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Diurnal Variations and Heat Stress: Physiological and Biochemical Resilience in White Fulani Cattle

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ABSTRACT

This study evaluated the effects of diurnal variations under heat stress conditions on physiological, biochemical and haematological parameters in White Fulani (*Bunaji*) cattle raised under semi-intensive system of management. Twenty (20) White Fulani cattle of mixed sexes were exposed to heat stress conditions with Temperature-Humidity Index (THI) and body temperature monitored both in the morning and afternoon. Blood samples were also collected to measure haematological, biochemical and antioxidant indices. The THI exceeded 80, indicating significant heat stress during both morning and afternoon periods. Elevated body temperatures were recorded in the muzzle, left ear, and neck, with significant differences (P < 0.05) between morning and afternoon readings. Biochemical indices showed elevated (but non-significant changes, suggesting physiological adaptation. Antioxidant and enzyme levels remained stable despite elevated heat stress. Haematological indices revealed a significant decrease in Red Blood Cell counts from morning to afternoon (P = 0.0001), while other parameters remained stable. Correlation analysis showed that temperatures at the left ear and neck significantly correlated with THI, indicating their sensitivity to heat stress. These findings highlight underlying physiological costs masked by short-term stability of White Fulani cattle's physiological and metabolic functions under prolonged heat stress conditions.

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INTRODUCTION

There has been a 1.0°C increase in environmental temperatures since the 1800s, and projections indicate another 1.5°C increase between 2030 and 2052 (IPCC 2018). Rising ambient temperatures result in stress that affects the adaptive capabilities of livestock (Nussa et al., 2018). Animals are exposed to a range of stressors, such as physical, nutritional, chemical, psychological, and temperature-related stress. When considering various stress factors, heat stress stands out as the most significant challenge in the context of ongoing climate change (Silanikove and Koluman 2015), and it is regarded as one of the primary stressors in the tropical (Nardone et al., 2010), arid (Silanikove 1992), and semiarid (Silanikove 2000; Al-Dawood 2015) parts of the globe. Heat stress is the cumulative effect of both internal and external factors that elevate an animal's body temperature and trigger a physiological response (Yousef 1985). According to Polsky and von Keyserlingk (2017), heat stress is responsible for considerable welfare issues and economic losses in the global dairy cattle sector, primarily because it is negatively correlated with production (Sammad et al., 2020), reproduction (Schuller et al., 2014), health and immunity (Das et al., 2016; Wang et al., 2019), and longevity (Burdick Sanchez et al., 2013). Heat stress induces the production of reactive oxygen species (ROS), which are by-products of oxygen metabolism generated during cellular respiration. When ROS production exceeds the capacity of antioxidative defenses, oxidative stress develops. This oxidative stress in heat-stressed cows disrupts metabolic processes and compromises lactation performance (Gao et al., 2017; Guo et al., 2021). As homeothermic animals, cows typically maintain a body temperature between 38.5 and 39.5 °C and operate within a thermoneutral zone of –1.1 to +25 °C (Collier et al., 2015). When cows are exposed to temperatures that surpass this thermoneutral range, they exhibit various physiological responses—such as elevated body temperature, as well as metabolic, endocrine, and inflammatory adjustments (Cincovic et al., 2011; Liu et al., 2019). Ultimately, maintaining homeothermy under hot conditions depends on the animal’s ability to balance internal heat production (thermogenesis) with heat dissipation (Carabano et al., 2019; Becker et al., 2020).

Ambient temperatures are generally higher during the day because of solar radiation and begin to drop after sunset. However, over the past 50 years, significant increases in the daily minimum temperature have narrowed the diurnal temperature range (Braganza et al., 2004). This range is a critical indicator of climate change and plays an essential role in heat stress. When ambient temperature and humidity do not fall sufficiently at night compared to daytime levels, dairy cattle are unable to shed the heat accumulated during the day, leaving them in a sustained state of heat stress (Becker et al., 2020).

Cattle accumulate heat during the hottest hours (midday), increasing core body temperature, respiration rate, and metabolic stress. Without sufficient nighttime relief, animals enter the next day under cumulative stress, exacerbating chronic health and productivity declines. Assessing physiological indicators under these diurnal conditions can help identify animals with superior coping mechanisms. The Temperature-Humidity Index (THI), which integrates both ambient temperature and relative humidity, is used to estimate the effects of heat stress on production (Ravagnolo and Misztal, 2000). Generally, a THI below 70 is deemed comfortable, between 75 and 78 is stressful, and above 78 is considered hazardous (Kadzere et al., 2002). Additionally, diurnal fluctuations in temperature and humidity can lead to variations in cow blood parameters (Zhang et al., 2023). Therefore, evaluating the impact of heat stress on the physiological health of dairy cows should also consider the broader spectrum of blood biochemical changes associated with these diurnal variations (Zeng et al., 2023).

