal temperature, respiratory frequency, and heart rate values. The negative shift in PC1 is interpreted as a tendency for slightly lower thermoregulatory activity than the female group, caused by male physiological adaptation to heat through increased heat dissipation efficiency. Under the same environmental conditions, bulls generally have a higher heat stress threshold and a more stable cooling mechanism, resulting in fewer individual variations [15].

The non-pregnant female group occupies a midpoint between the male and pregnant female groups, with a relatively small ellipse and little overlap. This pattern indicates that non-pregnant females have the most stable and efficient physiological condition among the three groups. The absence of pregnancy-related metabolic stress allows physiological parameters such as RT and RR to remain within the normal adaptive range with low variation. Therefore, this group's position is considered a normal physiological reference point for comparing reproductive states

3.5 Precision Selection Framework for Physiological Adaptation of Bali Cattle

Based on the results of the PCA analyses, a precision selection framework for physiological adaptation in Bali cattle under environmental conditions can be formulated. The first principal component (PC1), which explains 34.5% of the total variation, is designated as the leading indicator of heat adaptation, as it describes thermoregulatory efficiency through body surface temperature. FT and RT have the highest loadings on PC1, so they are designated as the main selection criteria for cattle with efficient heat dissipation capabilities. Individuals with a PC1 score $> +1$ indicate high thermoregulatory efficiency, indicating better adaptation to environmental heat stress.

The second component (PC2) explains an additional 20.7% of the variation and is influenced by the RR, HR, and RCT parameters. This component describes the internal physiological response to heat stress, so it can be used as an additional predictor in assessing stable physiological conditions in the body. Applying this selection framework allows for identifying Bali cattle individuals with optimal heat adaptation capabilities without complex equipment. In addition, the implementation of an intensive housing

system also plays a significant role in reducing heat stress levels through adequate shade and good air circulation. Individuals with high PC1 and low PC2 scores are recommended as candidate broodstock and adaptive bulls for breeding programs in tropical regions.

4 Conclusion

This study shows that physiological status and reproductive condition significantly influence the adaptability of Bali cattle to tropical environments, while sex did not significantly differ among individuals. PCA analyses identified two principal components (55.2%), with PC1 reflecting thermoregulatory efficiency and PC2 reflecting internal physiological stability. FT and RT are the primary indicators of physiological adaptation, while RR, HR, and RCT serve as supporting variables for heat stress. Individuals with high PC1 and low PC2 scores are recommended as adaptive cattle candidates because they demonstrate an optimal balance between heat loss and metabolic stability. These findings provide a scientific basis for developing a precision selection framework for Bali cattle that focuses on heat stress resistance to improve selection efficiency, animal welfare, and productivity.

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