MATERIAL and METHOD

Ethical Statement

The ethical approval for this study was obtained from the Department of Animal Production and Health, Faculty of Agriculture and Life Sciences, Federal University Wukari, through the Institutional Animal Care and Use Committee (approval number FUW/AGR/APH/037), prior to the commencement of the research. All procedures involving animals were conducted in accordance with the guidelines of the Nigerian Institute of Animal Science regarding location, experimental design, and management.

Study Area and Experimental Animals

The study was conducted at the Cattle Unit of the Teaching and Research Farm, Federal University Wukari, Nigeria during the peak of hot-dry season (Feb-March) in the Southern Guinea Savannah zone of Nigeria. Twenty (20) White Fulani cows aged 24-36 months were selected for the study. The study was conducted in a well-ventilated cattle pen that was oriented east-west and featured a galvanized metal roof and a concrete floor. All animals were vaccinated and confirmed to be clinically healthy before inclusion. Furthermore, the cattle grazed on pasture daily within a semi-intensive system.

Thermoregulatory Measurements

Body temperatures of the experimental animals were taken bi-weekly, in the mornings (10.00 am) prior to grazing and afternoons (3.00 pm) after grazing using an infrared thermometer (ARTLIGHT ZK-YK1028, Greece) at three different regions of the body (left ear, muzzle and neck) positioned at approximately 5 cm from the target spot on the animal. Using a digital thermo-hygrometer (HTC-1), temperature and relative humidity were measured at 10:00 am and 3:00 pm every day of the experimental period. These measurements were then used to determine the THI for both the morning and afternoon, following the method described by NRC (1971):

$$THI = (1.8 \times T_{db} + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T_{db} - 26.8)] \quad (1)$$

Where, THI = temperature-humidity index; T_{db} = dry-bulb or ambient temperature in °C and RH= Relative Humidity.

Blood Collection and Analyses

Two (2) ml of blood each were randomly collected from five animals in the morning (10.00 am) and afternoon (3.00 pm) on the same day at the end of the experimental period from the jugular vein into collecting tubes containing Ethylene Diamine Tetra Acetate (EDTA) and no Ethylene EDTA for haematological and serum biochemical analyses respectively. The collected samples were transferred in an ice packed flask to the laboratory and kept at 4℃ in a refrigerator until analysis. Serum parameters (serum glucose, triglycerides, cholesterol, High Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL) and Very Low-Density Lipoprotein (VLDL)) were determined. Anti-oxidative indices were also determined using commercial kits. For the liver function, alanine transaminase (ALT) aspartate transaminase (AST), alkaline phosphatase (ALP) was also determined.

Data Analyses

Data collected from the experiment were subjected to t-test using Fit Y by X function of JMP 10.0. Graphs were prepared using GraphPad Prism 6. Pearson’s correlation coefficients were computed to assess the relationships among variables, using the "CORREL" function in MS Excel. Results were expressed as the mean ± standard error of the mean (SEM), with $P < 0.05$ considered statistically significant.

RESULTS and DISCUSSION

Temperature-Humidity Index (THI)

Graph showing the morning and afternoon THIs (Figure 1) indicate both THIs were above the thermoneutral limit, indicating the presence of heat stress both in the mornings and afternoons. Combining temperature and humidity into THI provides a useful metric to gauge livestock comfort in varying environmental settings. An increased THI value translates to heightened heat stress on cattle. For instance, beef cattle perform best at a THI under 74, encounter mild stress between 75 and 78, moderate stress from 79 to 83, and face severe stress when THI exceeds 84 (Bulitta et al., 2015). Additionally, the observed data indicate that during the day, high THI levels placed cows under significant heat stress, disrupting their thermoregulatory mechanisms and normal body temperatures, which can adversely affect overall productivity (Habeeb et al., 2018).

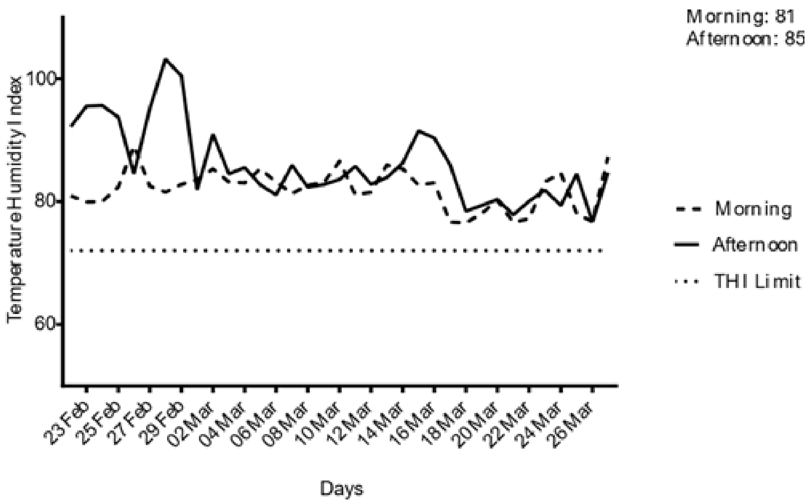

Figure 1. Daily THI at the cattle unit during the experimenal period (Febb-Mar)

Body Temperature

Body temperature of the White Fulani taken at three different regions of the body (Table 1) indicated significant ($P<0.05$) differences between the morning and afternoon temperatures. Afternoon readings show elevated body temperatures for the muzzle, left ear and neck. During periods of high ambient temperature, any increase in an animal's body temperature is determined by both the accumulation of heat and its simultaneous loss to the environment (Lees et al., 2019). The absorption of external heat by the animal's body surface elevates its temperature, which then forces the animal to activate thermoregulatory processes to sustain homeostasis by venting out surplus heat (Terrien et al. 2011; Renaudeau et al. 2012; Mota-Rojas et al., 2021). One key mechanism involves the skin acting as a radiator, whereby heat is emitted from the body surface into the air (Collier et al., 2019). Moreover, animals employ vasodilation and sweating as heat loss mechanisms; vasodilation, in particular, is initiated by an increased heart output that directs blood to the capillaries near the skin's periphery to aid in dissipating body heat (Habeeb et al., 2018; Shilja et al., 2016; Wang et al., 2020). If the body's natural cooling mechanism fails to lower its temperature or when ambient temperatures remain high, the brain triggers sweat production on the skin, enabling additional heat loss through the evaporation of liquid sweat (Habeeb et al., 2018).

Table 1. Body Temperature of White Fulani Cattle exposed to heat stress

Parameters	Morning	Afternoon	SEM	P value
Muzzle (°C)	35.24[b]	37.79[a]	0.23	<0.0001*
Left Ear (°C)	35.72[b]	37.71[a]	0.25	<0.0001*
Neck (°C)	36.33[b]	38.33[a]	0.21	<0.0001*

[a, b] Means with different superscript on the same row differ significantly ($P < 0.05$), SEM: Standard error of mean

Biochemical Indices

Biochemical indices of White Fulani cattle exposed to heat stress (Table 2) indicate elevated ($P>0.05$) values for almost all indices measured. Although not significant, afternoon values were elevated compared to the morning values. Blood parameters can exhibit diurnal variations due to the body's circadian rhythms and the stress response. These observed changes in biochemical indices between morning and afternoon although not statistically significant, the trends provide insight into how cattle might be subtly adjusting their physiology in response to diurnal heat variations. This suggests potential buffering capacity in the cattle to maintain metabolic stability under prolonged heat stress conditions. These small metabolic shifts, whether reflected in stress-mediated hyperglycaemia, haemoconcentration (as evident from total protein and urea levels), alterations in protein synthesis (albumin), or lipid metabolism adjustments (cholesterol, triglycerides, HDL) are consistent with broader observations in literature (Silanikove 2000; Kadzere et al., 2002; West 2003; Renaudeau et al., 2012; Baumgard and Rhoads 2013).

Table 2. Biochemical indices of White Fulani Cattle exposed to heat stress

Parameters	Morning	Afternoon	SEM	P value
Glucose (mg/dl)	51.6	54.8	3.07	0.4827
Total Protein (g/dl)	7.02	7.32	0.44	0.6416
Urea (mg/dl)	19	20.4	2.29	0.6765
Albumin (g/dl)	3.1	2.86	0.27	0.5469
Creatinine (mg/dl)	1.64	1.24	0.18	0.1531
Cholesterol mg/dl	66.6	68.8	3.14	0.6343
Triglycerides mg/dl	105.2	121.8	6.19	0.0946
HDL mg/dl	79	87.6	5.89	0.3323

SEM: Standard error of mean

Antioxidant Indices

The analysis of antioxidant and enzyme levels between morning and afternoon readings in cattle under heat stress conditions revealed no significant ($P>0.05$) differences in any of the measured parameters. Specifically, there were no statistically significant changes in Malondialdehyde (MDA), Glutathione (GSH), Catalase, Superoxide Dismutase (SOD), Alkaline Phosphatase (ALP), Aspartate

Aminotransferase (AST), and Alanine Aminotransferase (ALT) levels. This suggests that the cattle's antioxidant defence mechanisms and enzyme activities remained stable throughout the day under continuous heat stress conditions. These findings provide insight into early-stage compensatory responses that may mask emerging physiological strain. Oxidative stress readily damages biological macromolecules, thereby disrupting key metabolic and physiological pathways (Zhang et al., 2017).

Table 3. Antioxidative indices of White Fulani Cattle exposed to heat stress

Parameters	**Morning**	**Afternoon**	**SEM**	**P value**
MDA (nMol/l)	6.76	6.78	0.76	0.987
GSH (µ/l)	5.44	4.96	0.4	0.4206
Catalase (µ/l)	5.45	5.09	0.69	0.7279
SOD (µ/l)	5.04	5.35	0.48	0.664
ALP µ/l	19.4	23	1.91	0.2183
AST µ/l	76.4	72.8	4.11	0.553
ALT µ/l	12.4	15.4	1.83	0.2805

MDA= Malonaldehyde; GSH= Glutathione, SOD= Superoxide Dismutase; ALP= Alkaline Phosphatase; AST=Aspartate Transaminase; ALT= Alanine Transaminase; SEM: Standard error of mean

Haematological Indices

The t-test analysis of the blood parameters revealed a significant difference in the RBC counts between morning and afternoon readings. The RBC count significantly decreased from 5.86 ($x10^{12}/1$) in the morning to 3.12 ($x10^{12}/l$) in the afternoon (P = 0.0001), indicating a noteworthy change. Conversely, no significant differences were observed in other parameters, including Packed Cell Volume, Hemoglobin, WBC, Neutrophils and Lymphocyte, with P values greater than 0.05. These findings suggest that while RBC count shows a significant diurnal variation, other blood parameters remain relatively stable throughout the day. Also referred to as packed cell volume (PCV), haematocrit measures the percentage of erythrocytes in 100 mL of blood (Reece et al., 2015). Values obtained in this study is within the recommended range (Constable et al., 2017). Variations in haematocrit values, which reflect corresponding changes in erythrocyte counts (Merdana et al., 2020), alter blood viscosity. Both high and low haematocrit levels can affect blood flow dynamics and, consequently, impact cardiac function. Although no significant changes in hemoglobin were observed in this study, a decrease in hemoglobin in the afternoon compared with the morning values were observed. Both readings are below recommended levels for hemoglobin in cattle (Merck Manual 2012).

According to Lemerle and Goddard (1986), hemoglobin concentration is regulated in response to changing environmental conditions and is inversely related to environmental temperature. The pronounced decline in hemoglobin levels is likely attributable to higher water consumption in the summer (Shehab-el-deen et al., 2010), resulting in hemodilution as additional water is transported in the circulatory system for evaporative cooling during periods of extreme heat (El-Nouty et al., 1990). RBC levels reported in the afternoon is outside the recommended range for cattle (Constable et al., 2017). Typically located in red blood cells, hemoglobin is a protein complex containing iron that plays a vital role in delivering oxygen to body tissues (Chi et al., 2021). Recognized as critical indices, both haematocrit (HCT) and hemoglobin (HGB) reflect the oxygen delivery capacity of RBCs (Lima et al., 2011). Rising ambient temperatures lead to a reduction in blood constituent concentrations through hemodilution, provided that cool water is available (Wood and Quiroz-Rocha, 2010).

Table 4. Haematological indices of White Fulani Cattle exposed to heat stress

Parameters	Morning	Afternoon	SEM	P value
Packed Cell Volume %	29.8	26.8	1.96	0.3106
Haemoglobin g/dl	9.9	8.9	0.65	0.3062
WBC x109/1	6.08	6.58	0.76	0.6555
RBC x1012/1	5.86a	3.12b	0.27	0.0001*
Neutrophils %	32.6	27.4	4.09	0.3947
Lymphocyte %	64.2	72.6	3.9	0.1661

a, b Means with different superscript on the same row differ significantly ($P < 0.05$), SEM: Standard error of mean

Srikandakumar and Johnson (2004) report that heat stress may lead to the lysis of erythrocytes by generating excess free radicals in their lipid-dense membranes. As a result, hemoglobin levels can drop or the synthesis of hemoglobin may be compromised due to reduced nutrient intake when animals consume less feed. According to Lawrence et al. (2017), hemoglobin levels are linked to the number of erythrocytes, so a reduction in erythrocyte count leads to lower hemoglobin levels. Hemoglobin synthesis occurs during the initial phase of erythrocyte development; therefore, any interference with erythrocyte formation leads to disrupted hemoglobin production. While a similar and significant drop in RBC was also observed, hemolysis occurring in vitro—caused by vigorous handling during transport could explain the observed ~50% decline in RBC counts.

White blood cells, frequently referred to as leukocytes, are pivotal in shielding the body against infections. Cattle experiencing heat stress have shown elevated levels of these cells (Das et al., 2016), and an increase in THI may also cause changes in the balance of white blood cell types.

Relationship Between Body Temperature and THI of White Fulani Cattle Exposed to Heat Stress

The correlation analysis of temperature at different body regions with the Temperature-Humidity Index (THI) revealed that muzzle temperature showed a weak positive correlation with THI (r = 0.22; P = 0.066). This relatively low correlation suggests that the muzzle temperature may be influenced by extrinsic factors such as direct solar radiation and evaporative cooling, which can mask the underlying core body heat changes. Baumgard and Rhoads (2013) and West (2003), both noted that facial or peripheral measurements can sometimes be less reliable due to environmental interference. The temperature at the left ear demonstrated a moderate positive correlation with THI (r = 0.4; P = 0.0006), indicating a significant relationship between left ear temperature and THI. The left ear which has a rich vascular network, is likely to be a more sensitive indicator of thermal load and internal body temperature fluctuations. This finding agrees with previous research by Kadzere et al. (2002) and Rhoads et al. (2009), who observed that the auricular region more accurately reflects changes in systemic temperature during heat stress. Neck temperature also exhibited a moderate positive correlation with THI (r = 0.3; P = 0.0120), suggesting a significant relationship between neck temperature and THI. The neck region is characterized by abundant blood flow and relatively less exposure to direct cooling, making it a good proxy for internal temperature dynamics. The neck is an area with prominent vascularization and a reliable indicator of core temperature variations (Gaughan et al., 2007; Bernabucci et al., 2010). These findings indicate that while the temperature at the muzzle does not significantly correlate with THI, the temperatures at the left ear and neck show significant positive correlations with THI; suggesting that the left ear and neck temperatures might be more sensitive indicators of heat stress in cattle compared to the muzzle temperature.

Table 5. Correlation between THI and body temperatures of White Fulani Cattle exposed to heat stress

Parameters	Temp	THI	Cor	P value
Muzzle	36.51	83.19	0.22	0.066
Left Ear	36.72	83.19	0.4	0.0006*
Neck	37.33	83.19	0.3	0.0120*

$^{a, b}$ Means with different superscript on the same row differ significantly (P < 0.05), SEM: Standard error of mean

CONCLUSION and RECOMMENDATIONS

This study demonstrated that White Fulani cattle experience significant heat stress throughout the day, as evidenced by elevated THI values and increased body temperatures. Despite the severe stress conditions, most biochemical and antioxidant indices remained stable, indicating potential physiological adaptation mechanisms. However, the significant decrease in RBC counts highlights a specific vulnerability under continuous heat stress. The correlation of temperatures at the left ear and neck with THI suggests these body regions as sensitive indicators of heat stress. The findings underscore the importance of effective management and nutritional strategies to support cattle health and productivity under heat stress conditions, providing valuable insights for enhancing livestock welfare and performance in hot climates.

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Availability of Data and Materials

The datasets during and/or analyzed during this study is available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no competing interests.

Authors Contributions

NDI analyzed and interpreted the haematological and biochemical data. DJS, HNH, LBB were involved in data collection. SL and HNH participated in literature review. ZGS and ARJ were involved in animal management. ALA and OKJ contributed to analyzing and interpreting the thermoregulatory data and drafting the manuscript. All authors read and approved of the final manuscript.

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